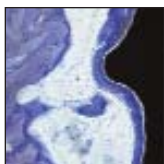




Time Sequence of Bone Healing Around Two Implant Systems in Minipigs: Preliminary Histologic Results



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The aim of this study was to evaluate the early phases of bone healing around two different implant surfaces. For this purpose, four minipigs were used. Implants with rough titanium surfaces (ITI sandblasted/acid-etched and Brånemark TiUnite) were placed in the maxillae of the animals and sacrifice was scheduled in such a way that healing times of 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, and 7 weeks were obtained. Histologic analysis revealed that a similar pattern was observed in the phases of bone healing around both types of implants between 3 days and 7 weeks. This pattern consisted of the replacement of blood clot and bone debris with a provisional connective tissue in the first few weeks and with mineralized tissue and marrow spaces later on. Both rough surfaces allowed for "contact osteogenesis" to take place. (Int J Periodontics Restorative Dent 2009;29:549–555.)

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Primary stability and optimal loading time of dental implants seem to be key characteristics for osseointegration and, consequently, success in implant dentistry.¹ The traditional protocol for implant therapy described by Adell et al² implied that abutment connection followed implant placement after a healing time of 3 to 4 months in the mandible and 5 to 6 months in the maxilla. Loading was initiated 2 weeks later. Nowadays, clinicians often focus on loading implants earlier than originally recommended.³

In a literature review, Esposito et al⁴ concluded that "while it is possible to successfully load oral implants immediately after their placement in mandibles of adequate bone density and height of carefully selected patients, it is yet unknown how predictable this approach is." *Early loading* of implants implies that the prosthetic phase is initiated in the first 3 to 4 weeks after implant placement, while *immediate loading* implies that the prosthetic device be placed within 24 hours of implant placement.³ Szmukler-Moncler et al⁵ reported that success in immediate/early loading of implants was possible, provided that extensive



Fig 1 Clinical image of a Brånemark implant and an ITI implant at the time of insertion.

micromotion at the interface between bone and implants was prevented.

In a literature review concerning the efficacy of different implant systems that was based on randomized controlled clinical trials, Esposito et al⁶ found no clinical differences among implant systems. Histologic studies also exist reporting on the performance of different implant systems—specifically concerning the percentage of bone-to-implant contact (BIC), with or without loading, observed at specific time points.^{7–9} Nevertheless, knowledge about the histologic characteristics during peri-implant healing can be improved. Abrahamsson et al,¹⁰ in an experiment in beagle dogs, evaluated the rate and degree of osseointegration of titanium implants during the early phases of healing (between 2 hours and 12 weeks after implant placement). Primary bone formation started after the first week, and parallel-fibered and/or lamellar bone was present after 4 weeks.

The aim of the present study was to evaluate the phases of bone healing around 2 different rough titanium

implant surfaces in minipigs to describe the histologic features of early bone formation around implants and the level of osseointegration at the time when dental implants are often loaded.

Method and materials

Four male minipigs, about 8 to 9 months old and weighing an average of 45 kg, were used. The experimental design was approved by the Italian Ministry of Health. For all procedures, the animals were sedated with intramuscular injections of ketamine hydrochloride (10 mg/kg), 2% xylazine hydrochloride (2 mg/kg), diazepam (0.2 mg/kg), and atropine sulfate (2 mg/kg). Anesthesia was obtained through a mouth-nose mask delivering a mixture of isoflurane and oxygen. Anesthesia was maintained through orotracheal intubation with the same gas mixture at 3.5% concentration. The animals received 4 mL enrofloxacin (Baytril, Bayer) intramuscularly at the time of surgery and once a day for the following 4 days.

