The Relationship of Some Histologic Parameters, Radiographic Evaluations, and Periotest Measurements of Oral Implants: An Experimental Animal Study

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The objective of this study was to analyze the efficacy and correlation between clinical and histologic parameters used to evaluate oral implants. After extraction of the premolars and a healing time of 4 months in 16 Dutch goats, four Branemark implants were placed in the maxillary left and right premolar regions. After a healing time of 6 months, followed by another 4 months with the permucosal abutments, the goats were sacrificed and the jaws were block-resected. Before histologic preparation, long-cone radiographs were made and Periotest scores of the implants were recorded. Bone level measured histomorphometrically were found to be 0.85 mm more apically, compared to that measured radiologically (P = .001). Furthermore, statistically significant correlations (P > 0.2) were not found between the Periotest values of the calcium-phosphate-coated and uncoated implants for (1) the first thread in contact with bone, or (2) with the total number of threads in contact with bone. It was concluded that the radiologic data overscored the real marginal bone level around screw-shaped oral implants, and that the Periotest device is neither able to discriminate between the first thread nor between the total number of threads in contact with bone.

(INT J ORAL MAXILLOFAC IMPLANTS 1997;12:380-386)

Key words: bone level, histomorphometry, oral implant, Periotest, radiology

The best way to ensure that an oral implant is anchored by a firm union with vital bone is to demonstrate direct bone apposition to the implant surface at the light microscopic and ultrastructural levels. Because this is only possible in the case of implants placed for experimental purposes, several diagnostic methods have been developed for clinical evaluation of the implant status.

At present, only crude clinical parameters are available for evaluating the biologic success of an oral implant, namely, absence of mobility and stable marginal bone level. Visual implant mobility as an indication of failing bone apposition is generally known. Although clear visible mobility can usually be correlated with an interposition of fibrous tissue, sometimes mobility is hardly detectable or is subclinical and thus represents the actual problem.

Long-cone radiographs are of little help in this respect because the discrimination accuracy of radiographs is limited. The resolution level of an optimal radiographic technique is close to 0.1 mm. Given that the size of a fibroblast is at least 10 times smaller, it becomes evident that radiographs cannot be used to exclude the possibility of intervening soft tissues.
Therefore, dynamic measurement methods using implant percussion were investigated to assess clinical mobility of oral implants. This has resulted in the development of an electronic device, the Periotest (Siemens AG, Bensheim, Germany), which has been reported to provide for an objective quantitative and reproducible clinical measurement of the stability of bone-implant anchorage. The Periotest device was originally developed to dynamically measure the periodontal reaction after an impact force was applied to the tooth. The Periotest method is based on the empirical finding that the greater the implant solidity, the higher the deceleration of the tapping rod that touches the implant, and thus the higher the damping effect of the surrounding tissues. This damping effect is not expressed in the measured contact time of the rod, but it appears on a digital scale with values ranging from ~8 to +50, called the Periotest value. The reproducibility of the device is said to be ±1 Periotest unit.

Besides lack of implant mobility, the second clinical parameter for assessing implant integrity is stable marginal bone level. This can be rated by means of accurate long-cone radiographs. However, irradiation hygiene and the fact that radiographs reveal marginal bone level mesiodistally only encourage many clinicians to look for other methods. Therefore, monitoring changes in attachment level by means of the periodontal probe is proposed by others through analogy with teeth, although the prognostic value of the technique has been questioned. Others deny the value of the probe in monitoring implant health.

The aim of the present study was to analyze the correlation between the histologic and radiologic bone level around oral implants placed in the maxillae of goats. In addition, Periotest values were correlated to the actual bone contact around implants of the same design, but with differing surface layers.

**Materials and Methods**

**Experimental Design.** Sixteen female adult Dutch goats with a mean weight between 50 and 80 kg and an age of 24 to 28 months were used to investigate the bone reaction around different calcium-phosphate–coated (Ca-P–coated) screw-shaped implants.

