Assessment of dental implant stability using resonance frequency analysis and quantitative ultrasound methods

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Abstract

Purpose: Quantitative ultrasound (QUS) and resonance frequency analyses (RFA) are promising methods to assess the stability of dental implants. The aim of this in vivo preclinical study is to compare the results obtained with these two techniques with the bone-implant contact (BIC) ratio, which is the gold standard to assess dental implant stability.

Methods: Twenty-two identical dental implants were inserted in the tibia and femur of 12 rabbits, which were sacrificed after different healing durations (0, 4, 8 and 13 weeks). For each implant, the ultrasonic indicator (UI) and the implant stability quotient (ISQ) were retrieved just before the animal sacrifice using the QUS and RFA techniques, respectively. Histomorphometric analyses were carried out to estimate the bone-implant contact ratio.

Results: UI values were found to be better correlated to BIC values ($R^2=0.47$) compared to ISQ values ($R^2=0.39$ for measurements in one direction and $R^2=0.18$ for the other direction), which were shown to be dependent on the direction of measurements. Errors realized on the UI were around 3.3 times lower to the ones realized on the ISQ.

Conclusions: QUS provide a better estimation of dental implant stability compared to RFA. This study paves the way for the future clinical development of a medical device aiming at assessing dental implant stability in a patient-specific manner. Clinical studies should confirm these results in the future.

Keywords: Implant stability, Bone-Implant contact, Quantitative ultrasound, Resonance frequency analysis, Dental implants

1. Introduction

The clinical assessment of dental implant stability is a difficult problem (1) because it depends on many parameters such as the implant properties, the surgical protocol and the patient behavior and bone quality (2). Moreover, there is a lack of standardization of the surgical procedures, in particular concerning the choice of the duration between implant insertion and loading, which may vary from 0 up to 6 months (3). An early implant loading may stimulate osseointegration phenomena (4), but may also degrade the consolidating bone-implant interface (BII) (5) in the case of unstable implants (6). Meanwhile, shortening the implant loading time is important to (i) avoid tissue losses and to (ii) limit the social impact of facial disfigurement. Therefore, an accurate evaluation of dental implant stability could help the surgeon to adapt his/her strategy and more specifically the choice of the healing period in a patient specific manner.

Different methods have been suggested to assess the implant stability. Most surgeons still rely on their proprioception (6) and accurate quantitative methods are required. Magnetic Resonance Imaging (7) and X-ray based techniques (8) are not adapted due to artifacts generated by the presence of titanium. Impact methods like the Periotest (Bensheim, Germany) (9) present a poor reproducibility (10).

Resonance frequency analysis (RFA) is the most commonly used technique to measure implant stability, and consists in measuring the first bending resonance frequency of a rod screwed into the implant (11). The Osstell® (Gothenburg, Sweden) is based on the RFA technique to measure the harmonic response of an implant through the implant stability quotient (ISQ), which can provide an assessment of the stiffness of the bone-implant structure (12), of the cortical bone thickness (13, 14), of the implant anchorage depth into bone (15) and of the marginal bone level (16). However, the properties of the BII (17) cannot be directly identified through RFA, and the orientation of the device was found to significantly affect the ISQ score (18). The correlation between the ISQ and bone implant contact (BIC) is relatively weak and remains a subject of debate (19-23). Moreover, sensitivity issues of ISQ to changes of periprosthetic bone tissue have been raised, due to the fact that only the first bending mode is considered (24).

Quantitative ultrasound (QUS) (25) represents a promising alternative to RFA in order to investigate the properties of the BII and therefore to assess dental implant stability. The set-up developed by our group consists of an ultrasonic echographic transducer screwed inside the implant that can measure the amplitude of the signal reflected by the bone-implant system. Different works have been carried out in silico, in vitro and in vivo in order to validate the approach. The interaction between an ultrasonic wave and a cylindrical implant was modeled numerically (26, 27) at the scale of the implant. Then, a realistic 3-D geometry was considered (28) and two studies carried out at the microscopic scale and taking into account the implant surface roughness and the implant threading allowed to quantify the effect of osseointegration phenomena on the ultrasonic response of the BII (29, 30).

