Assessment of Osteointegrative Response Around Dental Implants Using Technetium 99-Methylene Diphosphate Scintigraphy: A Comparison of Two Implant Surfaces in a Rabbit Model

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Purpose: *To compare the metabolic activity at the bone-implant interface of implants with machined and rough surfaces using bone scintigraphy during the in vivo process of osseointegration in a rabbit model, as well to establish a correlation between activity index (AI) and the bone-implant contact percentage (%BIC).* **Materials and Methods:** *Twenty-four implants were placed (12 with a machined surface and 12 with a rough titanium oxide surface) in 12 New Zealand White rabbits. Preoperatively and during the postoperative period (at 15 days and at monthly intervals), animals underwent bone scintigraphy with technetium 99m-methylene diphosphate (Tc-99m-MDP), and the AI for each implant was calculated by planar and pinhole collimator scintigraphy. A total of 240 AIs were obtained; after animal sacrifice at 105 days postsurgery, the %BIC was measured by scanning electron microscopy in 10 samples of each implant surface type.* **Results:** *The activity-time curve showed a similar morphology for both implant types and both scintigraphy techniques. The maximum mean AI appeared after 15 days of implantation and was higher in machined implants. Significant differences were not found in the %BIC according to implant type. A significant correlation between the mean activity registered in the first postoperative scintigraph and the mean %BIC at the end of the study was observed for machined implants only.* **Conclusions:** *Tc-99m-MDP is useful for the assessment of osseous metabolic activity associated with different microsurfaces. The association between mean AI and %BIC was only demonstrated for machined implants in the first postoperative scintigraphy image.* Int J Oral Maxillofac Implants 2012;27:561–565.

Key words: *bone scintigraphy, dental implants, implant surface, osseointegration*

Treatment of implant microsurfaces improves the speed and quality of the osseointegration process. This has been confirmed by different diagnostic methods, particularly by histomorphometric parameters, for which a tissue sample is required. Bone scintigraphy may be of interest because it allows observation of in vivo osseous activity. By means of autoradiography, it has been shown that the incorporation of a radioactive tracer (technetium 99m-methylene diphosphate [Tc-99m-MDP]) into bone is performed by ion exchange of neoformed hydroxyapatite crystals.[1] This dynamic process allows assessment of in vivo osseous activity, which in turn can be determined more accurately with the incorporation of a collimator in the gamma camera (pinhole or multiple-channel).

It has been shown that certain types of implant surfaces stimulate absorption of adhesion proteins, becoming "bioactive surfaces" that accelerate osseointegration.[2–4] As an example, the porous surface achieved by anodic oxidation has a strong capacity to absorb proteins and fluids (bioactive), in contrast to machined-surfaced titanium implants.[5] Although there is a large body of evidence regarding the use of bone scintigraphy as a useful technique to assess the course of in vivo osseointegration of dental implants,[6–11]

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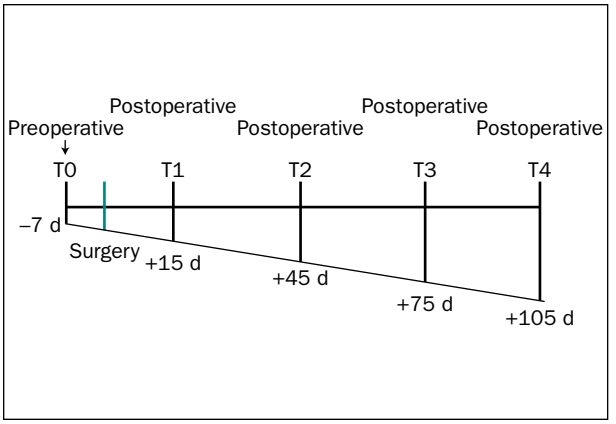

Fig 1 Sequence of acquisition of bone scintigraphy images.