The first and second premolars of the maxillary dentition were extracted bilaterally under general anesthesia and the alveoli were allowed to heal for a period of 4 months. Special care was taken to avoid both root and bone frag­ment fractures. Following completion of the bone healing at the extraction sites, four commercially pure titanium screw-designed implants with a diameter of 3.75 mm and a length of 10 mm (Mk II type, Nobel Biocare AB, Göteborg, Sweden) were placed under general anesthesia in the edentulous left and right premolar regions. Three of the four implants were coated with a Ca-P plasma-spray coating (fluorapatite, hydroxyapatite, and heat-treated hydroxyapatite). The implants were placed with Nobel Biocare drilling equipment using a very gentle surgical technique. A total of 64 implants was placed, with a distance of 7 mm between the implants, according to a balanced split-plot design. The implants were covered with a cover screw of pure titanium, and the mucoperiosteal flaps were closed with Vicryl (Ethicon GmbH, Norderstadt, Germany) 2–0 sutures. To avoid postsurgical infection, antibiotic (Albipen, Mycofarm, de Bilt, The Netherlands) coverage was applied for 3 days. The implants were buried for 6 months to permit healing of the alveolar bone. At the end of this period, the animals were subjected to a second operation for abutment connection (length 4 mm, diameter 4.5 mm). After placement, the abutments were covered with a plastic healing cap. The animals were sacrificed 4 months later by an overdose of Narcovet (Afarmo, Arnhem, The Netherlands).

**Clinical Evaluation.** The damping characteristics of the implants were determined using the Periotest device. After animal sacrifice, the entire maxilla was harvested and the Periotest values were determined immediately, before fixation of the tissue specimens. The jaws were positioned so that the implants were perpendicular to the floor, and the tapping handpiece of the Periotest was held in a horizontal position at a distance of about 2 mm from the abutment surface. Percussion by the Periotest rod occurred just below the edge of the coronal platform of each abutment. The same Periotest device was used during all measurements, and it was calibrated before the start of any of the measurements. All measurements were performed by the same investigator and were accomplished within 30 minutes of animal sacrifice. The mean Periotest value of two measurements for each implant was calculated. Data were accepted only when two consecutive values that differed by no more than one Periotest unit were obtained.

Radiographic examination was performed using a long-cone radiographic technique. To improve the standardization of the procedure, block biopsy specimens of the left and right halves of the maxilla, including the two implants and the adjacent tooth with the surrounding soft and hard tissues, were obtained. The block biopsy specimens were aligned perpendicular to the cone of the apparatus, and one radiograph per biopsy specimen was made. Kodak radiographic films (size 2), all from the same batch, were exposed using a General Electric x-ray machine.
with exposure factors of 65 kV and 15 mA, and a focal-film distance of 36 cm. All films were developed with a Dür-Periomat (Dür Dental-D-7120, Bietigheim-Bissingen, Germany) automatic dental film processor. On the radiographs, two reference points were identified mesial and distal from the implants: the bone crest (BC); and the top of the abutment (AB) (Fig 1). The distance BC-AB was measured mesially and distally from the implant, and the mean was calculated. All measurements were performed by the same examiner using a vernier caliper (Mitutoyo Digimatic 500) with a scale up to 0.01 mm. Finally, a correction factor was applied to compensate for possible small errors between the radiologic and actual lengths of the implants.

**Histologic Procedure.** Following the clinical evaluation, each implant was resected with the surrounding tissues out of the block biopsy specimens and was fixed in 10% buffered formalin solution. After dehydration by alcohol series, the implant tissue specimens were embedded in methyl methacrylate resin. The implant and tissue block were mounted in a modified inner circular saw microtome, and 10-μm-thick serial sections were prepared. The sections were made in a buccopalatal direction parallel to the long axis of the implant surface. The sections were stained with methylene blue and basic fuchsin for histomorphometric evaluation.

