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The comparison between implant stability quotient and bone-implant contact revisited: an experiment in Beagle dog

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Abstract

Background: Resonance frequency analysis (RFA) is applied clinically for the assessment of implant stability, and the relevance of this application is widely accepted. However, the relationship between resonance frequency (RF) and other parameters of implant stability, such as the histomorphometrical bone-to-implant contact (BIC) parameter, has become controversial in the last decade.

Objective: To analyse and clarify the controversial relationship between RF and histomorphometrical BIC measurements.

Material and methods: A total of 36 dental implants (9 mm length, Ø 4.0 mm; Biohorizons® Implant Systems Inc., Birmingham, AL, USA) with a soluble blasting media (sandblasting with soluble particles) surface were implanted in six beagle dog mandibles. RFA assessments were performed with a magnetic Ostel Mentor® device at the time of implant installation, and during the monitoring period at weeks 1, 2, 4, 6 and 8, before implant retrieval. The dogs were sacrificed and the implants were removed in block after 8, 6, 4, 2, 1 and 0 weeks, respectively. One group was obtained at time 0, immediately after the implantation. The samples were embedded in methyl methacrylate polymers (Technovit®) and cut along their long axis. BIC values were assessed by a non-subjective and systematic method based on backscattered scanning electron microscopy (BS-SEM) images. BIC% at the different time points was compared with the corresponding implant stability quotient (ISQ) values of the RFA assessment.

Results: No statistically significant correlation between BIC and ISQ values (Osstell Mentor®) was identified. The absence of a relationship between these two parameters is in agreement with several previous studies in humans and experimental animals.

Conclusions: The lack of correlation between BIC and ISQ values suggests that ISQ as determined by RFA is not able to identify the relationship between RF and histomorphometrical data.

For the successful osseointegration of an implant, a structural and functional connection between bone and implant surface is necessary. Primary stability at the time of installation involves the mechanical engagement of an implant to the surrounding bone (Lioubavina-Hack et al. 2006). Secondary stability follows, which is the biological stability gained with bone regeneration and remodelling around the implant, that is, osseointegration.

Implant stability is the main parameter that influences the schedule of implant loading and the outcome of the treatment (Jaffin & Berman 1991; Chiapasco et al. 1997; Lazzara et al. 1998; Szmukler-Moncler et al. 1998;

Testori et al. 2002). Factors affecting primary stability would be bone quantity and quality, surgical technique (including the skill of the surgeon) and type of implant (geometry, length, diameter and surface characteristics). The mechanical properties of bone are determined by the composition of the bone at the implant site and may increase during healing because soft trabecular bone tends to undergo a transformation to dense cortical bone at the vicinity of the implant surface (Sennerby & Meredith 2008). On the other hand, factors affecting secondary stability would be primary stability, bone modelling and remodelling, and implant surface conditions (Meredith 1998b; Atsumi et al. 2007).

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A number of approaches have been developed to measure implant stability using both invasive and non-invasive means. Among the invasive approaches, histomorphometrical assessment of implant–bone interface provides reliable data on the strength of the interface and the quality of implant anchorage in peri-implant bone. Bone-to-implant contact (BIC), bone volume density (BVD) and effective implant length (EIL) are some of the histomorphometrical parameters used for that purpose *ex-vivo*. The drawback of these measurement approaches is that they are only applicable in an experimental environment. On the other hand, for the quantitative assessment of bone mineral density *in vivo*, quantitative computed tomography (QCT) has been recently introduced. QCT is an accurate and reproducible method for the quantitative analysis of the mineral density of the residual bone. However, QCT does not offer the spatial resolution for an accurate analysis of the bone structure for which typically bone histomorphometry is used (Martinez et al. 2001). Other drawbacks of QCT are (i) a certain radiation exposure and (ii) results are severely impacted by metal components in the vicinity of the desired location.

Clinicians need non-destructive techniques and supporting objective guidelines to determine on an individual basis the stability of a given implant in its peri-implant bone. Currently, several non-invasive methods, such as torque measurements during implant insertion, radiographic assessment, the Periotest[®] instrument and resonance frequency analysis (RFA), have been clinically used (Meredith 1998a,b; Aparicio et al. 2006; Atsumi et al. 2007).

The torque value is the final twisting force of implant insertion. Although torque measurement during implant insertion is simple, this is not applicable in the process of treatment and follow-up (Friberg et al. 1999). Periotest[®], which was originally used for measurement of natural tooth mobility, has also been applied to test implant stability (Schulte & Lukas 1993; Aparicio 1997; Isidor 1998). The Periotest[®] assesses the damping capacity of the implant, although it is not useful to evaluate mesiodistal stability. In addition, Periotest[®] seems to be insufficient to detect small changes in implant stability, because the direction and position of the Periotest[®] probe affect the value. On the other hand, Meredith et al. (1996) have described a clinical non-invasive approach, named RFA, for the assessment of implant stability, which consist of evaluating bone anchorage around an implant by measuring the resonance fre-

quency of a transducer coupled to the implant (Meredith et al. 1996, 1997a,b). The instrument is a piezo-electric element with a frequency spectrum of 3500–8000 Hz. The frequency with the highest amplitude is used to calculate the so-called implant stability quotient (ISQ) on a scale from 0 to 100. According to the manufacturer, an almost linear relationship exists between the resonance frequency and the ISQ value.

In the process of implant treatment and the follow-up of the treatment, resonance frequency (RF) is superior to other analyses (radiographical analysis, cutting and insertion torque, and Periotest[®]). Measurement of implant stability through RFA is supposed to reduce the observer-dependent errors occurring with the Periotest[®], as a transducer is screwed on to the implant and measurements are completely automatized. The torque used during fixation of the transducer to the implant has been shown not to alter the results of RFA measurements, and the results obtained from this type of measurement were highly reproducible (Meredith et al. 1996). Currently, several versions of the RFA devices are available: the original is an electronic apparatus (Osstell[®]), whereas the more recent ones are wireless magnetic devices (Osstell Mentor[®] [Integration Diagnostics, Gothenburg, Sweden] and Osstell ISQ[®]). The use of the magnetic apparatus compared with the predecessor was thought to result in improved accuracy of the RFA technique (Atsumi et al. 2007).

A number of experimental and clinical studies showed increasing RFA values during healing after implant placement. The RF changed according to the stiffness of the excited abutment–implant system. Thus, changes in implant RF could indicate changes in anchorage (Meredith et al. 1997a, b; Sennerby et al. 2005) and therefore may be useful in documenting clinical implant stability (Meredith et al. 1996; Meredith 1998a; Zix et al. 2008). In the last fifteen years, several studies have been published (Meredith et al. 1997b; Nkenke et al. 2003; Gedrange et al. 2005; Schliephake et al. 2006; Scarano et al. 2006; Huwiler et al. 2007; Zhou et al. 2008; Ito et al. 2008; Abrahamsson et al. 2009; Stadlinger et al. 2009; Stadlinger et al. 2012; Jun et al. 2010; Blanco et al. 2011; Abdel-Haq et al. 2011; etc.) trying to establish the validity of RFA in the assessment of implant–bone anchorage by correlation of ISQ values and histomorphometrical parameters (Table 1).