Based on this concept, an in vitro preliminary study showed that the
reflection coefficient of an ultrasonic wave interacting with the BII significantly decreases as a function of healing time (31). A QUS device was developed to assess dental implant stability and was validated first ex vivo using cylindrical implants (32), and then in vitro using dental implants in a biomaterial (33) and in bovine bone tissue (34). The performance of RFA and QUS techniques were compared in vitro with implants inserted in bone mimicking phantoms (35). The QUS technique led to a significantly better estimation of different parameters related to the implant stability compared to the RFA technique (35).

An in vivo study (36) showed that QUS measurements were significantly sensitive to healing time, but the positioning of the ultrasonic probe on the implant abutment screw was performed manually, leading to reproducibility issues. Another in vivo study (37) considered a new version of the QUS probe. The results showed that the QUS technique led to more important variations of the indicator as a function of healing time compared to the RFA technique. However, the results obtained with the QUS and RFA devices were not compared to the bone-implant contact (BIC) ratio, which is the gold standard to assess dental implant stability.

The aim of this study is to evaluate the performance of the RFA and QUS techniques to assess dental implant stability by comparing the results obtained with both methods to those obtained via histomorphometric analysis, which leads to an estimation of the BIC ratio. Our strategy consists in using dental implants in rabbits and to consider stability measurements with both techniques (RFA and QUS) at different healing times.

2. Materials and methods

2.1. Animals

Twelve New Zealand White 5-months-old male rabbits (Charles River, L’Arbresle, France) with an average weight of 4.360 kg were used in this study. Animals were housed in a metal hutch in an environment with the European guidelines for care and use of laboratory animals. The study was carried out according to the EU Directive 2010/63/EU and has been approved by the ethical committee of the Alfort National Veterinary School (ENV A, project #20287). Temperature was maintained at 19°C and humidity at 55%. Artificial cyclic lightening and air conditioning system were used in the animal housing facility. Commercial food and water were provided ad libitum.

2.2. Surgical procedure

Twenty-two identical conical dental implants manufactured by Zimmer Biomet® (Warsaw, IN, USA) under the reference TSVT4B10 were used in this study. The implants were made of titanium alloy (Ti6Al4V), were 10 mm long and had a diameter of 4 mm.

Similarly to what was done in previous studies (38-40), each dental implant was placed in the femur or in the tibia of the rabbits, as shown in Figure 1. The surgical procedure described in more details by Pearce et al. (41) was reproduced. Briefly, a single skin incision was performed on each rabbit leg around the knee joint. The lateral condyle of each bone was drilled in a stepwise fashion in order to create 10-mm deep and 4.0-mm wide conical cavities, using surgical drills manufactured by Zimmer Biomet® (2.3, 2.8, 3.4 mm diameter). Before inserting the implants, an isotonic saline solution was used to rinse the cavities in order to remove bone fragments. Three rabbits were sacrificed at 0, 4, 8 and 13 weeks after initial implant surgery. The QUS and RFA measurements were realized just before the animal sacrifice. A total number of implant comprised between 1 and 2 was inserted in each rabbit.

2.3. Resonance frequency analysis

The Osstell device (Ostell, Göteborg, Sweden) was used to measure the RFA response of each implant in ISQ units (on a scale from 1 to 100). As recommended by the manufacturer, measurements were realized using a smart peg screwed into the implant. Moreover, as indicated in Figure 2, each measurement was performed in two perpendicular directions denoted 0° and 90°, and was repeated three times in order to assess the reproducibility of the measurements. The values obtained when positioning the axis of the device parallel (respectively perpendicular) to the bone axis were considered to be in the 0° direction (respectively in the 90° direction) and were denoted ISQ0 (respectively ISQ90). For each sample #i, the average and standard deviation of the three values of ISQ0 (respectively ISQ90) were denoted $ISQ0_{\text{avg}}$ and $ISQ0_{\text{std}}$ (respectively $ISQ90_{\text{avg}}$ and $ISQ90_{\text{std}}$).