**Histomorphometric Evaluation.** The complete series (sections 1 to 11) of histologic sections of each implant were gathered from five randomly chosen implants. The screw threads of each implant were numbered from 1 (most apical thread) to 12 (most coronal thread). For the complete series of histologic sections (1-11), the first screw thread showing bone contact was rated using a light microscope. In these sections, it was possible to determine the first thread showing bone contact at the mesial as well as distal sides of the implant. In addition, through evaluation of all of the histologic sections of these five implants, the path of the bone level could be defined (Fig 2).

Subsequently, of the buccopalatal sections of all implants, the midsection of each implant was used to rate the bone level, defined as the distance from the top of the abutment to the bone crest in contact with the implant surface. Also, the number of screw threads and the first screw thread in contact with bone were measured in these midsections. To rate the first screw thread with bone contact, the threads were numbered in ascending order from the most apical screw thread (1) to the most coronal screw thread (12) (Fig 2).

**Results**

During abutment placement, it was observed that 10 of the 64 implants were lost or were too mobile to connect with the abutment; these were removed. Furthermore, another six implants failed during the 4 months of the pergingival phase. Finally, 10 months after placement, 48 implants could be used for further examination.

Of the five randomly chosen implants, the bone height observed in the 11 histologic sections per
Histologic sections of the implants were made in a buccopalatal direction, parallel to the long axis of the implants. The first and last sections give mesial and distal views of the implant. The screw threads were numbered from 1 (most apical) to 12 (most coronal). The following histomorphometric data were determined from the histologic sections: (1) the number of the first screw thread with bone contact and (2) the number of screw threads with bone contact. From five randomly chosen implants, the path of the bone level was evaluated in these sections (n = 11).

Figs 2a and 2b

Histologic sections of the implants were made in a buccopalatal direction, parallel to the long axis of the implants. The first and last sections give mesial and distal views of the implant. The screw threads were numbered from 1 (most apical) to 12 (most coronal). The following histomorphometric data were determined from the histologic sections: (1) the number of the first screw thread with bone contact and (2) the number of screw threads with bone contact. From five randomly chosen implants, the path of the bone level was evaluated in these sections (n = 11).

Fig 3  Mean radiologic and histologic scores ([buccal + palatal]/2) of the bone level and differences (histologic/radiologic bone level) of 28 individual implants with optimal radiographs. The values under the bold line represent the values where the radiologic values were greater than the histologic values; those above the line represent values that were less.

The implant was rated at the same level. Thus, there was no difference in the first thread showing bone contact at the mesial or distal and buccal or palatal sides of the implant (Fig 2).

Although a standardized radiologic technique was used, only 28 of the 48 radiographs were acceptable for radiologic examination. These same 28 implants used for the radiologic measurements were also used for the histomorphometry. The mean values of the radiologic and histomorphometric scores of the bone level are given in Fig 3. On average, the histomorphometric-rated bone level was located 0.85 mm more apically, compared to the radiologic-measured bone level. Statistical testing of these data using the one-way analysis of variance (ANOVA; from the Statmost package Datamost, Salt Lake City, UT) and a paired t test revealed that this difference was statistically significant (P = .001).

Table 1 shows the mean Periotest scores and histomorphometric data for the different implant materials. A paired t test revealed a significantly higher Periotest value only for the titanium implants com-
Table 1 Mean ± SD Periotest Values and Histomorphometrical Data for Various Implant Materials

<table>
<thead>
<tr>
<th>Material*</th>
<th>Periotest value</th>
<th>No. of screw threads with bone contact</th>
<th>No. of first screw thread with bone contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti</td>
<td>10.1 ± 5.4</td>
<td>5.25 ± 4.9</td>
<td>3.25 ± 3.2</td>
</tr>
<tr>
<td>HA</td>
<td>7.4 ± 7.3</td>
<td>11.67 ± 7.0</td>
<td>7.22 ± 3.8</td>
</tr>
<tr>
<td>HAHT</td>
<td>4.3 ± 4.9</td>
<td>11.56 ± 6.1</td>
<td>7.53 ± 3.1</td>
</tr>
<tr>
<td>FA</td>
<td>2.9 ± 3.3</td>
<td>13.06 ± 7.2</td>
<td>8.37 ± 3.8</td>
</tr>
</tbody>
</table>

*Ti = titanium; HA = hydroxyapatite; HAHT = heat-treated hydroxyapatite; FA = fluorapatite.