The comparison of the literature in this area is difficult because of the heterogene-

ity of the studies and nature of the tests: (i) different types of histomorphometrical analysis: BIC (total, buccal or lingual), BVD (bone volume density), EIL (effective implant length), etc.; (ii) *in vivo* vs. *in vitro* experiments; (iii) animal vs. human studies; (iv) cadavers vs. patients; (v) different animal models – rabbits, dogs, mini-pigs, sheeps, etc. – in mandible, tibia, femur, etc.; and (vi) variations in macro and micro implant design. Subsequently, conclusive data on the relationship between bone–implant interface and RFA values are still lacking. It is worth mentioning that all the histomorphometrical measurements of BIC in these studies have been carried out by the conventional procedure, analysing the stained samples with light microscopy. Regarding the equipment used for measuring RF, the assessment of the ISQ values was performed with the electronic Osstell[®] device. The more accurate magnetic Osstell Mentor[®] device was used only in four studies.

Therefore, the aim of the present study was to test the hypothesis that measurements of implant stability using RFA correlate with histomorphometrical data of BIC in a beagle dog experiment. Special attention was paid to the acquisition of the data to be correlated. In this study, BIC assessment has been performed by a non-subjective and systematic method based on BS-SEM images (Manresa et al. 2013), and RFA values were assessed using the improved magnetic Osstell Mentor[®] device. The data obtained from this animal experiment might be useful in clarifying the current controversial relationship between RF and histomorphometrical parameters.

Material and methods

Experimentation animals

According to the ARRIVE guidelines for reporting the animal experimentation (Kilkenny et al. 2010; Berglundh & Stavropoulos 2012), this animal study was approved by the Animal Experimentation Ethics Committee of the University of Barcelona (UB, Spain). To perform this study, the number of animals was reduced to a minimum, according to the “3Rs” (Replacement, Refinement and Reduction of animals in research) as defined by Kilkenny et al. (2010). Thus, six adult beagle dogs, weighing an average of 11.5 kg, were selected and installed in the animal experimentation service facility of Bellvitge’s Health Science Campus of the UB, under veterinary control. The design of the study is depicted in Fig. 1. All experiments were

Table 1. Review of the literature on the correlation between ISQ and BIC measurements

Authors	Experiment	RFA device	Correlation ISQ-BIC	Correlation of RFA with other parameters
Meredith et al. (1997b)	Rabbits (tibiae)	Osstell®	No	Yes: EIL
Nkenke et al. (2003)	Cadaver	Osstell®	Yes (buccal side)	No: ITV
Gedrange et al. (2005)	Cadaver	Osstell®	Yes	No: Bone mineral density
Schliephake et al. (2006)	Dogs (mandible)	Osstell®	No	No: Bone density (radiographic evaluation)
				No: ITV
				No: BVD
Scarano et al. (2006)	Clinical study	Osstell®	Yes	
Huwiler et al. (2007)	Clinical study	Osstell®	nd	No: BVD
				No: BTC
Zhou et al. (2008)	Rabbits	Osstell®	Yes	No: Bone scintigraphy
Ito et al. (2008)	Mini-pigs (tibiae)	Osstell®	No	
Abrahamsson et al. (2009)	Dogs (mandible)	Osstell®	No	No: Bone density
				No: EIL
				No: ITV
Jun et al. (2010)	Cadáver	Osstell® Osstell Mentor®	No	No: PTV
				Yes: BVD
Stadlinger et al. (2009 and 2012)	Mini-pigs (mandible)	Osstell®	Yes	
Blanco et al. (2011)	Rabbits (femur)	Osstell Mentor®	Yes	
Abdel-Haq et al. (2011)	Sheep (tibiae)	Osstell Mentor®	No	
Antunes et al. (2013)	Dogs (mandible)	Osstell Mentor®	Yes	Yes: BA

EIL, effective implant length; ITV, insertion torque value; BVD, bone volume density; nd, not determined; BTC, bone trabecular connectivity; PTV, periosteal value; BA, bone area in threads.



Fig. 1. Design of the study.

performed according to the Spanish Government guide (Royal Decree 1201/2005 of October 10th, Spanish Official Gazette 252, October 21st, 2005) and the European guide (European Union Council Directive of November 24th, 1986, 86/609/EEC) for animal use and care. Throughout the experimental study, all animals were fed with a soft diet, and mechanical cleaning of teeth and implants was performed daily.

Surgical procedure

All mandibular premolars were extracted bilaterally (Fig. 2a,b). After a healing period of 3 months, three implants (9 mm length, Ø 4.0 mm; Biohorizons® Implant Systems Inc., Birmingham, AL, USA) with a soluble blasting media (SBM) (sandblasting with soluble particles) surface were placed in each hemimandibular premolar region, according to the protocol suggested by the manufacturer (Biohorizons).

The implants were placed at 7 mm distance from each other. A total of 36 implants were placed (Fig. 2c). All surgical procedures were performed by the same operator (C. M.). The surgical approach occurred under general anaesthesia and was supervised by a veterinary surgeon. Once anaesthetized, buccal and lingual full thickness flaps were reflected. Implants placement procedure was carried out according to the manufacturer's instructions and protocols, to ensure a standardized surgical procedure. For all implant installation, insertion torque was stabilized at 40 Ncm. Flaps were sutured using silk 4.0 interrupted sutures and removed after 10 days. After surgery, an intramuscular injection (prophylactically) of Terramycin 100® (Pfizer Laboratories, Alcobendas, Madrid, Spain, 25 mg/kg) was provided. The post-operative analgesia was carried out by the administration of meloxicam (Metamecam® injectable solution 5 mg/ml,

Rhein, Germany; 5 mg/20 kg/24 h). Finally, dogs were sacrificed at time points: 0, 1, 2, 4, 6 and 8 weeks after implant installation, by means of an overdose of sodium pentothal. The mandibles were dissected and each implant site was removed using a diamond saw, so samples could be obtained and prepared for histological analysis.

Resonance frequency measurements with Osstell Mentor® device

Immediately after implant installation, ISQ assessments of all implants were performed according to the manufacturer's instructions. The SmartPeg® (type 1, Integration Diagnostics AB, Gothenburg, Sweden) was directly screwed into the implants inside thread. The hand-held probe of Osstell Mentor® stimulates the SmartPeg magnetically, without being connected to it, and RFA was measured (Fig. 2d). Measurements were taken twice

