Osseointegration of Oral Implants After Delayed Placement in Rabbits: A Microcomputed Tomography and Histomorphometric Study

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Purpose: *This study compared osseointegration of implants placed 14 days after implant site preparation with that of immediately placed implants in rabbit femurs.* ***Materials and Methods:*** *Implants were placed bilaterally in the femoral condyles of 12 rabbits. On one side, the implants were placed 14 days after osteotomy, and the other side received implants immediately after osteotomy. Healing was assessed by microcomputed tomography and histomorphometry.* ***Results:*** *The delayed implants (placed 14 days after osteotomy) showed better osseointegration than the immediately placed implants. Bone-to-implant contact and bone volume, as assessed by histomorphometry and microcomputed tomography, were significantly higher for the implants placed after 14 days.* ***Conclusions:*** *From this study, it can be concluded that early osteotomy bed preparation and placement of implants after a 2-week delay predisposes to better bone-implant interface healing.* INT J ORAL MAXILLOFAC IMPLANTS 2013;28:1506–1511. doi: 10.11607/jomi.3133

Key words: *atraumatic implant placement, bone histology, histomorphometry, microcomputed tomography, osseointegration, osteotomy timing*

Dental implants have revolutionized contemporary dental treatment for the replacement of missing teeth. Endosseous dental implant therapy has become a widely accepted treatment modality for the replacement of missing teeth. Attempts to shorten the overall length of treatment have focused on approaches such as early or immediate loading following implant placement, immediate implant placement in fresh extraction sites, and immediate implant placement with early or immediate loading.[1,2]

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It has been reported that immediate implant placement can be adversely affected by the presence of infection[3,4] and by a lack of soft tissue closure and flap dehiscence over the extraction site.[5,6] To overcome this problem, alternative techniques have been described, calling for implant placement at various intervals following the initiation of wound healing subsequent to tooth extraction.[1,7–11] A systematic review concluded that sites with infection have low success rates comparable to those of uninfected sites.[12] However, this evidence was based on case series and animal studies. The review recommended that extraction sites be thoroughly debrided of infected material before implant placement and that systemic antibiotics be used postoperatively to ensure a successful outcome. In such situations, implant bed preparation can be performed along with the extraction, and a waiting time of 2 weeks can ensure complete elimination of the remaining infection at the implant site.

Surgical trauma during drilling and implant placement is considered to be a reason for implant failure because thermal, vascular, and mechanical factors contribute to the formation of necrotic tissue, thereby affecting maturation at the bone-implant interface.[13–16] Delayed implant placement after osteotomy gives the bone time to recover from surgical injury and allows for implant placement into an environment that is more conducive to healing and osseointegration.[17,18]

