Ex Vivo and In Vivo Biomechanical Test of Implant Attachment to Various Materials: Introduction of a New User-Friendly Removal Torque Equipment

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ABSTRACT

Objective: The removal torque (RTQ) analysis is commonly used for biomechanical evaluation of osseointegration. The overall aim of this study was to verify results obtained with a newly developed equipment for biomechanical testing of osseointegration.

Methods: Verification of the new equipment for biomechanical tests involved three experiments: Part I, comparison of RTQ between implants placed in four different types of dental synthetic plasters. Part II, comparison of RTQ between custom made, experimentally used implants to self-tapping, commercially available implants molded in the same type of dental plaster. Part III, comparison of RTQ between commercially pure titanium implants to Ti6Al4V implants placed in rabbit bone, 6 weeks after insertion. Briefly, for all experiments, the peak RTQ values and the removal process were recorded every 0.01 seconds up to 10 seconds. After the measurements, peak RTQ values were converted to shear strength.

Results: The developed equipment sensitively responded to the changes of properties related to the molding plasters, implant topographies, and materials. The monitored graphs corresponded well to the expected properties of the different implants and tested materials.

Conclusion: The new RTQ equipment proved to be accurate and could add new knowledge in understanding the biomechanical aspects of osseointegration.

KEY WORDS: ex vivo, in vivo, removal torque, shear strength

INTRODUCTION

The bone tissue attachment to an implant, that is the osseointegration of an implant, can be tested with various research equipments related to biomechanical tests. Such tests most often involve geometry-dependent torque tests converted to geometry-independent shear strength tests[1–3] as well as tensile tests (push and/or pull out tests).[4–6] The test method used is often dependent on the macro-design of the implants, that is screw- versus cylinder-type implants. It is our opinion that screw-shaped implants for in vivo tests of integration in bone beds cannot be tested with push- or pull-out tests, but they should be unscrewed with removal torque (RTQ). The RTQ data in Nmm can then be converted to shear strength data in N/mm^2 which is important for the understanding of the interfacial strength between the implant and the integrated bone tissue.

The advantage with in vivo three-dimensional tests is that they are quite rapid to conduct and the results are displayed more or less simultaneously compared with two-dimensional histomorphometrical tests performed on histologic stained cut and ground sections with the tested implant in situ.[7] However, the three-dimensional RTQ tests render a nonshear strength result in Ncm. The majority of published articles related to biomechanical

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test of osseointegration in preclinical test have used RTQ and the results are presented in Ncm.[8,9] Shear strengths can be calculated from RTQ-,[10] push-, and pull-out tests[11] if the area of the bone/implant interface is known. In order to convert RTQ data (Ncm) to shear strength data in N/mm^2, a section (or more) is needed from the implant with surrounding bone.[12] Albeit these types of additional data involve laboratory processing of the retrieved samples, we found it an added value to the biomechanical tests.[13] Unfortunately bone-implant contact measurements cannot be performed on RTQ-tested implants because of the interfacial rupture; however, bone area measurements can be performed.[14] This article will deal with RTQ tests of screw-shaped implants performed with a new custom made, user-friendly, research equipment. In our laboratories, RTQ tests have a long track record and in fact almost all doctoral theses from the laboratories that are involved in *in vivo* animal tests have been using the RTQ as one measure of osseointegration.[15–19] The equipments that have been used (and still are in use) have a great span, that is from hand testing instruments (15 BTG-N and ATG6CN, Tohnichi, Japan) where the accuracy is dependent on the user, who may interfere with the equipment and create incorrect results.[15,20] Such handling of the handheld torque gauge can result in higher values because of misalignment of the instrument as well as applying a too rapid unscrewing technique as well as in false low values because of a to slow unscrewing procedure.