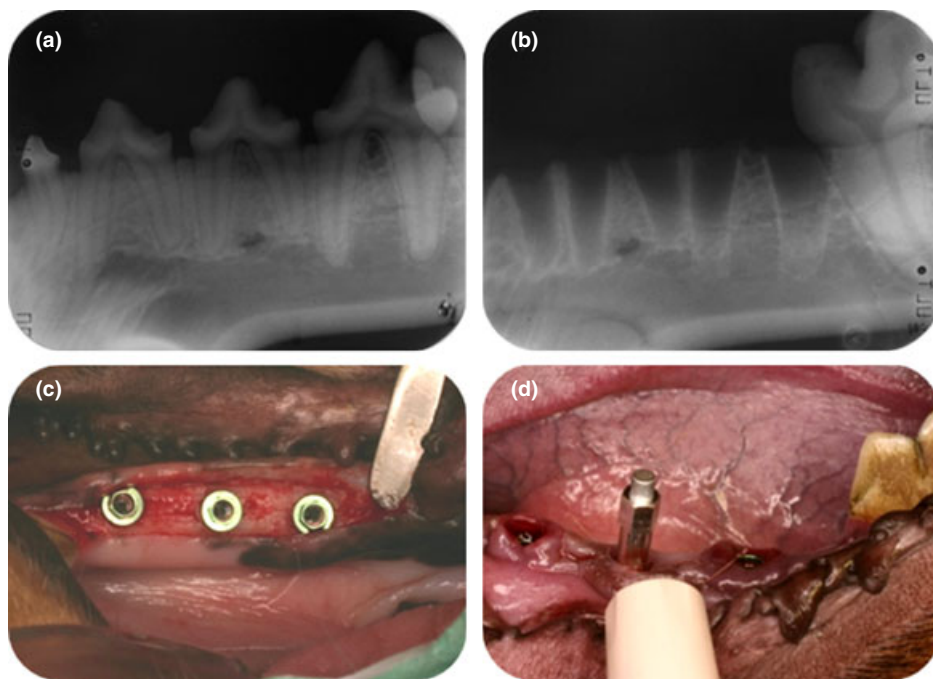


Fig. 2. Premolar extraction (a and b); Implant placement after 3 months of healing (c); Assessment of ISQ values (d).

with the hand-held probe in a direction perpendicular to the mandibular crest. The Osstell Mentor® device automatically transformed the RFA value (in hertz) for each assessment to ISQ units. Values between 1 and 100 were obtained, 100 being the highest degree of stability. The healing following implant installation was studied at 0, 1, 2, 4, 6 and 8 weeks, measuring implant stability at each time point.

Finally, dogs were sacrificed at time points: 0, 1, 2, 4, 6 and 8 weeks after implant installation, so samples could be obtained and prepared for histological analysis.

Preparation of samples

The biopsies were processed for ground sectioning (Donath & Breuner 1982; Donath 1985). The implant-bone specimens were fixed in 10% formaldehyde for 1 week and dehydrated in an ascending series of alcohol rinses before being embedded without decalcification in light-curing epoxy resin (Technovit®, Exakt-Kultzer, Wahrheim, Germany). Blocks were sectioned buccolingually with a diamond-edge band saw blade (Exakt micro-parallel-grinding System®, Exakt, Nordenstedt, Germany), and then ground and polished with 1200 and 4000 grain sandpaper to obtain a polished surface. For each implant, one section at each half longitudinally axis (buccal-lingual) was prepared. The blocks were coated with evaporated carbon and fixed with colloidal silver; four silver tracks were directed to the region of interest

in order to improve the conductivity of the specimen (Franch et al. 2000).

Histomorphometric analysis

Sample observation and BIC measurements were performed as described previously (Manresa et al. 2013) by BS-SEM (S-360; Leica, Cambridge, UK), at the Scientific and Technological Centers of the UB, acquiring 10–16 images per sample. Image processing and BIC determination was performed using the Fiji image processing package (<http://pacific.mpi-cbg.de/>). Images were stitched (Preibisch et al. 2009), filtered and thresholded to obtain a binary image of the whole implant that finally was dilated and outlined. The length of this outline was measured as the maximum possible BIC. In the present study, the measurements were performed along the total length of the implants, buccal and lingual. The regions of coincidence between this line and the bone were measured as the real BIC. The percentage of BIC was calculated by dividing the real BIC by the maximum possible BIC and multiplying by 100.

Statistical analysis

Data obtained from each section in the histological analyses and from each implant in the RFA were coalesced, and mean values were calculated for each healing time. Degree of osseointegration (BIC%) was compared with the ISQ values for the corresponding implant sites using the Spearman correlation coefficients. In the correlation analysis, the

implant was used as the statistical unit ($N = 36$). Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Good primary stability was achieved for all implants after installation. Healing was uneventful in all the 36 implant sites, and no implant exhibited clinical mobility at any time point.

ISQ values

At the time of implant installation, the ISQ mean values (Fig. 3; Table 2) were 80.91. From day 0 to 1 week, a small decrease in the RFA value was observed (79.75). The ISQ value increased from week 1 to week 2 (81.17). From week 2 to week 4, the RFA values remained stable (81.58), and then rose to 83.75 at week 6. Finally, after 8 weeks, a small decrease in ISQ data was noticed, the mean value being 81.08, very close to the initial value.

Histomorphometrical measurements (BIC%)

The results of the histological analysis of the ground sections are depicted in Fig. 4 and Table 3. Considering the mean values for each time point, BIC% amounted to 24.70% immediately following implant installation. After 1 week of healing, the BIC% decreased to 23.70 and then to 22.90% at week 2. From week 2 to week 6, the BIC% gradually increased, being 36.20 and 33.0, respectively, at 4 and 6, weeks. Finally, at week 8, the BIC% value was established as 45.50%. Fig. 5 shows the progression of bone-to-implant contact along time. Although only one spire is represented for each sample, the BIC measurement was carried out along the total length of the implant. Images corresponding to the earliest periods, from time 0 to week 2, showed that the calcified tissues around the implant were immature and surrounded by vascular spaces (Fig. 5a–c). Images at the 4th week showed a higher level of contact between the implant and a denser osseous structure with fewer and smaller vascular spaces and more mature bone tissue (Fig. 5d). At weeks 6 and 8, the increase in the BIC% was in parallel with the increase in maturity of the osseous tissue that gradually surrounded the implant surface (Fig. 5e,f).

ISQ and BIC correlation

Differences in BIC% were not reflected in the RFA during the 8-week monitoring period. There was no statistically significant

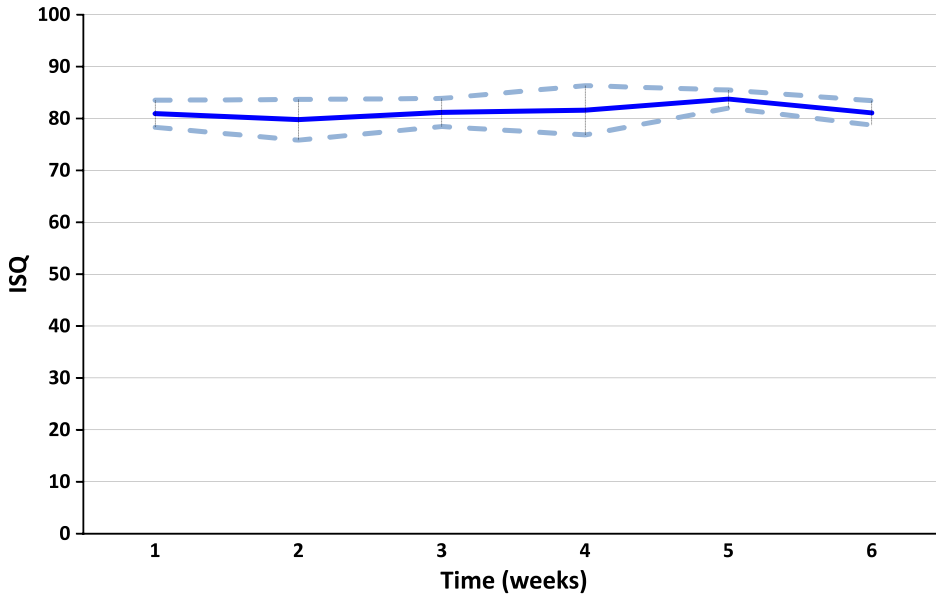


Fig. 3. Diagram of the Implant Stability Quotient (ISQ values) obtained with the Osstell Mentor® device from day 0 to week 8. Standard deviation (SD) shown in dashed lines.