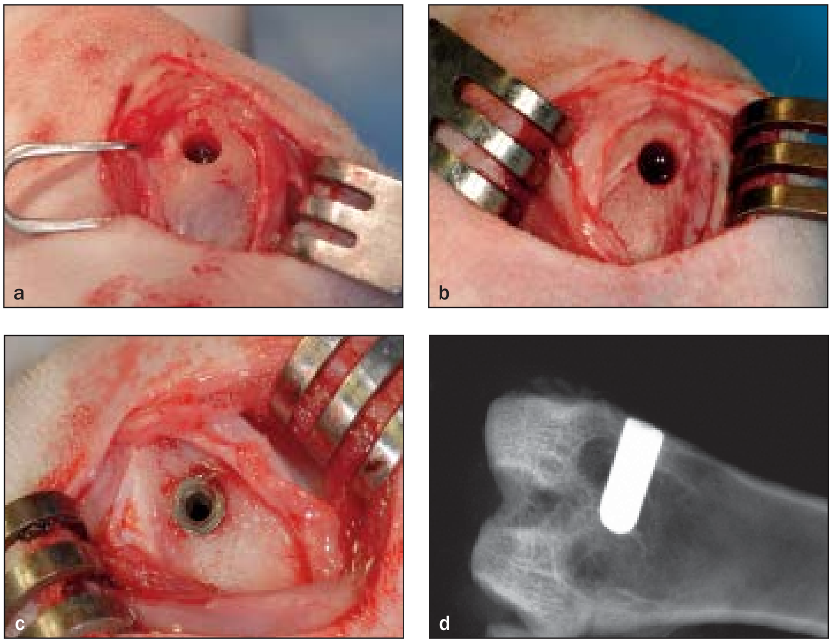

Fig 1a Implant site immediately after osteotomy.

Fig 1b The site 14 days after osteotomy.

Fig 1c The site with the implant in position.

Fig 1d Radiograph of the retrieved femoral condyle after 8 weeks.

Animal studies and a clinical report have been published to explain this procedure.[19–21] Kunnekel et al[19] reported, in a rabbit study, better osseointegration when implants were placed 14 days after osteotomy. However, in that study, osseointegration of the implants was assessed only by resonance frequency. A clinical study also confirmed the advantages of implant placement 2 weeks after osteotomy.[20] Although these pilot studies described the procedure, reports of the outcomes were mainly based on clinical parameters. Hence, the aim of the present study was to investigate the osseointegration of dental implants placed in rabbit femurs at 14 days after osteotomy by measurement of bone volume (BV) and bone-to-implant contact (BIC) through microcomputed tomography (micro-CT) and histomorphometry.

MATERIALS AND METHODS

Twelve New Zealand White rabbits (6 months old and weighing approximately 4 to 5 kg each) were used for this study. All rabbits were housed separately in standard cages under laboratory conditions. The study was approved by the ethical committee of the College of Dentistry of King Saud University. The procedure was based on a well-established bilateral femoral implant model in rabbits.

Surgeries were performed using aseptic routines and under general anesthesia by intramuscular injection of a combination of a dose of 35 mg/kg of ketamine and a dose of 5 mg/kg of xylazine. The surgical areas were shaved, disinfected, and then isolated with drapes. Infiltration anesthesia was performed at the experimental sites. The distal femoral condyles on both sides were accessed via incisions through the skin and fascia, and the bone surfaces were exposed using a periosteal elevator.

Twenty-four commercially pure titanium implants, with dimensions of 3.75×7 mm, were custom manufactured for the study. A pilot drill was used first, followed by a 3.25-mm drill and, finally, a 3.35-mm drill (Nobel Biocare). The surfaces of the implants had been roughened by a grit-blasting procedure. In each animal, delayed implantation was performed on one side, and the contralateral side served as the control. During the first surgical stage, surgical drilling was performed on one side of the femoral head. The osteotomy was created per the recommended protocol, and the site was closed with sutures (Fig 1). After 14 days, a second surgery was performed. The previous osteotomy site was reopened, and an implant was placed. On the contralateral side, an osteotomy was created and an implant with the same dimensions was placed immediately afterward. This implant served as the control. The wounds were closed and allowed to heal for 8 weeks.

Postsurgical pain was controlled by intramuscular administration of Fynadyne (Schering Plough Animal Health Benelux). To reduce the risk of postoperative infection, 10 mg/kg of enrofloxacin (Baytril, Bayvet Division, Chemagro) was administered. After 8 weeks, the animals were killed, and the femoral condyles were harvested for micro-CT and histology.