Being aware of such manmade created errors when using the handheld Tohnichi Torque Gauge device (Tohnichi Mfg. Co., Ltd., Tokyo, Japan), we decided to construct electronic equipment for RTQ tests that excluded the operator sensitivity. That equipment builds up the torque in a standardized way every time, and the peak value, that is the RTQ (Ncm) is registered on a display. This equipment was bench tested 1992 and has been used in house in the majority of scientifically published articles where RTQ tests are applied.

In order to monitor the loosening process, we have recently applied a computer program to the former electronic equipment that enabled us to study the torque process with better accuracy.[10] However, as the implant modification has become more complex, and as a result, the healing period has significantly shortened,[21,22] we have now experienced a need for higher accuracy in the complete RTQ process to distinguish such differences. Despite the accuracy of the former equipment, RTQ values below a few Ncm could not be obtained. There is also the problem that the former equipment was built on components that now is becoming obsolete. Yet another challenge is to develop a downsized, handy, and reliable equipment that can be transported to various laboratories since today, commercially available testing devices are often fixed, and quite big that cannot easily be moved and often need a specialist in charge.

This article describes and shows results obtained with a novel, user-friendly, custom-made, and portable equipment designed especially for biomechanical testing of implant integration. Both bench-testing ex vivo and in vivo test have been performed using the new RTQ equipment/prototype. The novel computer program display and monitor the curve related to the entire biomechanical testing process.

MATERIALS AND METHODS

RTQ Equipment

The torque equipment is assembled from an electrical motor and a strain gauge lever mounted on a gantry, in order to facilitate a straight and stable adoption to the implant.

The equipment is made of parts available on the market, however, installed in a customized rig. The torque is applied to the implant from the electrical motor which, controlled by a laptop computer, increases the current and thereby the torque in a controlled way. The gentle increase of torque power gives an optimal time span for the unscrewing sequence enabling us to measure the time and torque throughout the process. The available equipments on the market were not originally designed for measuring RTQ for laboratory animals and, therefore, we designed the current equipment specifically aimed for this kind of laboratory implant experiments.

A control unit increases, linearly over time, the electrical current to the motor and thereby the torque strength. The torque strength is increased with 22.5 Ncm/s from the start of the measurement (bench tests performed in house). The measurements from the strain gauge are transmitted via a control box to the computer with a frequency of 100 values per second. The computer collects, calculates, and displays the values in a way chosen by the operator. The removal of the implant can be monitored as curves giving the torque as a function over time. All 1,000 values (if choosing a running

TABLE 1 Prior to Testing, the Equipment was Calibrated with a Prescribed Weight (W, 1.083 or 0.5345 kg) and Lever Arms (L, 0–10 cm)

True Torque L [cm] × m = 1.083 kg F = 10.624 N	Equipment Value 1–100 Ncm		True Torque L [cm] × m = 0.5345 kg F = 5.243 N	Equipment Value 1–50 Ncm	
0 × F = 0	2,260	2,260	0 × F = 0	2,263	2,263
1 × F = 10.624	2,350	2,324	1 × F = 5.243	2,298	2,280
2 × F = 21.248	2,400	2,393	2 × F = 10.486	2,336	2,318
3 × F = 31.872	2,480	2,465	3 × F = 15.729	2,373	2,355
4 × F = 42.496	2,552	2,541	4 × F = 20.972	2,415	2,393
5 × F = 53.120	2,626	2,615	5 × F = 26.215	2,455	2,437
6 × F = 63.744	2,712	2,691	6 × F = 31.458	2,490	2,470
7 × F = 74.368	2,790	2,769	7 × F = 36.701	2,530	2,515
8 × F = 84.992	2,862	2,845	8 × F = 41.944	2,562	2,554
9 × F = 95.616	2,934	2,924	9 × F = 47.187	2,606	2,597
10 × F = 106.242	2,993	3,020	10 × F = 52.434	2,640	2,634

Variations in, for example, temperature and electrical power were mitigated through this calibration process.
The table shows the equipment values, which the computer interpolates into a function used for interpreting the strain gauge values to Ncm in both high (1–100 Ncm) and low (0–50 Ncm) range removal torque.
Note that the difference between the two calibrations using the same weight reveals an inaccuracy of ≤1% when repeated 4 times.
The true torque is calculated for the high and low weight, respectively, as follows:
$T = L \times F$ where $F = m \times g$.
T = torque, m = mass, L = lever arm, g = acceleration of gravity (9.81 m/s^2).

time of 10 seconds) are stored and can be exported to MS Excel files rendering possibilities of further data analyses not possible before. Additional programs for presentation of the results are also easily applied.