The maximum number of screw threads with bone contact is 24 (12 mesial and 23 distal).

The number of the first screw thread with bone contact is determined by starting at the apex of the implant and continuing to screw thread 12 at the top of the implant.

pared to the Ca-P–coated implants (P = .001). A simple linear regression analysis was performed to determine if there was a correlation between (1) the observed Periotest values and the first screw thread showing bone contact, and (2) the Periotest values and the total number of screw threads showing bone contact for the different implant surfaces. The correlation coefficient (r) revealed that a correlation (P > .2) did not exist between the Periotest value and the first screw thread with bone contact for the coated (r = −.11) and uncoated implants (r = .38), or between the Periotest value and the total number of screw threads in contact with bone for the coated (r = −.17) and uncoated implants (r = .42).

Discussion

There are two reasons that account for the finding that only 30 of the 64 implants could be used for radiographic evaluation in the present study. First, the animal model was selected primarily to study the influence of different implant materials on bone behavior in spongy bone, which is the condition found in maxillae of goats. During the months after placement, 48 implants were maintained for further investigation. The results of this part of the investigation are reported in another article that showed the positive effect of Ca-P coatings on implant success during the healing and remodeling period. Second, another 18 implants were excluded from further radiographic investigation because the readability of these radiographs hampered the exact interpretation of the marginal bone height. This was mainly the result of the absence of strict parallelism of both implants per block biopsy specimen. In this context, it has to be noted that only one radiograph per side (left/right) was made.

From a previously published study, Student’s paired t tests revealed no statistically significant difference between the palatal and buccal data for the various parameters for the histomorphometric evaluations also used in the present study. Therefore, all histomorphometric data presented in Fig 3 were grouped for analysis.

The most commonly used approach to diagnose bone behavior around oral implants is radiographic bone examination. For example, radiography is used to determine the occurrence of vertical bone loss. However, it is also known that radiography is hampered by some technical difficulties: first, the resolution level is limited; and second, it provides a two-dimensional representation of a three-dimensional structure. Therefore, to gain more insight into the clinical relevance of radiographs, the radiologic measured bone height around experimental Brånemark implants and the histologic marginal bone height were compared in the present study. The measurements revealed that there was a statistically significant difference between the radiologic- and histologic-rated bone level. On average, the radiologic data overscored the real marginal bone height by 0.85 mm. Confirmation of this observation was found in a recent study in which radiologic and histologic bone height measurements were performed on the same type of implants placed in the same implantation region of goats, but in unloaded conditions. Sewerin showed in an in vitro study that distortion of the buccal and lingual bone margins in clinical radiographs may result in overestimation of bone heights. He attributed the degree of overestimation to the buccolingual position of the implant. As a clinical consequence, one should be aware that even optimal long-cone radiography can lead to a too optimistic image of the actual situation. However, Ahlqvist et al reported in a clinical study that the threshold for marginal bone loss around Brånemark implants exceeds 0.47 mm; therefore, they suggest the use of implant threads as a measurement scale.
Interesting is the fact that the course of bone level around implants, at least in the present study, was equal mesiodistally and buccopalatally. This strengthens the value of the radiographic technique for extrapolating mesiodistal data to the buccopalatal data. However, it should be stressed that in this animal study, nonpathologic conditions such as dehiscences or fenestrations did not occur because of the favorable alveolar width–implant diameter ratio. From clinical observations, it is known that these conditions are not always met in the clinic.