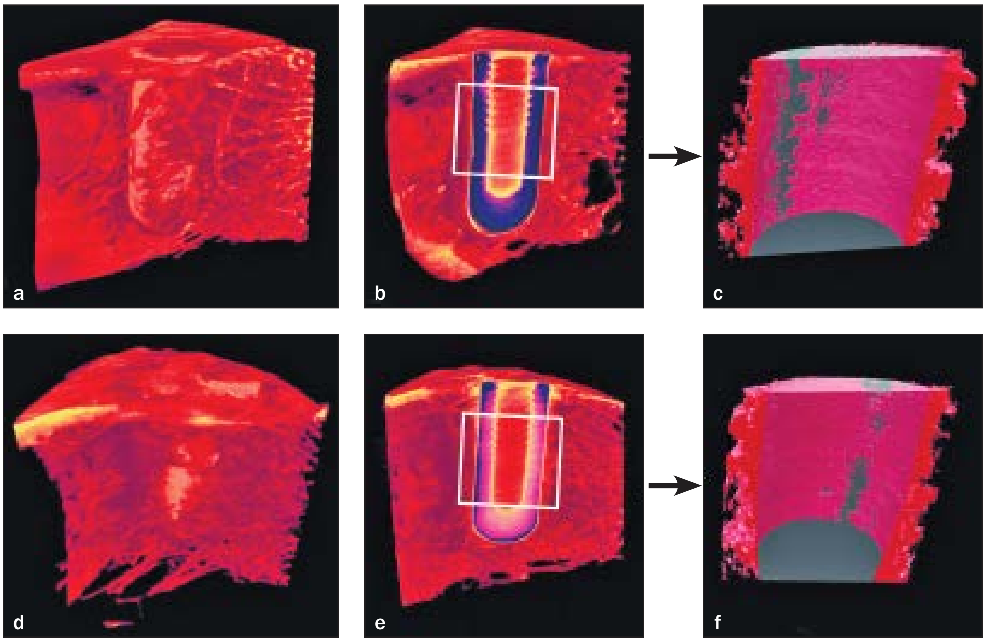

Fig 2 CT reconstructions of bone specimens. *(a, b, d, e)* 3D volume reconstructions; *(c, f)* 3D surface reconstructions; *(a, b, c)* immediate implantation; *(d, e, f)* delayed implantation. *(a, d)* The complete bone sample including the implant; *(b, e)* artificially cut sample; *(c, f)* cut-view visualizations of the bone volume within a 500-μm zone around the (transparent) implant.

Micro-CT Analysis

The femoral condyles, including the implants, were fixed in 10% formaldehyde and dehydrated in ethanol. Subsequently, the specimens were wrapped in Parafilm to prevent drying during scanning. All of the samples were scanned at an energy of 101 kV and an intensity of 96 μA with a pixel resolution of 37.41 μm, using an aluminum filter (1 mm) (Skyscan 1072 x-ray microtomograph, TomoNT, version 3N.5, Skyscan). The rotation step was 0.90 degrees, and the exposure time was 3.8 seconds. After cone beam reconstruction was performed (version 1.6.6, Skyscan), the data were analyzed using CT Analyzer (version 1.11, Skyscan). An area of 500 μm around the implant, surrounding it over a length of 4 mm, was chosen as the region of interest for the analysis. Three-dimensional (3D) volume reconstruction was performed using Skyscan CT-Vox (version 2.4.0), and 3D surface reconstructions were created using 3D-Doctor (version 4.0, Able Software). The bone volume in this area was determined and expressed as a percentage of the total volume of the region of interest (ROI) (Fig 2).

Histology

Subsequent to micro-CT scanning, the specimens were dehydrated in increasing concentrations of ethanol (70% to 100%) and embedded (nondecalcified) in modified methylmethacrylate for 5 days (300 mL methylmethacrylate, 30 mL dibutylphthalate, and 5 g 2,2'azabisisobutyronitrile 98%). After polymerization, thin sections (10 μm) were cut in a longitudinal direction (parallel to the axis of the implant) with an inner circular saw microtome (Leica SP-1600). These sections were stained with methylene blue and basic fuchsin and were used for morphologic evaluation and histomorphometric analysis.

Histomorphometry

Histologic evaluation was performed using a Zeiss Axio Imager (Zeiss Axio Imager Z1, Carl Zeiss Micro Imaging) transmission light microscope. Histomorphometry was performed using digital image analysis software (Qwin Pro, Leica Imaging Systems).