The computer program also supports the calibration of the equipment through interpolating a number of torque measurements when using known weights and lever arms. When the calibration is done along a chosen scale, for example from 10 to 100 Ncm, this ensures accurate values over the whole span in a RTQ evaluation. Through the improved calibration possibilities, the accuracy of this RTQ equipment is limited to the background noise of its components. Table 1 shows the calibration data obtained when calibrating with two different weights (1.083 and 0.5345 kg, respectively) and various length of the lever arm (0–10 cm). This calibration revealed an inaccuracy of ≤1% when repeated four times.

Part I: Comparison of RTQ between Implants Placed in Four Different Types of Dental Synthetic Plasters

Screw shaped commercially pure titanium (cpTi) implants, prepared in house, that is experimental implants (outer diameter 3.75 mm), with a total threaded length of 6 mm and a square head of 2 mm were caste/molded in 24-well polystyrene wells (NUNC, Denmark) using four different dental synthetic plasters with different hardness leaving the nonthreaded upper part visible ($n = 5$ for each group). The molding was made simultaneously and the hardening continued over night. The removal torque tests were conducted at the very same time-period.

Gypsum 1 (G1): Coecal TM Dental Stone Type III (GC America, Illinois; compressive strength: 306 kg/cm^2 = approximately 31 N/mm^2).

Gypsum 2 (G2): Molda (Heraeus, Kulzer GmbH, Germany; compressive strength not shown; Density 300 g/cm^3).

Gypsum 3 (G3): Giludur Synthetic Hard Plaster (BK Giulini Chemie GmbH, Germany; compressive strength 30 N/mm^2).

Gypsum 4 (G4): Fino Synthetic Rock (Fino GmbH, Germany, compressive strength 64 N/mm^2).

These gypsum materials were chosen because they have shown RTQ values within similar range compared with most in vivo results that we have obtained in various studies throughout the years.

Shear Strength. The peak torque results from the tests were converted to shear strength data using the geometrical formula which was first described in the article by Rubo De Rezende and Johansson[12] and further compared with a more complex formula, rendering similar results, used by Johansson.[23]

The torque is divided with the area of the implant multiplied with the lever arm. Because the length is measured along the entire implant contour, the lever arm is the mean distance from the implant center to the screw surface. In the more complex formula, the lever arm was integrated over the implant area contour. However, our tests have proven that the simple geometry formula is in agreement with the more complex one.[23]

T (torque in Nmm)/π × d (mean diameter of implant) × l (implant length in gypsum) × rl (lever arm = mean radius of implant). The additional area and lever from the circular bottom of the implant were also added. The lever is in this case 2/3rl.

$$\tau = T(Nmm)/[(\pi \times d \times l \times rl) + (\pi \times rl^2/2 \times 2rl/3)]$$

Part II: Comparison of RTQ between Custom Made, Experimentally Used Implants to Self-Tapping, Commercially Available Implants

Screw shaped cpTi implants, prepared in house, that is experimental implants, with an outer diameter of 3.75 mm and a total threaded length of 6 mm, were molded in gypsum type 3, in standard sized plastic wells (as above). The square head was left visible above the gypsum surface.

Moreover, commercially available cpTi implants with an apical groove, were also molded in gypsum type 3, leaving the nonthreaded part visible.

These two types of implants ($n = 5$ of each type) were tested with the RTQ equipment concurrently.