Table 1 Study design

	Time (d)				
	First surgery	Second surgery	Sacrifice	Healing time 1	Healing time 2
Animal 1	0	+7	+14	21	14
Animal 2	0	+4	+3	7	3
Animal 3	0	+14	+28	42	28
Animal 4	0	+14	+35	49	35

At the beginning of the experiment, the maxillary lateral incisors were extracted bilaterally. After 3 months, two implants—one ITI (sand-blasted, large-grit, acid-etched [SLA], 3.3 × 8 mm, Straumann) and one Brånemark (TiUnite Mk III, 3.75 × 8.5 mm, Nobel Biocare)—were inserted in one side of the mandible in all animals (Fig 1). Implant placement was performed according to the manufacturers' instructions. A crestal incision was performed in the edentulous region and a flap was raised. Implant site preparation was performed in the exposed area, and the implants were inserted. Cover screws were placed. The flaps were sutured; sutures were removed 10 days later. Implants on the other side of the mandible were inserted at different times. Sacrifice of the animals was scheduled to allow healing times of 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, and 7 weeks (Table 1). The animals were sacrificed with an overdose of sodium thiopental. Block biopsies were harvested from the experimental regions and placed in a



Figs 2 and 3 (Left) A Brånemark implant and (right) an ITI implant 3 days after insertion. The arrows indicate the outline of the implant bed. The gap between the implant (I) and implant bed is filled with blood clot (BC) and bone debris (BD) produced during the drilling procedure (toluidine blue; original magnification $\times 20$).



fixative (4% formalin). The single bone samples containing the implants were dehydrated in increasing grades of ethanols and subsequently infiltrated in methylmethacrylate, sectioned, ground, and polished to a final thickness of about 80 μm . From each tissue block, two sections were obtained,

stained in toluidine blue, and used for light microscopic examination under a Nikon Eclipse E600 microscope equipped with a program for image analysis and processing (Image J, NIH). BIC was expressed as the percentage of mineralized bone in contact with the implant surface.

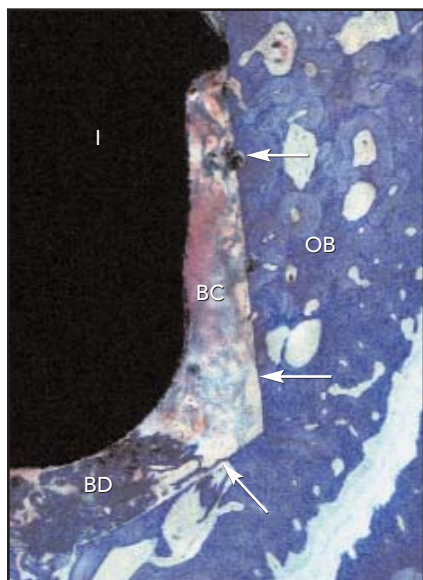


Fig 4 (left) ITI implant (I) 3 days after insertion. The arrows indicate the implant bed design. Note the presence of blood clot (BC) and bone debris (BD) filling the gap between the implant and original bone (OB) (toluidine blue; original magnification $\times 100$).

Fig 5 (right) ITI implant (I) 21 days after insertion. The arrows indicate the seam of mineralized tissue lining the titanium surface. Lateral to this, an area of provisional connective tissue (PCT) can be observed (toluidine blue; original magnification $\times 100$). OB = original bone.