Table 2. Implant stability quotient (ISQ values) for all RF assessment

Time (weeks)	ISQ values – Osstell Mentor®				
	Mean	SD	Q25	Median	Q75
0	80.91	3.26	80.25	81.50	83.13
1	79.75	4.92	75.62	79.75	84.25
2	81.17	3.37	81.00	81.50	82.75
4	81.58	5.96	83.13	83.75	84.38
6	83.75	2.19	82.88	84.50	85.00
8	81.08	2.94	80.25	81.50	82.75

SD, standard deviation; Q25, quartile 1 (25%); Q75, quartile 3 (75%).

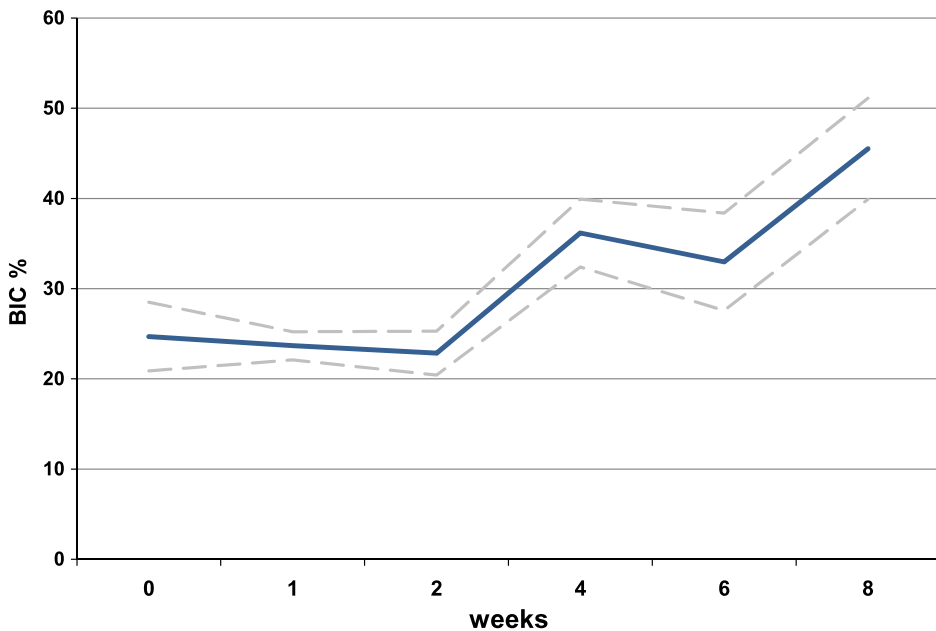


Fig. 4. Diagram of the degree (mean values) of osseointegration [bone-to-implant contact (BIC%)] from day 0 to week 8. Values obtained with backscattering scanner electron microscopy (BS-SEM). Standard deviation (SD) shown in dashed lines.

correlation between RFA and BIC values (Spearman correlation coefficient = -0.083 Fig. 6).

Discussion

Resonance frequency analysis is believed to be a potentially useful clinical tool for the prevention, diagnosis and prediction of implant failure and is helpful in the maintenance of viable implants (Meredith 1998a; Huang et al. 2002; Glauser et al. 2004; Sjöström et al. 2005; Zix et al. 2008). However, the validity of this rather new technique still has to be determined by correlating the results with other methods that assess the supportive character of an implant site, such as mechanical testing, radiological examination and, finally, histometric analysis.

Histological and histomorphometrical assessment is the most accurate method of observing morphological changes at the implant–bone interface. It has been suggested that RFA is related to the stiffness of the implant in the surrounding tissues (Meredith et al. 1996, 1997a,b). The stiffness of the implant–bone unit that is supposed to be assessed by RFA may be affected by the thickness of the bone layer on the implant surface and the density of the peri-implant bone. Hence, an increased bone–implant contact is supposed to result in higher structure stiffness and would increase interfacial strength (Gedrange et al. 2005; Sennerby et al. 2005). Different results on a possible relationship between RFA and BIC have been reported. The literature in this area is controversial and, without being exhaustive, some representative examples of studies on positive and negative correlations between RFA and histomorphometrical parameters, in clinical and experimental settings, are summarized in Table 1 and described below.

Five clinical studies on the correlation between RFA and histomorphometrical parameters, three in cadavers and two in patients, have been published with different outcomes. A study on human cadavers (Nkenke et al. 2003) found a weak correlation between BIC% at the buccal aspect of the implant and ISQ, but not on the lingual side. The study could not confirm any correlation between ISQ values and peak insertion torque data, nor between ISQ values and bone mineral density. A second study in cadavers performed by Gedrange et al. (2005) for the determination of the primary stability of orthodontic palatal implants stated a relationship between RF and BIC. Both Nkenke et al.

Table 3. Bone-to-implant contact percentage (BIC%)

Time (weeks)	BIC% (BS-SEM)				
	Mean	SD	Q25	Median	Q75
0	24.69	11.64	19.52	22.31	33.80
1	23.66	4.78	19.66	23.37	27.15
2	22.85	7.43	21.60	25.84	27.61
4	36.16	11.54	26.27	34.18	46.81
6	32.97	16.52	21.02	29.99	40.91
8	45.51	17.16	38.11	44.29	58.28

SD, standard deviation; BS-SEM, Backscattered scanning electron microscopy; Q25, quartile 1 (25%); Q75, quartile 3 (75%).