Two quantitative parameters were assessed (Fig 3):

1. BIC: Bone contact was analyzed along the total length of the implant, starting at the first coronal microthread up to the apex of the implant. BIC was defined as the percentage of the implant surface in direct contact with bone without an intervening fibrous tissue layer.
2. Percentage of peri-implant bone area (BV): The bone mass around the implant was analyzed in a rectangular ROI at the flat part of the implant. BV measurements were based on the quantification of the bone tissue in a specified ROI, which was set as a virtual cylinder (dimensions equal to those of the defect).

All measurements were performed on both sides of the implant for at least three histologic sections per implant.

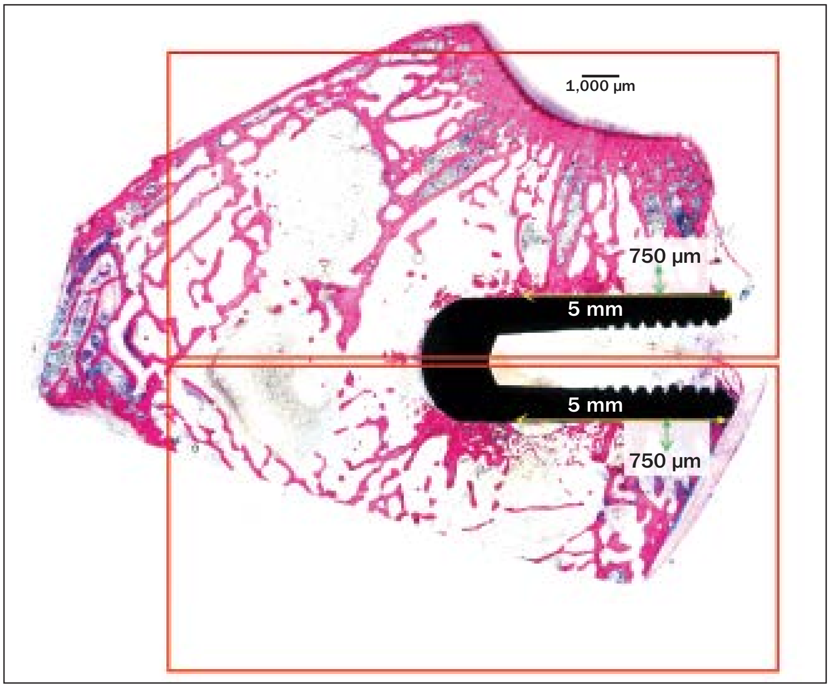

Fig 3 Demonstration of the method used to determine BIC and BV in histologic sections. The ROI and the BIC parameters are marked.

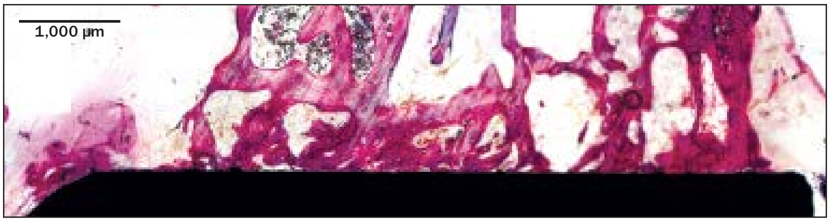

Fig 4 Demonstration of bone formation around a control implant (immediate placement) (methylene blue/basic fuchsin; bar = 1,000 μm).

Fig 5 Demonstration of bone formation around an experimental implant (delayed placement) (methylene blue/basic fuchsin; bar = 1,000 μm).

Statistical Analysis

All statistical analysis was performed with GraphPad Instat software (version 3.05, GraphPad Software). The Student *t* test was used to determine the statistical significance between the two groups.