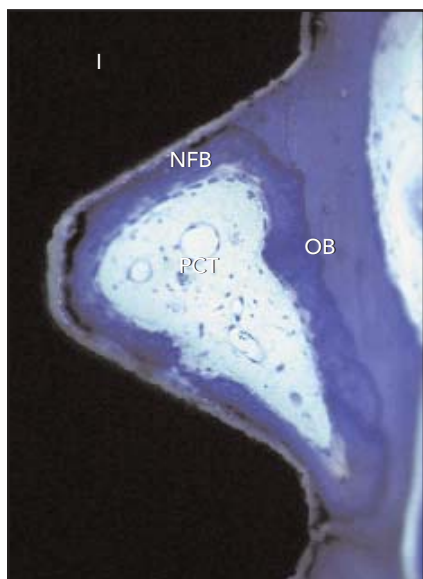
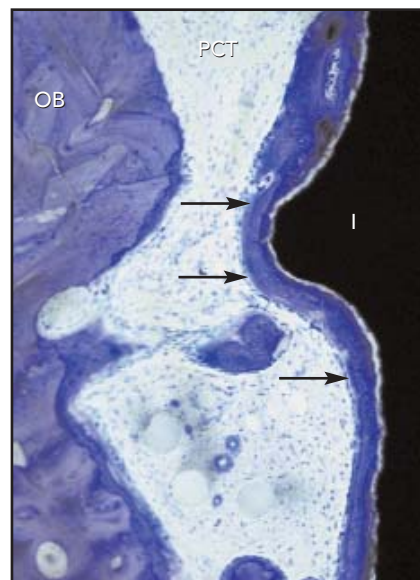
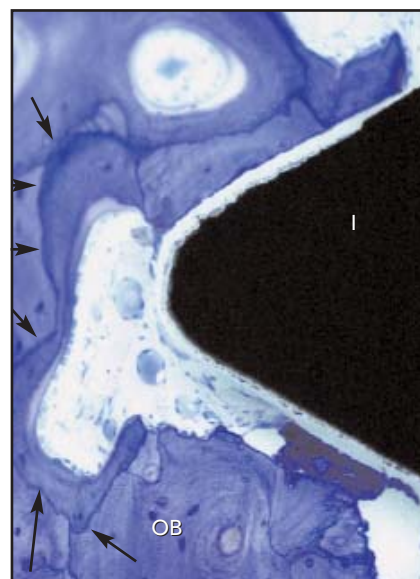


Fig 6 Brånemark implant (I) 21 days after insertion. The titanium surface is lined by newly formed bone (NFB) that is being remodeled. The space between the threads is occupied by provisional connective tissue (PCT) (toluidine blue; original magnification $\times 200$). OB = original bone.

Fig 7 Brånemark implant (I) 28 days after insertion. Lateral to the implant thread, remodeling of the newly formed bone can be observed. The arrows indicate osteoid apposition (toluidine blue; original magnification $\times 200$). OB = original bone.



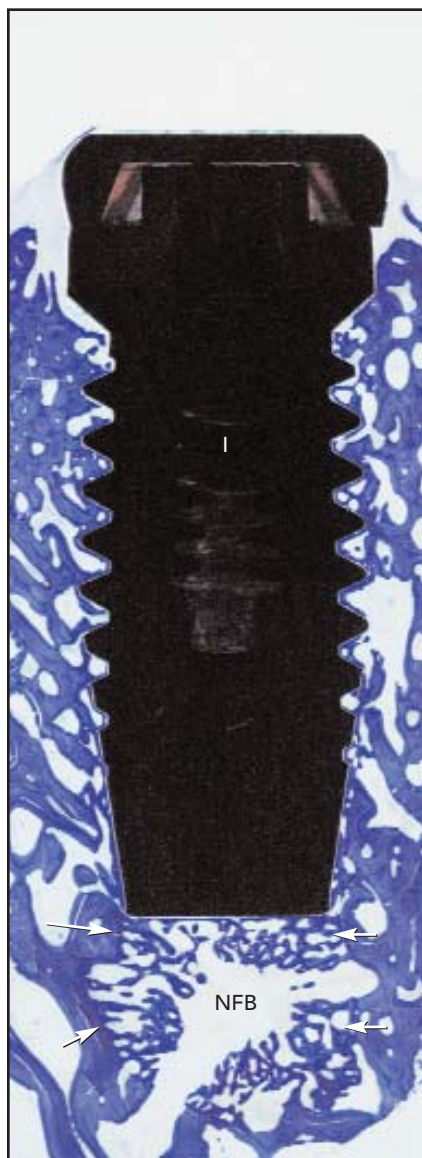
Results

All animals healed uneventfully after extractions and implant placement.