In addition to bone height measurements, the lack of stability of implants is an important parameter for monitoring implant success. The latter was indirectly investigated by the clinical efficacy and predictability of the Periotest device. Periotest values measured on the retrieved implant-bone specimens were related to the histologic bone-implant contact area. No correlation could be found. Considering these findings, interpretation of Periotest data for the evaluation of osseointegration needs to be clarified. The Periotest was originally designed to measure the damping properties of the periodontal ligament around natural teeth. The clinical functioning of implants is based on the achievement of direct bone apposition. However, the implant-bone interface will always consist of a mixture of fibrous and bone tissue. It is assumed that the Periotest data reflected only the mechanical properties of this fibro-osseous complex because the force of the rod of the Periotest transmitted to the implant can be expressed as an impulse. This impulse is transmitted to the bone. Deceleration of the rod is thus dependent on the damping capacities and Young’s modulus of the implant-surrounding tissues. The Periotest values found in this study were correlated neither with the bone level in relation to the implant body, nor with the amount of threads in contact with bone. Combined with other appropriate clinical data, the Periotest should provide the clinician with sufficient information about the implant support to arrive at a diagnosis. Lower Periotest values are assumed to be indicative of favorable and predictive implant-bone conditions. However, this supposed relationship between Periotest values and bone contact was not found in the present study. Although the mean values of the Periotest and bone data suggest a correlation, statistical testing did not confirm this finding. To illustrate this, examples from three different heat-treated hydroxyapatite-coated implants with Periotest values of +2, 0, and +4 exhibited a total of 7, 9, and 8 screws in contact with bone, respectively. An additional three heat-treated hydroxyapatite-coated implants with more or less the same Periotest values of +1, +2, and +3 exhibited double the total numbers of screws in contact with bone: 18, 18, and 19, respectively. This observation corroborates the findings of Carr et al. who investigated the relationship between Periotest value and torque value. They found that this relationship is not strong enough to use one parameter to predict another. Recently, Evans et al. concluded that the Periotest gives an objective measurement of implant stability, but it does not correlate with the degree of osseointegration. A low Periotest value will thus not exclude an implant anchored with little bone. This means that the reliability of one single Periotest examination is not quite substantial, but it is perhaps suitable in a consecutive series of measurements of a great number of implants as demonstrated in previous investigations.

Part of our results are in contrast with the findings of Carr et al. when different implant materials are considered. They reported that for unloaded implants assessed 3 or 4 months postplacement, the Periotest values are not different for the various implant materials used (commercially pure titanium, titanium alloy hydroxyapatite). However, in our study the implants were loaded for 4 months. This discrepancy in observations may be attributed to the loading situation wherein biomaterial-specific interactions become evident.

Because the Periotest device alone is not able to measure the amount of bone in contact with the implant, radiographs or probing remain necessary tools to follow the marginal bone level and/or attachment level over time. Quirynen et al. reported that the mean distance between the marginal bone level and the probing attachment level around Bränemark implants was 1.4 mm, and that 85% of the observations fell within 1 mm of the means. However, the values ranged from -2 to +5 mm.

Finally, it must be emphasized that the exclusion of 18 implants did not influence all the aforementioned observations. First, in a second series of experiments, a similar discrepancy between histomorphometric and radiographic bone level was observed. Second, separate additional evaluation of the Periotest data and bone contact percentages of the excluded specimens did not enable the establishment of a relationship between Periotest and bone contact values. Also, a critical note is appropriate with respect to the high implant failure rate. Although most failures occurred in the noncoated group, this cannot interfere with the statistical significance of our findings, especially since the overall design of this study was not focused on demonstrating a relationship between material and Periotest value, but on Periotest value and actual bone contact percentages.
Conclusion

The present study revealed that radiologic data overscored the actual marginal bone level around screw-shaped oral implants, and that the Periotest device is not able to discriminate between the first thread, or between the total threads in contact with bone. Studies that investigate the diagnostic value of evaluation techniques, based on an understanding of their sensitivity and specificity compared to established clinical parameters and on a standardization of histology data addressing the biologic support for oral implants, are recommended.

Acknowledgments

These investigations were supported by The Netherlands Technology Foundation (STW). The authors thank Mrs A. Grosjean-Dreisbach (Pennsylvania) for her critical review of the English grammar of this article.

References