2.4. Quantitative ultrasound device

The QUS device used in the present study is composed of a planar ultrasonic monoelement transducer (Sonaxis, Besançon, France) that generates a broadband ultrasonic pulse with a central frequency of 10 MHz, which propagates perpendicularly to its active surface. Similarly to what was done in Vayron et al. (37), the ultrasonic probe was fixed onto a healing abutment made of titanium alloy, which was then screwed into the dental implant. This procedure was followed to avoid positioning problems between the transducer and the implant axis. A pulser-receiver was connected to the probe with a coaxial cable, and the radiofrequency (rf) signal was recorded using a transient recorder with a sampling frequency of 100 MHz. An ultrasonic measurement was performed for each implant, as shown in Figure 3, and the measurements were made instantaneously.

For all measurements, a controlled torque of 3.5 N.cm was applied when screwing the transducer into the implant, which is approximately 10 times lower than torque values recommended by implant manufacturers for the implant insertion (42). The reproducibility of the measurements was assessed by unscrewing the transducer and carrying again the measurement three times for each implant.

The method described in Vayron et al. (37) was used to derive an ultrasonic indicator UI, which was shown to be correlated with the implant stability. The envelop $S(t)$ of the radiofrequency signal $s(t)$ was first determined. Then, an indicator I that estimates the average amplitude of the signal between 20 and 120 μs was defined following:

$$I = \frac{1}{2000} \sum_{i=2000}^{12000} S(t_i)$$
where $T_s = 0.01 \mu s$ corresponds to the sampling period. In order to obtain values that (i) increase when bone quantity and quality increase around the implant and (ii) are comprised between 1 and 100, similarly to the ISQ, the ultrasonic indicator $UI$ was defined by:

$$UI = 100 - 10 \times I$$

For each sample $#i$, the average and standard deviation of the three values of $UI$ were denoted $UI_{i}^m$ and $UI_{i}^{std}$.

### 2.5. Histomorphometric analyses

After the RFA and QUS measurements were realized, the animals were sacrificed and histomorphometric analyses were performed. The samples were prepared following a procedure for non-decalcified histology described more extensively by Soffier et al. (43) and Chevallier et al. (44). The ratio of the implant surface in intimate contact with mineralized bone tissue was assessed manually by analyzing histomorphometrical images by classical microscopy. Two histological sections were studied for each sample, so that two histomorphometrical measurements of the BIC could be realized. For each sample $#i$, the average and standard deviation of the two BIC ratio was denoted $BIC_{i}^m$ and $BIC_{i}^{std}$.

### 2.6. Statistical analyses

Analyses of variance (ANOVA) were performed to evaluate the difference between the values of the BIC ratio, of ISQ0, of ISQ90 and of $UI$ obtained with different healing times (0, 4, 8 and 13 weeks). Moreover, linear regression analyses were carried out in order to determine the correlation between ISQ, $UI$ and the BIC ratio. All statistical analyses were carried out using the Microsoft Excel software (Redmont, WA, USA).

All the procedures detailed in the present work comply with the ARRIVE guidelines.

### 3. Results

#### 3.1. Sample analysis and BIC estimation

Figure 4 shows two images corresponding to 2 histological sections together with the corresponding value of the BIC ratio.

Table 1 shows the average value of $BIC_{0}$ and of $BIC_{90}$ obtained for all samples corresponding to the same healing duration and to all data pooled. The mean value of $BIC_{90}$ corresponds to an estimation of the average reproducibility of the BIC measurements. Table 1 also shows the standard deviation of $BIC$ obtained for all samples corresponding to the same healing duration and to all data pooled, which corresponds to the interspecimen variability. ANOVA test of the results obtained with all 22 implants showed a significant effect of healing time on the BIC ratio (p-value $= 5.8 \times 10^{-5}$ and F-statistic $= 14.0$), with BIC values first increasing as a function of healing time and then decreasing for healing times between 8 and 13 weeks. However, an important interindividual variability was also observed for samples with a healing time of 13 weeks. Moreover, the mean measurement error was comprised between 2.62 and 5.84 for the various values of healing durations.