The peak torque results from the experimental implants only, that is without apical grooves were converted to shear strength data using the geometrical formula defined in part I. The RTQ data obtained from the implants with the apical grooves were not converted to shear strength data because the geometry of the implant is more complex. Asymmetrical implants like the ones with apical grooves ads tensile components that cannot be transformed to shear strengths.

Part III: Comparison of RTQ between cpTi Implants to Ti6Al4V Implants Placed in Rabbit Bone

The in vivo experiment involved cpTi, and titanium alloy (Ti6Al4V) implants ($n = 4$ of each material) with an outer diameter of 3.75 mm and a total threaded length of 6 mm inserted in a rabbit using the in-house routine design for testing implant integration. This design involves insertion of one implant in each femur condyle region and three implants of the same material in each tibia (tuberositas tibia region) with the follow up of 6 weeks. The RTQ data was recorded for each implant and the peak torque value form each implant was used for shear strength calculations (for formula, see part I). The length in this case refers to the estimated bone length in close vicinity to the implant measured on cut and ground sections (see below) in the light microscope.

After RTQ tests were completed, the implants were left in the bone bed. Bone blocks with the implants in situ were immersed in fixative followed by routine handling of specimens for preparation of two-dimensional undecalcified cut and ground sections with the implant in situ.[7,24,25] The sections were histologically stained in 1% toluidine blue in 1% borax solution, mixed in a 4:1 proportion with 1% pyronin-G solution and inspected in the light microscope. Bone length in close vicinity to the implant was measured on both sides of the implant and a mean value was presented per section, and this length was used in the formula. All samples were measured and conversions were done for each of them.

RESULTS

Part I: Comparison of RTQ between Implants Placed in Four Different Types of Dental Synthetic Plasters

RTQ. The loosening torque of the implants molded in the different gypsums showed various biomechanical results as well as different shapes of the curves (Figure 1, A). The peak torque values were 19.0-, 5.6-, 17.7- and 41.6) Ncm for the G1, G2, G3 and G4, respectively, or 190-, 56-, 177- and 416 Nmm (Figure 1, B).

Shear Strength. The 6-mm implant had a total surface length of 12.8 mm (measured on a cut and ground section of an implant in the light microscope). This length was used for shear strength calculations (using the mean value for each test). The corresponding shear strength data for G1–G4 were 0.79, 0.23, 0.74 and 1.74 N/mm^2, respectively.

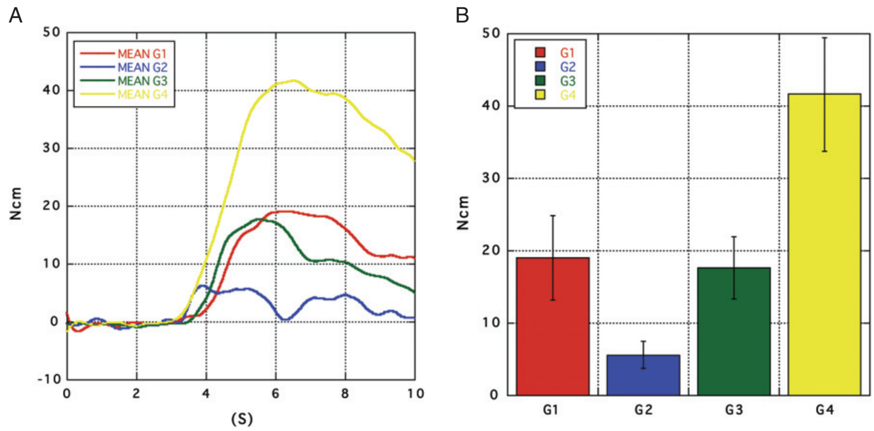

Figure 1 (A) Removal torque curves (mean values) for implants placed in different gypsums ($n = 5$; G1–G4). (B) Graph showing the mean peak values in Ncm and standard deviation (SD) for all groups (G1: 19.0 [5.9]; G2: 5.6 [1.9]; G3: 17.7 [4.3]; G4: 41.6 [7.8]).