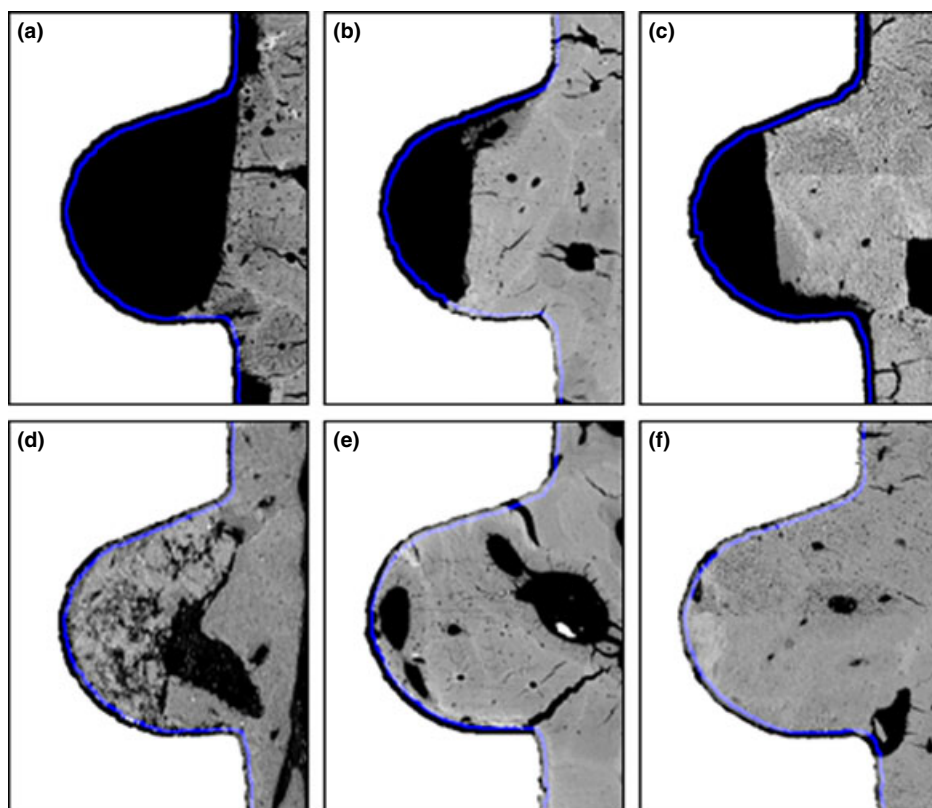


Fig. 5. Progression of bone-to-implant contact. Detail of six spires at 0 (a), 1 (b), 2 (c), 4 (d), 6 (e) and 8 (f) weeks, respectively. Implants are shown in white, bone in greys and the line where BIC was measured is coloured blue. The thickness of the line has been enlarged for imaging purposes. Note how the blue line increases the overlap with bone along time due to the increase in the bone filling up the spire. All images are at the same scale.

(2003) and Gedrange et al. (2005) found a positive correlation between the height of the crestal cortical bone and ISQ. An experiment in three human cadavers to evaluate the initial stability parameters (insertion torque value, ISQ and Periotest value) of implants inserted just after tooth extraction and to examine the relationship between initial stability parameters and BIC was undertaken by Jun et al. (2010). The initial stability parameters showed statistically significant correlation between each other, but no correlation was found between BIC and the initial stability parameters. In one of the two studies on patients, a statistically significant correlation has been reported (Scarano et al. 2006) between ISQ and BIC in a retrospective histological and

histomorphometrical study of seven titanium implants retrieved from humans. However, no significant correlation was found between BVD or bone trabecular connectivity (BTC) measurements and ISQ values in a study on humans jawbone characteristics (Huwiler et al. 2007). Curiously, in this study, no predictive value for losing implant stability was given to RFA, because the decrease in the value occurred after the fact.

Several animal experiments have been reported, also with different outcomes. A study in rabbits (Meredith et al. 1997b) failed to find a correlation between the degree of BIC and RFA measurements, although a strong correlation was observed between RF values and the EIL. Schliephake et al. (2006) could

not find any correlation between BIC% and ISQ values of 80 implants in 10 foxhounds after healing periods of 1 or 3 months. In a study, (Zhou et al. 2008) using two methods (bone scintigraphy and RFA) to evaluate the osseointegration ability in 30 rabbits, it was found that bone scintigraphy was more sensitive to the change of peri-implant bone than the digital radiographic examination, but it did not correlate with histomorphometrical data (BIC). However, it was found that RFA increased with the bone-to-implant contact during the healing phase and correlated with the histomorphometrical data. Ito et al. (2008) performed an experiment where 24 implants placed in the tibia of four mini-pigs were analysed with RF and histology after 1, 2 and 4 weeks. Although the correlation between RF and BIC, which was measured all around the implant, was not significant ($r = 0.221$), the correlation coefficient increased ($r = 0.361$) when BIC was measured at the neck of the implant. The study conducted by Abrahamsen et al. (2009) to evaluate the relationship between BIC and ISQ values during a 12-week healing period, in a beagle animal model experiment, did not find any correlation between the two parameters. Also, no correlation was found in the same experiment between ISQ values and bone density. However, studying the influence on early osseointegration of dental implants installed in rabbits with two different drilling protocols, Blanco et al. (2011) did find a positive correlation between the increase in ISQ values and BIC. On the other hand, in an experimental pilot study in sheep, aimed at comparing the early-term osseointegration characteristics of standard (SLA) and modified sand-blasted and acid-etched (modSLA) implants, no correlation was found between RFA and BIC (Abdel-Haq et al. 2011). A weak correlation between RFA and BIC was observed by Stadlinger et al. (2009 and 2012) using experimental coated (collagen and glycosaminoglycans) implants placed in the mandibles of 20 mini-pigs. Finally, a study conducted by Antunes et al. (2013) showed correlation between RFA versus BIC using deproteinized bovine bone mineral as grafting material to promote osseointegration and stability in implants placed in dog mandible bone defects.

In the current experiment, the correlation between RFA and BIC was investigated during healing periods of up to 8 weeks. RFA analysis was performed with an Osstell Mentor® magnetic device. A new standardized non-subjective and highly discriminative method was used for the determination of BIC data, based on BS-SEM images (Manresa et al. 2013).

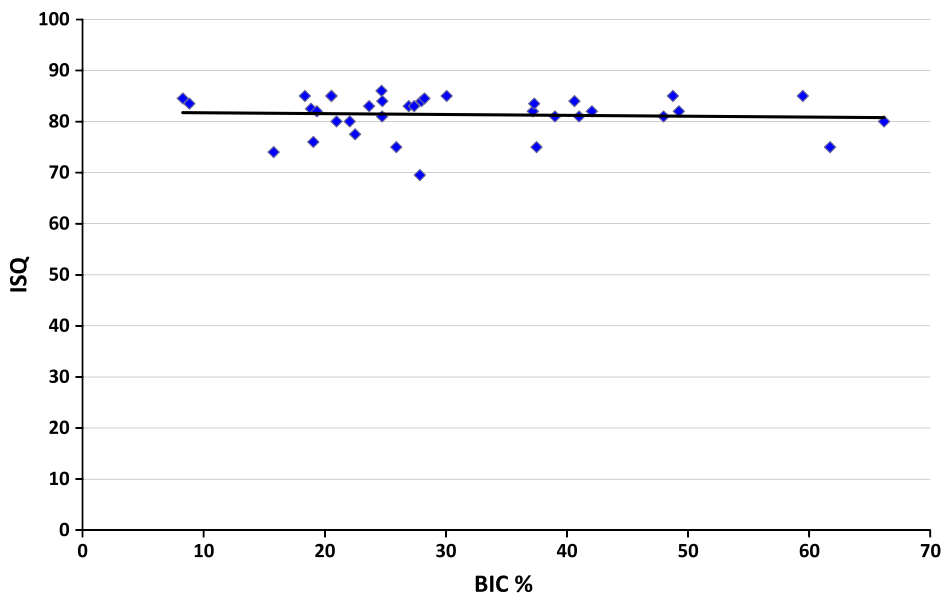


Fig. 6. Correlation of ISQ and BIC% values.