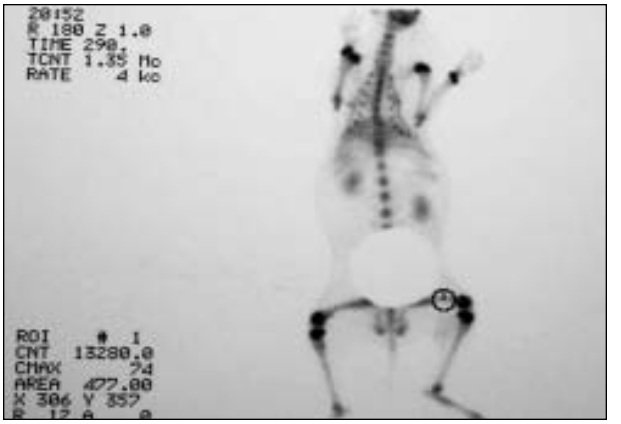

Fig 2 Planar scintigraphy image at a control site (animal #11) obtained at 45 days after placement of the implants.

no studies have yet been performed to analyze the relationship between metabolic activity around an implant and the bone-implant contact percentage (%BIC) and use the result to compare different implant surfaces.

Therefore, the objective of the present experimental study was to compare the metabolic activity at the bone-implant interface of implants with machined and rough surfaces using bone scintigraphy as a method to observe the in vivo process of osseointegration in a rabbit model, as well to establish a correlation between activity index (AI) and the %BIC. The working hypotheses were as follows: (*1*) scintigraphy may be useful to compare in vivo metabolic activity of different implant microsurfaces, and (*2*) there is a correlation between mean scintigraphic osseous activity and the %BIC assessed by scanning electron microscopy, and therefore greater scintigraphic activity will be detected for implants with a higher %BIC.

MATERIALS AND METHODS

Material

The study was approved by the Ethics Committee on Animal Research of the University of Barcelona. A total of 12 somatically homogenous male young-adult New Zealand White rabbits received 12 MkIII TiUnite implants (rough titanium oxide surface; Nobel Biocare) and 12 MkIII machined implants (Nobel Biocare). All implants were 3.75 mm wide and 10 mm long.

For each bone scanning procedure, each animal was administered 185 mBq of Tc-99m-MDP intravenously prior to induction of general anesthesia. Acquisition of static images of planar scintigraphy was performed with a digital ELSCINT SP-6HR wide-field scintillation gamma camera equipped with a low-energy, high-resolution collimator.

Scintigraphic Protocol

Tibial and femoral planar and pinhole scintigraphy scans were obtained preoperatively (7 days before implantation) and at 15, 45, 75, and 105 days after implant placement (Fig 1). Activity counts were obtained in the regions of interest (ROI), ie, where the implants were located, as well as in the contralateral ROI at the tibial and femoral levels; the latter sites served as controls (Fig 2). All ROIs were assessed by the same investigator. In planar scintigraphy imaging, image acquisition was limited to 1,000 activity counts. In pinhole collimator scintigraphy, image acquisition time was restricted to 500 s, and the activity in the ROI was recorded only in the paw in which implants had been placed but in two different regions: one corresponded to the implant and another to a more distant area, which was used as a control (Fig 3).

The AI was defined as the rate between the number of counts in the implant ROI and the number of counts in the control ROI: AI = counts ROI implants/counts ROI control.

Scintigraphic studies were performed under general anesthesia with ketamine 25 mg/kg (Ketolar, Parke-Davis, Pfizer) and xylazine 5 mg/kg (Rompun, Bayer). A 21-G Abbocath catheter (Becton Dickinson) was then inserted into a peripheral vein for the intravenous administration of the radioisotope exclusively.

Implant Placement

After induction of general anesthesia, a single dose of a nonsteroidal anti-inflammatory (meloxicam, 0.2 mL/5 kg body weight; Metacam, Boehringer-Ingelheim) was administered by the subcutaneous route. Implants were placed into the distal femoral diaphysis and the proximal tibial diaphysis, following the manufacturer's instructions. According to the order in which they underwent the surgical procedure, animals corresponding to odd

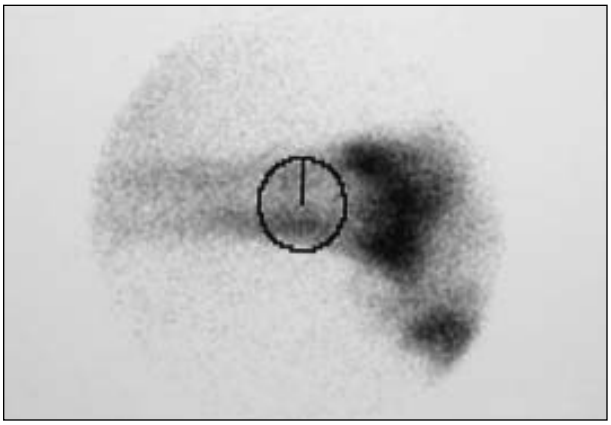

Fig 3 Pinhole collimator scintigraphy image in the femoral region of interest (animal #11) obtained at 75 days after placement of the implants.