Part II: Comparison of RTQ between Custom Made, Experimentally Used Implants to Self-Tapping, Commercially Available Implants

RTQ. The mean RTQ for the experimental 6-mm implants were 224 Nmm compared with 725 Nmm for the commercially available implants (Figure 2, A–D).

Shear Strength. Only the experimental implants were included in conversion to shear strength data. Measurements of the entire implant lengths were performed on one cut and ground section of the implant in the microscope (using a 2× objective) and revealed a total surface length of 12.8 mm. Taking the entire threaded implant length in consideration, that is 12.8 mm and a RTQ of 224 Nmm the shear strength was 0.94 N/mm^2.

Part III: Comparison of RTQ between cpTi Implants to Ti6Al4V Implants Placed in Rabbit Bone

RTQ. The data from the in vivo test performed after 6 weeks of follow-up in rabbit bone demonstrated higher loosening torques for the cpTi implants compared with the Ti6Al4V, mean 198 Nmm versus mean 132 Nmm, respectively. The RTQ curves and bars are presented in Figure 3, A and B.

Shear Strength. Measurements of the "true" bone length in close vicinity to the implant in the microscope revealed a mean of 1.95 mm for the cpTi and 1.82 mm for the Ti6Al4V. Using these actual lengths, the conversions to shear strengths demonstrated 5.43 and 3.88 N/mm^2, respectively.

DISCUSSION

The RTQ curves (torque as function of time) shows the building up of torque to the peak value when the screw is released, the removing torque. The equipment builds up the torque linearly with 22.5 Ncm/s and the maximum value for each RTQ test would follow this line if the screw is fixed in a material without any elasticity. The registered peak values, often appears to the right of the maximum torque line, which indicates that the material or the fixation of the specimen has a certain elasticity. The distance between the maximum torque line and the torque curve for each specimen may indicate a value of the elasticity of the anchoring in the bone bed; however, because numerous factors are involved, it would be difficult to fully clarify the mechanisms. The following part of the curve, after the maximum value, shows the torque resistance during the unscrewing of the implant. In the next generation of the RTQ meter, our task will be to minimize the inertia and elasticity of the components in order to get a value of the elasticity of the bone anchoring.

The conversion from RTQ to shear strength is of course pure geometry. If the geometry is "complex" and thus differs between implants as shown in part 2 of this study, no "true" shear strength comparison can be performed because of the design of the commercially available implant in the apical portion, that is undercut/grooves. This apical design is most likely the reason for the high RTQ values obtained.