Implants with a SBM (sandblasting with soluble particles) surface was used in this experiment because the evidence from histomorphometrical data and clinical studies suggests that rough implant surfaces exert a clinically significant influence on faster osseointegration (Cochran et al. 1998; Berglundh et al. 2003; Abrahamsson et al. 2004; Buser et al. 2004; Tavares et al. 2007). Implant surface parameters are supposed to stimulate the bone cell reaction, resulting in an enhanced healing response and improved BIC.

Resonance frequency values as assessed by the Osstell Mentor® device are depicted in Fig. 3 and Table 2. The analysis of the data revealed a similar evolution of the ISQ values to that reported in previous animal experiments (Glauser et al. 2004; Al-Nawas et al. 2006) and clinical studies on patients (Nedir et al. 2004; Balshi et al. 2005; Boronat-López et al. 2006; Huwiler et al. 2007). At implant installation, the ISQ was 80.91 (medium data), decreasing the first week of healing to 79.75. This was followed by a period (from week 2 to 6) of increasing values. And at week eight, the ISQ had almost returned to the initial value (81.08).

Bone-to-implant contact measurement values obtained by the BS-SEM methodology are depicted in Fig. 4 and Table 3. The results obtained with this methodology of image acquisition, processing and analysis showed an evolution along time very similar to that obtained by other authors in comparable studies (Klongnoi et al. 2006; Tavares et al. 2007; Abrahamsson et al. 2009; Ballo et al. 2009; Calvo-Guirado et al. 2011; Santis et al. 2011).

The analysis of the data revealed that only minor changes of RF values (ISQ) occurred during healing periods of up to 8 weeks. However, the BIC% (mean values) experimented a small decrease until week 2, after which it increased significantly until the last evaluated period (week 8). In the present study, we did not find a positive correlation between the increase in ISQ values and BIC (Spearman correlation coefficient = -0.083 , Fig. 6). However, given that there is just one dog for healing period, this study could be considered as mainly descriptive in nature. Our results parallel previous findings of the above studies, which also failed to correlate RFA with histomorphometrical parameters (Schliephake et al. 2006; Ito et al. 2008; Zhou et al. 2008; Abrahamsson et al. 2009; Jun et al. 2010; Abdel-Haq et al. 2011).

In several clinical studies (Frigberg et al. 1999; Becker et al. 2005), it has been stated that all implants, irrespective of their initial stability, tend to reach a similar level of stability. These results are supported by another study in rabbits (Andersson et al. 2008) which found that implants in soft bone with low primary stability showed a marked increase in stability compared with implants in dense bone. On the other hand, in the rabbit experiment by Meredith et al. (1997b), RF increased with time as a function of an increased stiffness, resulting from new bone formation and remodelling. Taking these observations into account, an explanation of the lack of correlation between BIC and RFA measurements, both in our experiment and others with similar outcomes, could be that the degree of bone contact does not necessarily reflect the

stiffness of the surrounding bone. In fact, in moderately rough implants, as used in this study (SBM, sandblasting soluble particles), their surface is often covered by a thin layer of bone, which is probably not determinant for the biomechanical support of implants. According to Ito et al. (2008), RFA with Osstell® measures stiffness, which is a combination of bone-implant contact and bone density around the implant. Considering this point, it is not surprising that RF does not correspond with histological implant-bone contact.

On the other hand, it has been hypothesized that the inconsistency in the previously reported correlation between ISQ and BIC could be due to the fact that implant stability and BIC% were measured after several weeks or even months of osseointegration, when the interface in the BIC area may become completely bounded. This is because in some studies, the initial stability of implants, which is crucial to the osseointegration ability, was not assessed (Huang et al. 2012).

Very recently, Hsu et al. (2013) found in artificial bone studies (Huang et al. 2012) that the initial implant stability as measured by RFA was strongly positively correlated with the 3-dimensional 3D BIC% assessed by high-resolution microcomputed tomography. Taking into account that this correlation was not found using the 2D BIC%, the authors stated that 3D BIC% should be more representative than the 2D BIC%, as only one or a few histological sections from 2D BIC, cannot represent the entire 3D BIC between the implant and bone (Ito et al. 2008; Liu et al. 2012). However, these studies are of limited value due to the use of synthetic bone models to mimic the advanced cellular structure of bone. As real bone may exhibit more complex biological properties than the synthetic bone, more research needs to be carried out to elucidate how primary implant stability is affected by the 3D BIC%.

Conclusions

The present experiment in beagle dog mandible failed to identify correlations between the histomorphometrical parameter of osseointegration BIC and ISQ values. This finding of an absence of relationship between these two parameters is in agreement with several previous studies in human and experimental animals.