#### 3.2. Resonance frequency analysis

Figure 5 shows the relation between i) ISQ0 and ISQ90 and ii) the BIC measured with histomorphometric analyses. A significant correlation was obtained between ISQ0 and the BIC, while no correlation was obtained between ISQ90 and the BIC. The vertical error bars correspond to the standard deviation obtained for the three measurements of the ISQ ($ISQ_{0}^{std}$ and $ISQ_{90}^{std}$) and indicate the reproducibility of each measurements. The horizontal error bars correspond to the standard deviation obtained for the two measurements of the BIC ($BIC_{0}^{std}$).

#### 3.3. Ultrasonic measurements

Table 1 shows the same parameters corresponding to $UI$ as the one shown for the BIC in subsection 3.1. An ANOVA test of the 22 implants demonstrated a significant effect of healing time on $UI$ (p-value $= 6.10^{-3}$ and F-statistic $= 5.8$), but no significant effect of healing time on ISQ0 (p-value $= 0.11$ and F-statistic $= 3.2$). Both ISQ0 and ISQ90 first increase as a function of healing time, but then decrease for healing times superior to 4 weeks. The interindividual reproducibility of ISQ0 decreases as a function of healing time. However, no global trend was observed in the evolution of this same parameter for ISQ90.

Figure 6 shows the relation between i) ISQ0 and ISQ90 and ii) the BIC measured via histomorphometric analysis. The vertical error bars correspond to the standard deviation obtained for the three measurements of the $UI$ ($UI_{0}^{std}$) and indicate the reproducibility of each measurements. The horizontal error bars correspond to the standard deviation obtained for the two measurements of the BIC ($BIC_{90}^{std}$). A significant correlation was obtained between $UI$ and the BIC.

Figure 7 shows the relationship between i) ISQ0 and ISQ90 and ii) the $UI$. A significant correlation was obtained between ISQ0 and $UI$, whereas no correlation was obtained between ISQ90 and $UI$. 

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**Fig. 3.** Measurement configuration of the ultrasonic indicator using the ultrasonic transducer screwed into a dental implant.

**Fig. 4.** Images of the histologic section of a dental implant (A) directly after implantation in a rabbit tibia and (B) after 8 weeks of healing time. Mineralized bone tissues correspond to the pink regions of interest of the image and were colored with Van Gieson’s stain. The BIC was respectively equal to (A) 24.1% and (B) 60.4%.
The maximal measurement errors for BIC values were obtained after 4 weeks of healing (see Table 1), which may be explained as follows. In the early period after implant insertion, bone resorption primarily occurs around the implant. However, after around 3 to 4 weeks of healing, bone formation increases and becomes predominant over bone resorption (50, 51). Therefore, after 4 weeks of healing, there is a high heterogeneity in the amount of bone in contact with the implant, which leads to the high value of BIC obtained in Table 1.

4.3. Correlation between ISQ and BIC values

Figures 5 and 6 show that ISQ0 and UI are correlated to BIC values, whereas no correlation between ISQ90 and BIC was obtained. The results shown in Fig. 6 are in agreement with those shown in Fig. 5 since ISQ0 and UI are significantly correlated, whereas no correlation was obtained between ISQ90 and UI. These results highlight that ISQ values highly depend on the direction of the measurements, which is in agreement with results from Pattijn et al. (18). Moreover, the correlation obtained between ISQ and BIC measurements in the present study was relatively weak. Note that there is also controversy in the literature regarding the dependence of BIC and ISQ, since some studies conclude with a significant correlation between ISQ and BIC (45-47) with P-values varying between 0.016 and 0.024, while other studies showed there was no correlation between the two aforementioned parameters (19-23).