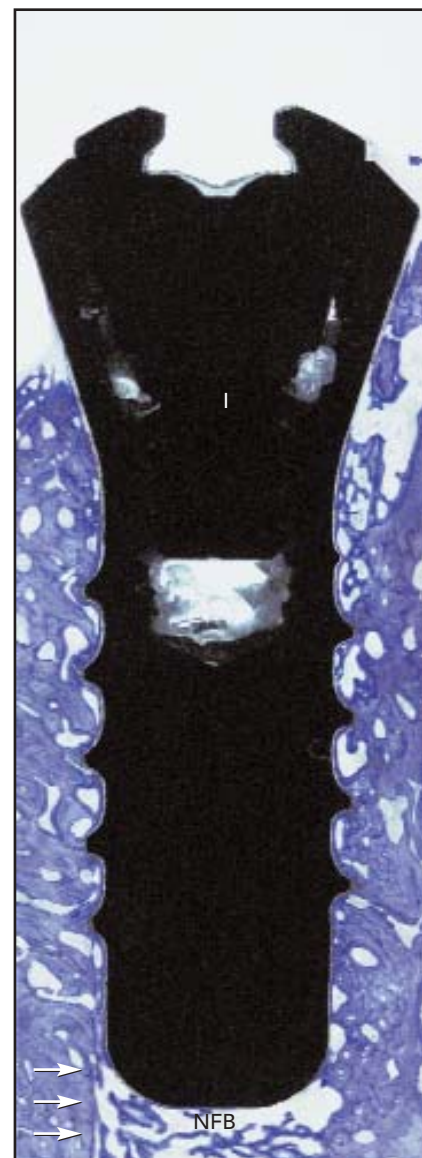
Gross histologic observations

Figures 2 and 3 provide histologic overviews of one Brånemark and one ITI implant, respectively, after 3 days of healing. At this time, the tissue compartment adjacent to the implants was characterized by the presence of a

blood clot and bone debris (Fig 4). The design of the implant bed was easily viewed, mainly in the apical portion of the implants (Fig 4). In the coronal portion, the implants seemed to be in tight contact with the original bone. In the first 3 to 4 weeks, a thin seam of newly formed bone tissue lining the



Figs 8 and 9 (Left) Brånemark implant and (right) ITI implant at 42 and 35 days after insertion, respectively. The prepared implant bed (arrows) is still detectable. The gap between the implant (I) and the implant bed is now filled with newly formed bone (NFB), and mineralized tissue is present along most of the length of both implants (toluidine blue; original magnification $\times 16$).



surface of both implants could be observed (Fig 5). Lateral to this osseous tissue, portions of nonmineralized tissue (provisional connective tissue) were often seen in the compartments between the threads (Figs 5 and 6). Signs of tissue remodeling were evident adjacent to the implants (Fig 7). Specimens from later healing times (Figs 8 and 9) were characterized by

the presence of mineralized bone, both woven and lamellar, still undergoing remodeling. In some samples, the implant bed design was still visible after up to 42 days. The gap between implant and bone appeared to have been filled with newly formed bone. The spaces between the threads were often occupied by mineralized tissue of the woven and lamellar type.

Table 2 Bone-implant contact percentages at different healing times

	3 d	7 d	14 d	21 d	28 d	35 d	42 d	49 d
ITI	37.0	57.0	86.1	93.7	84.3	92.6	93.7	85.1
BRA	84.4	62.9	91.7	79.5	87.9	73.1	84.5	74.1

ITI = ITI SLA implant; BRA = Brånemark TiUnite implant.

Histometric results

The measurements of BIC in the two implant systems at different healing times are shown in Table 2. The percentage of contact between mineralized bone and implants showed an increasing pattern for both implant systems over time. BIC increased from 37.0% at day 3 to 85.1% at 7 weeks for the ITI implants. The corresponding values for the Brånemark implants were 84.4% and 74.1%. The highest BIC value was obtained after 21 days for the ITI implants (93.7%) and after 14 days for the Brånemark implants (91.7%).