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References

- Abdel-Haq, J., Karabuda, C.Z., Arisan, V., Mutlu, Z. & Kürkcü, M. (2011) Osseointegration and stability of a modified sand-blasted acid-etched implant: an experimental pilot study in sheep. *Clinical Oral Implants Research* **22**: 265–274.
- Abrahamsson, I., Berglundh, T., Linder, E., Lang, N.P. & Lindhe, J. (2004) Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. *Clinical Oral Implants Research* **15**: 381–392.
- Abrahamsson, I., Linder, E. & Lang, N.P. (2009) Implant stability in relation to osseointegration: an experimental study in the Labrador dog. *Clinical Oral Implants Research* **20**: 313–318.
- Al-Nawas, B., Wagner, W. & Grötz, K.A. (2006) Insertion torque and resonance frequency analysis of dental implant systems in an animal model with loaded implants. *International Journal of Oral and Maxillofacial Implants* **21**: 726–732.
- Andersson, P., Verrocchi, D., Viinama'ki, R. & Sennerby, L. (2008) A one-year clinical, radiographic and RFA study of Neoss implants. *Applied Osseointegration Research* **6**: 23–26.
- Antunes, A.A., Oliveira Neto, P., De Santis, E., Caneva, M., Botticelli, D. & Salata, L. (2013) Comparisons between bio-oss[®] and straumann[®] bone ceramic in immediate and staged implant placement in dogs mandible bone defects. *Clinical Oral Implants Research* **24**: 135–142.
- Aparicio, C. (1997) The use of the periotest value as the initial success criteria of an implant: 8-year report. *International Journal of Periodontics and Restorative Dentistry* **17**: 151–161.
- Aparicio, C., Lang, N.P. & Rangert, B. (2006) Validity and clinical significance of biomechanical testing of implant/bone interface. *Clinical Oral Implants Research* **17**: 2–7.
- Atsumi, M., Park, S.H. & Wang, H.L. (2007) Methods used to assess implant stability: current status. *International Journal of Oral and Maxillofacial Implants* **22**: 743–754.
- Ballo, A.M., Akca, E.A., Ozen, T., Lassila, L., Vallittu, P.K. & Närhi, T.O. (2009) Bone tissue responses to glass fiber-reinforced composite implants. A histomorphometric study. *Clinical Oral Implants Research* **20**: 608–615.
- Balshi, S.F., Allen, F.D., Wolfinger, G.J. & Balshi, T.J. (2005) A resonance frequency analysis assessment of maxillary and mandibular immediately loaded implants. *International Journal of Oral and Maxillofacial Implants* **20**: 584–594.
- Becker, W., Sennerby, L., Bedrossian, E., Becker, B.E. & Lucchini, J.P. (2005) Implant stability measurements for implants placed at the time of extraction: a cohort, prospective clinical trial. *Journal of Periodontology* **76**: 391–397.
- Berglundh, T., Abrahamsson, I., Lang, N.P. & Lindhe, J. (2003) De novo alveolar bone formation adjacent to endosseous implants: a model study in the dog. *Clinical Oral Implants Research* **14**: 251–262.
- Berglundh, T. & Stavropoulos, A. (2012) Preclinical *in vivo* research in implant dentistry. Consensus of the eighth European workshop on periodontology. *Journal of Clinical Periodontology* **39**: 1–5.
- Blanco, J., Alvarez, E., Muñoz, F., Liñares, A. & Cantalapiedra, A. (2011) Influence on early osseointegration of dental implants installed with two different drilling protocols: a histomorphometric study in rabbit. *Clinical Oral Implants Research* **22**: 92–99.
- Boronat-López, A., Peñarocha-Diago, M., Martínez-Cortisoso, O. & Mínguez-Martínez, I. (2006) Resonance frequency analysis after the placement of 133 dental implants. *Medicina Oral, Patología Oral y Cirugía Bucal* **11**: E272–E276.
- Buser, D., Broggin, N., Wieland, M., Schenk, R.K., Denzer, A.J., Cochran, D.L., Hoffmann, B., Lussi, A. & Steinemann, S.G. (2004) Enhanced bone apposition to a chemically modified SLA titanium surface. *Journal of Dental Research* **83**: 529–533.
- Calvo-Guirado, J.L., Mate-Sanchez, J., Delgado-Ruiz, R., Ramirez-Fernández, M.P., Cutando-Soriano, A. & Peña, M. (2011) Effects of growth hormone on initial bone formation around dental implants: a dog study. *Clinical Oral Implants Research* **22**: 587–593.
- Chiapasco, M., Gatti, C., Rossi, E., Haefliger, W. & Markwalder, T.H. (1997) Implant-retained mandibular overdentures with immediate loading. A retrospective multicenter study on 226 consecutive cases. *Clinical Oral Implants Research* **8**: 48–57.
- Cochran, D.L., Schenk, R.K., Lussi, A., Higginbottom, F.L. & Buser, D. (1998) Bone response to unloaded and loaded titanium implants with a sandblasted and acid-etched surface: a histometric study in the canine mandible. *Journal of Biomedical Materials Research* **40**: 1–11.
- Donath, K. (1985) The diagnostic value of the new method for the study of undecalcified bones and teeth with attached soft tissue (sage-schliff [sawing and grinding] technique). *Pathology Research and Practice* **179**: 631–633.
- Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The sage-schliff (sawing and grinding) technique. *Journal of Oral Pathology* **11**: 318–326.
- Franch, J., Pastor, J., Franch, B., Durall, I., Manzanares, M.C. & Feline, J. (2000) Back-scattered electron imaging of a non-vertebral case of hypervitaminosis A in a cat. *Journal of Feline Medicine and Surgery* **2**: 49–56.
- Friberg, B., Sennerby, L., Meredith, N. & Lekholm, U. (1999) A comparison between cutting torque and resonance frequency measurements of maxillary implants: a 20-month clinical study. *International Journal of Oral and Maxillofacial Surgery* **28**: 297–303.
- Gedrange, T., Hietschold, V., Mai, R., Wolf, P., Nicklisch, M. & Harzer, W. (2005) An evaluation of resonance frequency analysis for the determination of the primary stability of orthodontic palatal implants. A study in human cadavers. *Clinical Oral Implants Research* **16**: 425–431.
- Glauser, R., Sennerby, L., Meredith, N., Réé, A., Lundgren, A., Gottlow, J. & Hämmerle, C.H.F. (2004) Resonance frequency analysis of implants subjected to immediate or early functional occlusal loading. Successful vs. failing implants. *Clinical Oral Implants Research* **15**: 428–434.
- Hsu, J.T., Huang, H.L., Tsai, M.T., Wu, A.Y.J., Tu, M.G. & Fuh, L.J. (2013) Effects of the 3D bone-to-implant contact and bone stiffness on the initial stability of a dental implant: micro-CT and resonance frequency analyses. *International Journal of Oral and Maxillofacial Surgery* **42**: 276–280.
- Huang, H.M., Lee, S.Y., Yeh, C.Y. & Lin, C.T. (2002) Resonance frequency assessment of dental implant stability with various bone qualities: a numerical approach. *Clinical Oral Implants Research* **13**: 65–74.
- Huang, H.L., Tsai, M.T., Su, K.C., Li, Y.F., Hsu, J.T., Chang, C.H., Fuh, L.J. & Wu, A.Y.J. (2012) Relation between initial implant stability quotient and bone-implant contact percentage: an *in vitro* model study. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* doi: 10.1016/j.oooo.2012.01.037. (in press).
- Huwiler, M.A., Pjetursson, B.E., Bosshardt, D.D., Salvi, G.E. & Lang, N.P. (2007) Resonance frequency analysis in relation to jawbone characteristics and during early healing of implant installation. *Clinical Oral Implants Research* **18**: 275–280.
- Isidor, F. (1998) Mobility assessment with the periotest system in relation to histologic findings of oral implants. *International Journal of Oral and Maxillofacial Implants* **13**: 377–383.
- Ito, Y., Sato, D., Yoneda, S., Ito, D., Kondo, H. & Kasugai, S. (2008) Relevance of resonance frequency analysis to evaluate dental implant stability: simulation and histomorphometrical animal experiments. *Clinical Oral Implants Research* **19**: 9–14.
- Jaffin, R.A. & Berman, C.L. (1991) The excessive loss of branemark fixtures in type IV bone: a 5-year analysis. *Journal of Periodontology* **62**: 2–4.
- Jun, S.H., Chang, B.M., Weber, H.P. & Kwon, J.J. (2010) Comparison of initial stability parameters and histomorphometric analysis of implants inserted into extraction sockets: human fresh cadaver study. *The International Journal of Oral and Maxillofacial Implants* **25**: 985–990.
- Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. & Altman, D.G. (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *Journal of Pharmacology and Pharmacotherapeutics* **1**: 94–99.
- Klongnoi, B., Rupprecht, S., Kessler, P., Thorwarth, M., Wiltfang, J. & Schlegel, K.A. (2006) Influence of platelet-rich plasma on a bioglass and autogenous bone in sinus augmentation: an explorative study. *Clinical Oral Implants Research* **17**: 312–320.
- Lazzara, R.J., Porter, S.S., Testori, T., Galant, J. & Zetterqvist, L. (1998) A prospective multicenter study evaluating loading of osseointegrated implants two months after placement: one-year results. *Journal of Esthetic Dentistry* **10**: 280–289.
- Lioubavina-Hack, N., Lang, N.P. & Karring, T. (2006) Significance of primary stability for osseointegration of dental implants. *Clinical Oral Implants Research* **17**: 244–250.