RESULTS

General Observations

The healing of wounds was uneventful in all cases. All the animals survived the 8 weeks of healing, and all 24 implants were retrieved after 8 weeks of healing. Radiographs obtained before micro-CT analysis showed that all implants had osseointegrated. The bone morphology was normal around the implant sites.

Microscopic examination of methylene blue/basic fuchsin–stained sections of the implants and surrounding tissue demonstrated that all the sections generally showed bone apposition, bone remodeling, and ingrowth of newly formed bone into the threads of the implants (Figs 4 and 5). The bone adjacent to the implants contained large numbers of osteocytes, which had been laid down adjacent to the implant surface. In both the delayed and immediately implanted specimens, a thick layer of lamellar trabecular bone was formed on the implant surface.

Micro-CT Findings

Micro-CT analysis showed excellent osseointegration in all the specimens. The 3D model analysis revealed a mean BV of 36.66% ± 10.62% in the delayed implantation

Table 1 BV and BIC Measurements (Means ± SDs) in Control (Immediate) and Experimental (Delayed) Groups

Time of implant placement	BV*	BV†	BIC†
Delayed	36.66 ± 10.62	40.49 ± 10.60	50.31 ± 10.24
Immediate	26.23 ± 8.58	30.47 ± 8.22	40.25 ± 12.01
P value	.0149	.0176	.0385

*Micro-CT measurements; †histomorphometric measurements.

sites, compared to a mean BV of 26.23% ± 8.58% in the immediately implanted sites (Table 1). The delayed implants showed significantly greater volume than the immediately placed implants ($P < .05$).

Histomorphometry

The BIC and BV are listed in Table 1. BIC was found to be significantly greater in the delayed group compared to the immediately implanted group. The mean BIC in the delayed group was 50.31% ± 10.24%, compared to 40.25% ± 12.01% in the immediate group.

BV calculated from the histologic sections showed the same trend as the micro-CT measurements, ie, a mean of 40.49% ± 10.60% for the delayed sites and 30.47% ± 8.22% for the immediate implants.

DISCUSSION

This study was conducted to analyze the osseointegration of dental implants placed 14 days after osteotomy and to compare it with the osseointegration of implants placed immediately in rabbit femora. Micro-CT and histomorphometric analysis revealed better osseointegration of the implants that were placed 14 days after preparation of the surgical site. The observations from this animal study are in agreement with earlier clinical and animal studies.[19–21] However, this is the first report that analyzed osseointegration with the help of micro-CT and histology. These findings could be attributed to the favorable implant bed at the time of implant placement. One of the mechanisms that can explain this phenomenon might be injuries and heat generation at the immediate implant site, which were reported in several earlier investigations.[22,23] Futami et al[24] suggested that a microspace between the host bone and the implant enabled the migration of osteogenic cells from the bone marrow toward the implant surface, thus favoring rapid and extensive osteogenesis.

One of the limitations of this study was the use of a rabbit model. Compared to other species, such as primates and some rodents, rabbits undergo faster skeletal changes and bone turnover.[25,26] Although it might be difficult to extrapolate the results of rabbit studies to the likely human clinical response, the contralateral design of the present study renders the data more reliable. Moreover, previous clinical observations also support the findings of the histologic data.[19–21] However, a healing period of 2 weeks after osteotomy in rabbits requires further investigation in an animal model more similar to humans.

CONCLUSION

Delayed implantation after osteotomy could be helpful in achieving better osseointegration. A waiting period of approximately 14 days promoted the activity of surrounding tissue to its maximum level because collagen formation and neoangiogenesis indicated a relaxed healing implant bed configuration. Therefore, enhancement of alveolar bone–implant bonding can be achieved by delaying implant placement.[20,21]

It is also imperative to analyze critically the indications for this modified implantation protocol. Implantation sites with infection and compromised bone conditions are among the situations that might benefit from delayed implant placement after osteotomy. However, further studies are necessary to support the present observations.

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