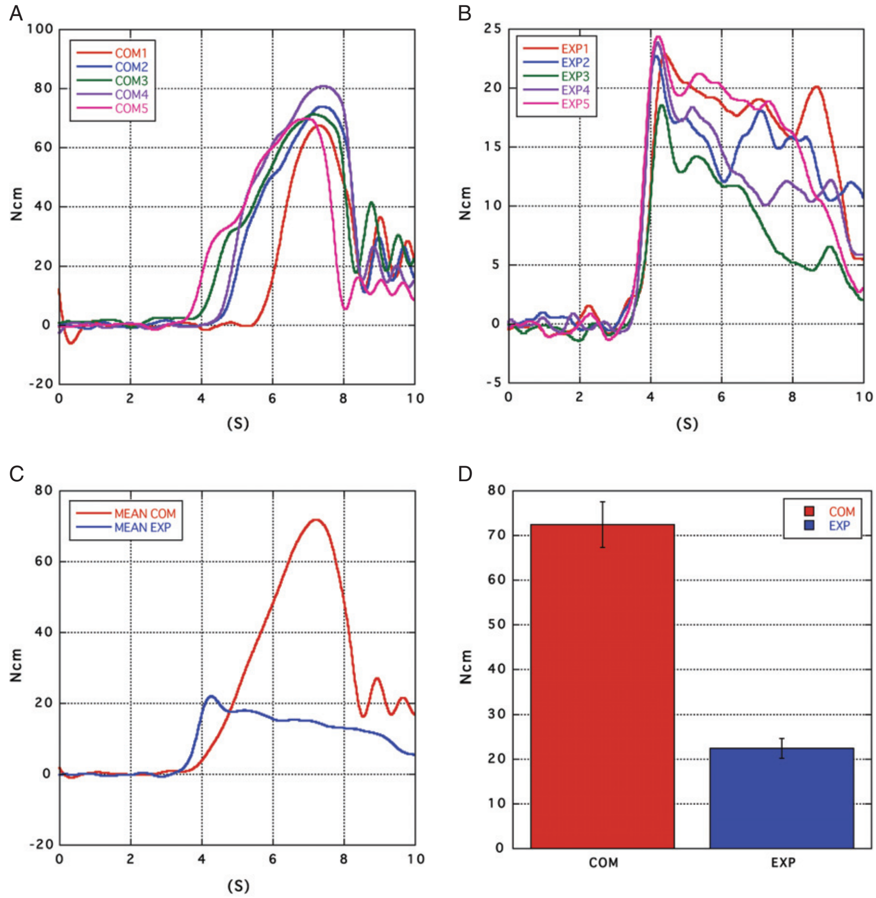

Figure 2 (A) Removal torque curves for all commercial implants placed in gypsum type 3 ($n = 5$; COM1-COM5). (B) Removal torque curves for all experimental implants placed in gypsum type 3 ($n = 5$; EXP1-EXP5). (C) Removal torque curves (mean values) for the commercial implants and the experimental implants. (D) Graph showing the mean peak values in Ncm and SD for the two groups (COM: 72.50 [5.1]; EXP: 22.40 [2.2]).

Experimental implants used in part 1 and part 2, molded in the same gypsum type 3 revealed mean RTQ of 17.7 and 22.4 Ncm, respectively. Although similar type of implant was molded in the same type of gypsum, a few Ncm difference was noted between different gypsum batches. The obtained differences are probably related to the different molding times rendering a small inaccuracy in the hardness of the material. However, the

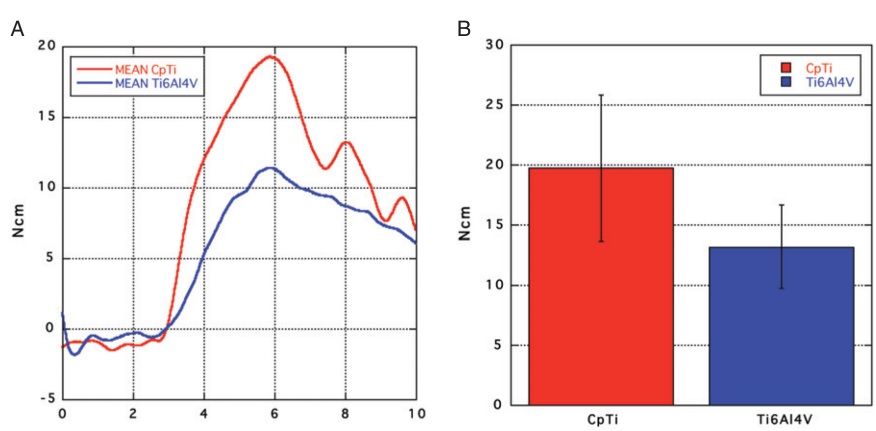

Figure 3 (A) Removal torque curves (mean values) for the cpTi implants and the Ti6Al4V implants placed in rabbits after 6 weeks ($n = 4$). (B) Similar data presented as graphs with SD. The mean values (Ncm) for cpTi: 19.75 and for the Ti6Al4V: 13.20.

intention was not to compare results from part 1 with part 2 tests; hence, it can be said that the differences would not challenge the accuracy of the new equipment.