- Liu, C., Tsai, M.-T., Huang, H.-L., Chen, M.Y.-C., Hsu, J.T., Su, K.-C., Chang, C.-H. & Wu, A.Y.-J. (2012) Relation between insertion torque and bone-implant contact percentage: an artificial bone study. *Clinical Oral Investigations* **16**: 1679–1684.
- Manresa, C., Bosch, M., Manzanares, M.C., Carvalho, P. & Echeverría, J.J. (2013) A new standardized-automatic method for Bone-to-Implant-Contact histomorphometric analysis base on Backscattered Scanning Electron Microscopy images. *Clinical Oral Implants Research* doi: 10.1111/clr.12129 (in press).
- Martinez, H., Davarpanah, M., Missika, P., Celletti, R. & Lazzara, R. (2001) Optimal implant stabilization in low density bone. *Clinical Oral Implants Research* **12**: 423–432.
- Meredith, N. (1998a) Assessment of implant stability as a prognostic determinant. *The International Journal of Prosthodontics* **11**: 491–501.
- Meredith, N. (1998b) A review of nondestructive test methods and their application to measure the stability and osseointegration of bone anchored endosseous implants. *Critical Reviews in Biomedical Engineering* **26**: 275–291.
- Meredith, N., Alleyne, D. & Cawley, P. (1996) Quantitative determination of the stability of the implant-tissue interface using resonance frequency analysis. *Clinical Oral Implants Research* **7**: 261–267.
- Meredith, N., Book, K., Friberg, B., Jemt, T. & Sennerby, L. (1997a) Resonance frequency measurements of implant stability *in vivo*: a cross-sectional and longitudinal study of resonance frequency measurements on implants in the edentulous and partially dentate maxilla. *Clinical Oral Implants Research* **8**: 226–233.
- Meredith, N., Shagaldi, F., Alleyne, D., Sennerby, L. & Cawley, P. (1997b) The application of resonance frequency measurements to study the stability of titanium implants during healing in the rabbit tibia. *Clinical Oral Implants Research* **8**: 234–243.
- Nedir, R., Bischof, M., Szmukler-Moncler, S., Bernard, J. & Samson, J. (2004) Predicting osseointegration by means of implant primary stability: a resonance-frequency analysis study with delayed and immediately loaded ITI SLA implants. *Clinical Oral Implants Research* **15**: 520–528.
- Nkenke, E., Hahn, M., Weinzierl, K., Radespiel-Tröger, M., Neukam, F.W. & Engelke, K. (2003) Implant stability and histomorphometry: a correlation study in human cadavers using stepped cylinder implants. *Clinical Oral Implants Research* **14**: 601–609.
- Preibisch, S., Saalfeld, S. & Tomancak, P. (2009) Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics* **25**: 1463–1465.
- Santis, E.D., Botticelli, D., Pantani, F., Pereira, F.P., Beolchini, M. & Lang, N.P. (2011) Bone regeneration at implants placed into extraction sockets of maxillary incisors in dogs. *Clinical Oral Implants Research* **22**: 430–437.
- Scarano, A., Degidi, M., Iezzi, G., Petrone, G. & Piattelli, A. (2006) Correlation between implant stability quotient and bone-implant contact: a retrospective histological and histomorphometrical study of seven titanium implants retrieved from humans. *Clinical Implant Dentistry and Related Research* **8**: 218–222.
- Schliephake, H., Donnerstag, F., Berten, J.L. & Lönquist, N. (2006) Palate morphology after unilateral and bilateral cleft lip and palate closure. *International Journal of Oral and Maxillofacial Surgery* **35**: 25–30.
- Schulte, W. & Lukas, D. (1993) Periotest to monitor osseointegration and to check the occlusion in oral implantology. *The Journal of Oral Implantology* **19**: 23–32.
- Sennerby, L. & Meredith, N. (2008) Implant stability measurements using resonance frequency analysis: biological and biomechanical aspects and clinical implications. *Periodontology 2000* **47**: 51–66.
- Sennerby, L., Persson, L.G., Berglundh, T., Wennerberg, A. & Lindhe, J. (2005) Implant stability during initiation and resolution of experimental peri-implantitis: an experimental study in the dog. *Clinical Implant Dentistry and Related Research* **7**: 136–140.
- Sjöström, M., Lundgren, S., Nilson, H. & Sennerby, L. (2005) Monitoring of implant stability in grafted bone using resonance frequency analysis. A clinical study from implant placement to 6 months of loading. *International Journal of Oral and Maxillofacial Surgery* **34**: 45–51.
- Stadlinger, B., Bierbaum, S., Grimmer, S., Schulz, M.C., Kuhlisch, E., Scharnweber, D., Eckelt, U. & Mai, R. (2009) Increased bone formation around coated implants. *Journal of Clinical Periodontology* **36**: 698–704.
- Stadlinger, B., Hintze, V., Bierbaum, S., Möller, S., Schulz, M.C., Mai, R., Kuhlisch, E., Heinemann, S., Scharnweber, D., Schnabelrauch, M. & Eckelt, U. (2012) Biological functionalization of dental implants with collagen and glycosaminoglycans—A comparative study. *Journal of Biomedical Materials Research - Part B Applied Biomaterials* **100B**: 331–341.
- Szmukler-Moncler, S., Salama, H., Reingewirtz, Y. & Dubruille, J.H. (1998) Timing of loading and effect of micromotion on bone-dental implant interface: review of experimental literature. *Journal of Biomedical Materials Research* **43**: 192–203.
- Tavares, M.G., Tambasco De Oliveira, P., Nanci, A., Hawthorne, A.C., Rosa, A.L. & Xavier, S.P. (2007) Treatment of a commercial, machined surface titanium implant with H₂SO₄/H₂O₂ enhances contact osteogenesis. *Clinical Oral Implants Research* **18**: 452–458.
- Testori, T., Del Fabbro, M., Feldman, S., Vincenzi, G., Sullivan, D., Rossi, R., Jr, Anitua, E., Bianchi, F., Francetti, L. & Weinstein, R.L. (2002) A multicenter prospective evaluation of 2-months loaded Osseotite[®] implants placed in the posterior jaws: 3-year follow-up results. *Clinical Oral Implants Research* **13**: 154–161.
- Zhou, Y., Jiang, T., Qian, M., Zhang, X., Wang, J., Shi, B., Xia, H., Cheng, X. & Wang, Y. (2008) Roles of bone scintigraphy and resonance frequency analysis in evaluating osseointegration of endosseous implant. *Biomaterials* **29**: 461–474.
- Zix, J., Hug, S., Kessler-Liechti, G. & Mericske-Stern, R. (2008) Measurement of dental implant stability by resonance frequency analysis and damping capacity assessment: comparison of both techniques in a clinical trial. *International Journal of Oral and Maxillofacial Implants* **23**: 525–530.