4.4. Correlation between UI and BIC values

The correlation found between UI and BIC values is in good agreement with results obtained in our previous in vivo study (36), where a determination coefficient of R² = 0.45 was found for the correlation between BIC and the UI. Vayron et al. (36, 37) showed that the indicator UI increased during healing, which is consistent with the present results since the BIC also increases during healing time (13, 52). Moreover, in silico (27-29) and in vitro (34, 35) studies also showed that UI increased when bone quantity around the implant increases, which may be explained as follows. When the BIC is low, the implant surface is mostly in contact with fibrous tissues, which leads to a stronger gap of mechanical properties at the implant surface than for higher BIC, which corresponds to a situation where the implant is mostly in contact with bone tissue. Consequently, the transmission coefficient at the BII is lower for lower values of the BIC (29, 31) and acoustic energy leakage out of the implant is therefore lower. As a result, the acoustic energy recorded at the upper surface of the implant is lower when there is more bone in contact with the implant, and the UI thus increases. However, the correlation between the BIC and the UI found herein is only moderate (R² = 0.47), which may be explained by experimental errors on the BIC estimation (see Table 1). Furthermore, the BIC is an indicator of bone quantity and not of bone quality, which also influences QUS measurements since the UI was shown to increase while (i) trabecular density increases and (ii) cortical thickness increases (28, 35). Note that the dependence of the UI on bone quality was also shown in silico (28).

4.5. Comparison between RFA and QUS techniques

Previous studies showed that the QUS technique is more sensitive to variations of in vitro implant stability (35) and to healing time (37) compared to the RFA techniques, which is in agreement with the present study. Besides a better correlation of the UI with BIC values compared to ISQ, ultrasonic measurements were also more reproducible than ISQ measurements, with a mean standard deviation on UI values equal to 0.51 while the mean standard deviation on ISQ values was equal to 1.69 (see Table 1 and Fig. 4 and 5). The better sensitivity of QUS compared to RFA to variations of the BIC can be explained physically. The ISQ is related to the resonance frequency of the bone-implant system, which depends on properties of the entire host bone that vibrates when excited mechanically (24). However, QUS measurements are only sensitive on bone tissue located at a distance lower than around 30 µm from the implant surface (28-30), which corresponds to the region of interest where osseointegration phenomena are known to occur (31, 53). Therefore, QUS are likely to

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**Table 1.** Average value, mean measurement error and interindividual variability obtained for the BIC ratio, ISQ0, ISQ90 and UI values for each healing duration and for all data pooled.

| Healing time (weeks) | Number of implants | Number of animals |
|---------------------|--------------------|-------------------|
| 0                   | 4                  | 6                 |
| 4                   | 5                  | 5                 |
| 8                   | 6                  | 6                 |
| 13                  | 3                  | 3                 |
| All data            | 22                 | 12                |

| Healing time (weeks) | Mean value of BICi (measurement error) | Mean value of BICi (measurement error) | Standard deviation of BICi (interindividual variability) |
|---------------------|---------------------------------------|---------------------------------------|----------------------------------------------------------|
| 0                   | 18.91                                 | 45.53                                 | 58.50 49.51 42.22                                         |
| 4                   | 3.32                                  | 5.84                                  | 3.87 3.68                                                  |
| 8                   | 12.56                                 | 5.57                                  | 4.94 15.14 18.52                                          |
| 13                  | 7.41                                  | 6.01                                  | 17.14 15.14 18.52                                         |
| All data            | 7.41                                  | 6.01                                  | 17.14 15.14 18.52                                         |

| Healing time (weeks) | Mean value of ISQ0i (measurement error) | Mean value of ISQ0i (measurement error) | Standard deviation of ISQ0i (interindividual variability) |
|---------------------|----------------------------------------|----------------------------------------|----------------------------------------------------------|
| 0                   | 63.00                                  | 74.52                                  | 73.88 70.67 70.41                                         |
| 4                   | 1.18                                   | 2.23                                  | 1.67 1.95 1.29                                            |
| 8                   | 9.15                                   | 3.82                                  | 3.55 1.63 7.11                                            |
| 13                  | 71.38                                  | 84.07                                  | 77.40 74.0 76.35                                         |
| All data            | 71.38                                  | 84.07                                  | 77.40 74.0 76.35                                         |