One interesting question when applying new methods and new equipments in research is how to evaluate and test the accuracy of these devices. All equipments must be calibrated and validated, as in the case of the new tool used in the present study by using known weights and "reference materials" (similar screws but different hardness of gypsum for example).

The ex vivo test of the loosening torque of implants being molded and cured in gypsum with different hardness can be regarded as an easy, cost–effective, and reliable bench test. The results from these tests show clear differences in loosening torque between softer and harder gypsums where the former revealed very low values albeit clearly observed and monitored. In the past, it was considered very difficult to accurately measure such low torques. There are other materials to be used, such as the commercially available three-dimensional-structured artificial bone with various porosity (mimicking cortical and cancellous bone), which was used in the article by Tabassum and colleagues.[26] However, this study reflected the surgical technique used rather than the RTQ alone of an implant in dead material, because implants were placed in press-fit and nonpress fit prepared holes. Hence, the data obtained in the current study cannot be compared with these results.

Commercially pure titanium implants with various outer diameters (varying from 3–6 mm) have been tested in a similar animal model as the one used in the present article, using the in-house RTQ equipment.[2] The greater the implant diameter, the higher was the RTQ. However, converting the RTQ data to shear strengths, using the same formula as in the present article, showed no significant differences between the various implants.

These results point to the importance of not only present RTQ (geometry dependent) values in Ncm. The added value by converting to shear strength (geometry independent) is important as well. Implants with various designs (both macro- and micro-design) are sometimes involved in the very same study.[27] We find it difficult to judge/interpret such studies. It is impossible to determine what is actually measured if the tested implants do not have the same macro-design. Therefore, the results of the RTQ from the commercial implants with apical groves in this study (part 2) were not further converted to shear strength data because of the design of the implant where the apical portion most likely had a great input to the elevated torques obtained.

The present in vivo part of the study involved different materials (with similar macro- and micro-"designs") albeit they demonstrated different integration. Both the RTQ and the shear strength values were greater for the cpTi implants compared with the Ti6Al4V. Earlier in-house in vivo studies comparing these materials using various equipments have demonstrated similar findings.[10,28,29] In one study, using the Tohnichi Torque Gauge device, a 30% difference was observed between these materials after 12 weeks of follow up: mean 23 Ncm for cpTi and 16 Ncm for the alloy implants, respectively. Another in vivo test performed using the former electronic equipment, comparing cpTi and Ti6Al4V implants with surface alterations, that is two different surface roughness (prepared by blasting with TiO_2 particles of two different diameters) also revealed differences between the materials. The loosening torques of the implants was compared after 12 weeks of follow-up, using the rabbit model as in the present and previous[1] studies. The study also showed about 30% difference between cpTi and alloy implants, the former being significantly firmer integrated in bone compared with the latter. Converting to shear strength values also revealed similar data, that is the cpTi presented greater shear strength values. Yet, another in-house study comparing machined cpTi to alloy implants with various time of follow-up in rabbit bone demonstrated also differences and the cpTi implants presented higher RTQ values compared with the alloy implants at 1, 6, and 12 months of follow up.[29] These data, from various in-house studies using different RTQ equipments have all shown differences between bone integration of implants made of cpTi and titanium-6 aluminum-4 vanadium. The tests conducted on the new equipment in the present study demonstrated similar results: the cpTi implants were better integrated in rabbit bone compared with the alloy implants. Therefore, it is indicated that the accuracy of the new equipment and the former equipments are reliable, albeit the new equipment used in the present study will render much more information not possible with the former equipments. However, very small/low RTQ values such as below a few Ncm, presumably render nonaccurate values, that is background noise related to the equipment and therefore the next generation with higher sensitivity may resolve these issues and may further contribute to the evaluation of integration of implant materials in the future.

CONCLUSION

The various tests performed with the new equipment have shown a good repeatability and accuracy. This combined with the monitoring program enables us to follow the course of events during the building up of the RTQ and the following detachment. Presently, a great amount of data obtained with the new device is being analyzed, and the added value from the interpretations of the curves is ongoing.

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