Discussion

The present study showed that a similar pattern in bone healing around SLA and TiUnite surfaces was observed between 3 days and 7 weeks; this pattern consisted of the replacement of blood clot and bone debris with a provisional connective tissue in the first weeks, followed by mineralized tissue and marrow spaces later on; both rough surfaces allowed for "contact osteogenesis" to take place. Further, it was observed that implant bed preparation and implant positioning do not always match perfectly.

These findings are in agreement with those previously reported by Abrahamsson et al,¹⁰ who compared the pattern of early bone formation at SLA and turned implant surfaces in dogs between 2 hours and 12 weeks. The authors observed the presence of erythrocytes and fibrin in the first phases of healing, followed by the proliferation of vascular structures and migration of mesenchymal cells, which resulted in woven bone formation after 1 to 2 weeks.

A similar pattern of bone formation was described concerning the healing of extraction sockets in dogs; Cardaropoli et al¹¹ observed blood clot in extraction sites during the first 3 days of healing. This was later replaced by a provisional matrix. After 2 weeks, woven bone was detected, and after 1 month the extraction sockets were mainly occupied by mineralized tissue.

The features of implant osseointegration reported by Davies¹² can be recognized in the series of events described in the present study. Davies¹² stated that osseointegration happens thanks to three different mechanisms: osteoconduction, de novo bone formation, and bone remodeling, through distance and contact osteogenesis. After the fibrin clot

has formed and adhered to the implant surface, as observed in the first days after implant placement (see Figs 2 to 4), osteogenetic cells start producing bone from the implant bed walls toward the device (distance osteogenesis), while migrating cells start lining the implant surface (contact osteogenesis) (see Figs 5 and 6). De novo bone formation is thus initiated and will be completed by bone remodeling (see Fig 7).

In the present study, linear measurements were performed to assess the proportion of the titanium surface that was invested in bone and in contact with mineralized and nonmineralized tissue. Such assessments were carried out on two sections from one sample per healing time only, and therefore no conclusions could be drawn; nor could statistical analyses be performed. Nevertheless, the trend of the results concerning BIC seems to be in line with the descriptive phases of bone healing, which showed no relevant differences between SLA and TiUnite surfaces. Davies¹² speculated that rough surfaces favor osseointegration by increasing the surface available for fibrin attachment and by providing mechanical characteristics that prevent fibrin detachment (and thus the formation of a gap between fibrin

clot and titanium). Abrahamsson et al,¹⁰ comparing the rate of osseointegration of turned and rough implant surfaces, defined the SLA surface as an "osteophilic" surface, because it favored early osseointegration, with almost twice the bone contact observed on the turned surface. A comparison between the present findings and the results of Abrahamsson et al concerning BIC cannot be made because different species were used.

A gap was often observed at day 3 between the implant and the implant bed in sections from early healing times. This finding was also reported by Abrahamsson et al¹⁰ and is probably related to the surgical preparation. The gap was filled with bone debris produced during drilling and by the blood clot that had formed at the time of surgery. This discrepancy did not seem to impair or influence implant osseointegration over time; at later healing times, although the contour of the gap was still visible, it had been filled with mineralized tissue. In some areas, on the other hand, the implant surface appeared to be in tight contact with the original bone, thus providing enough stability for wound healing and proper osseointegration.

Healing in humans, dogs, and minipigs occurs at different rates, making correlations between biologic events in such species difficult. Raghavendra et al,¹ in an analysis of the existing literature on early peri-implant bone healing, assumed that "the critical time frame for implant healing in humans would be 2 to 3 weeks postplacement" and concluded that primary stability resulting from the interaction of remodeling and

osseointegration with functional loading is crucial to successful implant therapy. In the present study, after 3 to 4 weeks the implants were characterized by a thin seam of newly formed bone tissue lining their surface, and spaces of provisional connective tissue could be observed in the compartments between the threads. Signs of tissue remodeling were evident adjacent to the implants. Although it might be speculated that such a pattern corresponds to a delicate phase for bone healing, during which the interface should be prevented from being disturbed by micromotion and loading, more histologic studies comparing loaded and unloaded implants during the different phases of bone integration are needed to verify this hypothesis.

Acknowledgment

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