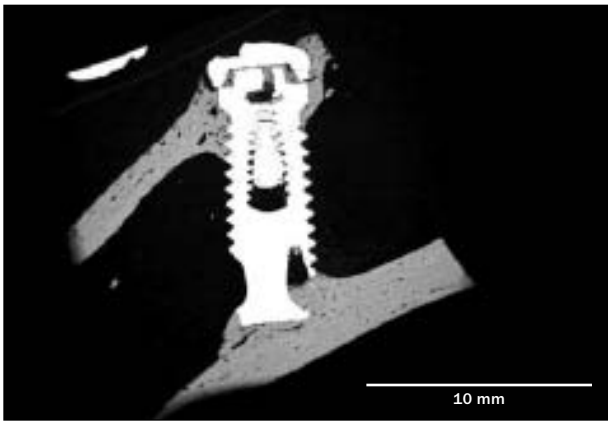

Fig 4 Scanning electron microscopic image of a tibial implant (animal #4).

numbers received machined implants in the tibial surface and rough implants in the femoral surface, whereas animals corresponding to even numbers received rough implants in the tibial surface and machined implants in the femoral surface. Intraoperatively, doses of penicillin-streptomycin 1 mg/10 kg body weight (Hipracilin Retard, Hipra) and buprenorphine 0.05 mg/7 kg body weight (Buprex, Schering-Plough) were administered by the intramuscular route. This treatment was continued for 1 week after surgery.

Sampling Processing Method

Animals were sacrificed when radioactivity in the surgical area was similar to that obtained at T0 (background preoperative activity). Therefore, all animals were sacrificed at the same time because an AI similar to T0 was observed at T4 (105 days after surgery). Samples were obtained and processed according to the method of Manzanares et al,[12] which was developed from the technique described by Donath and Breuner.[13] An EXAKT light polymerization unit was used for the polymerization process. The specimen was then fixed in colloidal silver and coated with evaporated carbon. Scanning electron microscopy samples were used for analysis of %BIC with a computer imaging program (IMAT) developed by Serveis Cientifico-Tecnics of the University of Barcelona. For the assessment of the contact area, only the threaded portion of the implant was considered; the neck and screw were excluded, even if they were surrounded by cortical bone (Fig 4).

Statistical Analysis

Data of 240 AI (planar scintigraphy, n = 120; pinhole scintigraphy, n = 120) were entered into a database. A two-factor analysis of variance was used to assess the interaction between type of implant (machined vs TiUnite) and site of implantation (femoral vs tibial metaphysis) for %BIC according to scintigraphic activity. The relationship between %BIC and mean AI in the planar and pinhole scintigraphic scans at different time points was analyzed with the Pearson correlation coefficient. Statistical significance was set at $P < .05$. The analysis was performed using SAS software (SAS Institute), version 8.0 for Windows.

RESULTS

There were statistically significant differences between the implant types, with mean (± standard deviation [SD]) AI values being lower for TiUnite implants than for machined implants (femur: 1.17 ± 0.04 vs 1.29 ± 0.04, respectively; $P = .037$; tibia: 1.93 ± 0.09 vs 1.29 ± 0.04, respectively; $P = .026$). For both types of implants, AI values at the femoral level were significantly lower than AI values at the tibial level ($P = .013$). With respect to the different time points, preoperative activity was similar for both implant types ($P = .814$). Only at the first assessment (T1, 15 days postoperatively) were the differences in AI of TiUnite and machined implants statistically significant in the planar ($P = .004$) and pinhole ($P = .008$) images. No differences were observed at other time points.

Two TiUnite implants and two machined implants were excluded because of technical failures that prevented assessment of %BIC of the longitudinal axes of the implants (animal #3 tibia, animal #5 femur, animal #9 femur, animal #10 femur). Therefore, 20 specimens (10 TiUnite, 10 machined) were available for evaluation, 11 from the femoral position and 9 from the tibial position. The mean %BIC was 47.6% for TiUnite implants and 55.6% for machined implants. There were no interactions between type of implant (machined vs TiUnite) and site of implantation (femoral vs tibial

metaphysis) for %BIC according to scintigraphic activity (type of implant, $P = .105$; site of implant, $P = .431$; type × location, $P = .495$). In contrast, the correlation between %BIC and scintigraphic activity was significant only for pinhole scans at T1 (15 days after surgery) ($r = 0.693$, $P = .026$).