| Healing time (weeks) | Mean value of ISQ90i (measurement error) | Mean value of ISQ90i (measurement error) | Standard deviation of ISQ90i (interindividual variability) |
|---------------------|----------------------------------------|----------------------------------------|----------------------------------------------------------|
| 0                   | 66.01                                  | 74.53                                  | 80.42 82.47 75.71                                         |
| 4                   | 0.75                                   | 1.82                                  | 2.73 3.08 2.08                                           |
| 8                   | 12.39                                  | 3.86                                  | 6.31 7.61 9.16                                           |
| 13                  | 11.18                                  | 4.41                                  | 2.79 1.63 9.00                                          |
| All data            | 11.18                                  | 4.41                                  | 2.79 1.63 9.00                                          |
Fig. 5. Relationship obtained between the ISQ measured in the directions 0° (black points) and 90° (grey points) and the BIC. The solid lines correspond to a linear regression analysis. The error bars denote the reproducibility of the measurements. The determination coefficients are indicated.

Fig. 6. Relationship obtained between UI and the BIC. The solid lines correspond to a linear regression analysis. The error bars denote the reproducibility of the measurements. The determination coefficient is indicated.

Fig. 7. Variation of the i) ISQ measured in the directions 0° (black points) and 90° (grey points) and ii) the UI. The solid lines correspond to a linear regression analysis. The error bars denote the reproducibility of the measurements. The determination coefficients are indicated.
be more sensitive to the properties of the BII and to osseointegration phenomena.

4.6. Limitations

The present study has several limitations. First, only one type of implant was considered and the comparison between RFA and QUS should be done with other implant types. The dimensions of the implant are likely to affect both the RFA results (54) and the QUS results. Therefore, slightly different results might be obtained for a different implant design. Here, we chose to focus on a single implant design to limit the number of parameters in our study, especially since the sample number was already small. A future study could confirm the better correlation between QUS results and the BIC for different implant designs.

Second, uncertainties on the estimation of BIC values was high because (i) only two BIC measurements could be realized for each implant and (ii) BIC measurements were realized on 2D histological sections, and can therefore only approximate actual BIC values on the entirety of the 3D implant.

Third, a relatively low number of rabbits was considered herein, which is justified by ethical reasons and because it corresponds to the range of sample numbers used in previous studies using the Oststell device (15, 22). Studies with more animals should be performed in the future. Fourth, the only parameter representing the progress of osseointegration considered herein was the BIC, which is not representative of the evolution of bone quality during healing.

Fifth, the exact position of the implant in rabbit bone was not controlled and a bi-cortical fixation may sometimes be obtained, which is usual for the present animal model, which has been used in (38-40, 55). However, obtaining a bi-cortical fixation does not jeopardize the results obtained herein because the aim of this paper was not to assess the evolution of the healing process itself (as it was the case in (38) for instance), but to compare the sensitivity of the QUS and RFA methods on these changes. On the contrary, for the aforementioned objective, it is interesting to consider a wide range of situations in order to obtain an important variability in terms of implant stability. Note that we considered implants in femoral and tibial bone, which have different properties with different ratio of cortical layer and trabecular bone, in order to obtain a wide range of implant stability to be able to compare the QUS and RFA technique for various healing conditions.

5. Conclusion

The present study allows to assess the performances of RFA and QUS techniques to assess dental implant stability by comparing the results obtained for the same samples with both methods to BIC ratio measurements. A better correlation between the BIC and the UI was found compared to the ISQ, which was shown to be dependent on the direction of measurements. Moreover, the errors realized on the UI were 3.3 times lower to the ones realized on the ISQ. These results may be explained by the reproducibility and by the principle of measurements of both methods. Future works should now focus on the development of an ultrasonic device that could be used in clinical practice in the future to estimate dental implant primary and secondary stability. In particular, clinical studies could help to define a target value for the UI above which an implant is considered to be stable enough to be loaded.

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Conflict of interest

The authors declare no conflict of interest.

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