DISCUSSION

The choice of the rabbit as the model for the present study is supported by numerous previously published studies using the same model in which implants were placed in the same locations, although treatments of the implant surface that may increase the strength of osseointegration have not always been described.[14–20]

Significant differences were observed in %BIC at the end of the study between machined implants and implants with a rough titanium oxide surface. Differences in %BIC according to site of implantation were not found. Differences in the level of scintigraphic activity were observed depending on the location of the implants: AI values at the femoral level were significantly lower than at the tibial level for both implant surfaces. The usefulness of Tc-99m-MDP bone scintigraphy in assessing osteoblastic activity around dental implants has been documented by others.[21] The integration process of dental implants has been evaluated by different techniques, including bone single-photon emission computed tomography (SPECT) and bone scintigraphy, which have consistently shown distinct phases of osteoblastic activity with a maximum peak within the first month after placement of dental implants.[7–9,22] In the authors' experience, AI returned to preimplantation levels after approximately 14 weeks, which is a more prolonged period than the passive osseointegration process established for the rabbit and data reported in histologic or clinical studies of osseointegration.[15,20] However, peak AI values obtained in the present experimental model cannot be compared with previous data because of a lack of similar published studies. In human studies, such as those carried out by Bambini et al[9] and Khan et al,[22] peri-implant osteoblastic activity returned to preimplantation levels after 4 months or between 3 and 5 months, depending on the specific metabolic properties of the jaw.[7]

In the present experimental model, the different implant surfaces achieved similar %BIC during the study period. Interestingly, the theoretically more bioactive surface (TiUnite) did not show a higher rate of scintigraphic activity. This may be explained by the milder inflammatory reaction and therefore greater biocompatibility[23]; alternatively, machined implants undergo more biologic activity to obtain the same %BIC. Khan et al[22] demonstrated that endosseous dental implantation is associated with a 30% increase in the activity of dental alveoli, as compared with a drilled hole that did not receive an implant.

Histomorphometric studies that use light or electron microscopy to compare, for example, %BIC, the number and characteristics of osteocytes, and characteristics of the collagen near the implant surface, are very useful for comparison of the performance of different implant surfaces.[24–27] However, the requirement that a large number of animals must be sacrificed at different time points to follow the course of osseointegration over time is a disadvantage of these types of studies. In the present study, histologic specimens were prepared to be observed under scanning electron microscopy with a single goal of quantifying the %BIC. In this respect, the histologic result was evaluated only at the end of the period of scintigraphic activity, rather than with respect to different %BIC at various time intervals. No statistically significant differences were found between implants according to their location or surface characteristics. However, a higher %BIC was recorded at the tibial level for both types of implants.

Albrektsson and Johansson[27] reported a higher %BIC for TiUnite implants placed in the femur; they explained that this was a result of the higher trabeculation of the rabbit femoral metaphysis. In the present study, AI was significantly lower in the femoral location for both types of implants, as observed by both planar and pinhole bone scintigraphy. This may be explained by the fact that rough surfaces in general—and the titanium oxide surface in particular—showed a comparatively high %BIC in the early periods of osseointegration as a result of greater stability and osseoconductivity. In the present study, all animals were evaluated at the same time point, and from a histologic point of view, this phenomenon could not be assessed, as only the final result was evaluated.

A positive correlation between bone activity, as registered with pinhole collimator scintigraphy, and higher %BIC for machined implants was seen at the first examination after surgery. The same findings at the same time point and for the same implant type with planar scintigraphy were not found. The clinical generalizability of these results, however, is limited, given that the %BIC of machined and titanium oxide surfaces did not show statistically significant differences and that four bone specimens were excluded because of technical failures in sample processing. Therefore, a study with a larger sample size would be desirable. In addition, bone area was not measured; future studies might investigate the relationship between AI and bone area around the implant.

CONCLUSIONS

Greater scintigraphic activity, as registered by pinhole collimator, was correlated with a higher bone-implant contact percentage, as assessed by scanning electron microscopy, for machined implants in the immediate postoperative period. However, this finding may not be clinically relevant because of the size and distribution of the study sample.

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