Simultaneous or Delayed Placement of Surface Modified and Fluoridated Dental Implants into Autogenous Block Bone Grafts: A Histologic and Biomechanical Study in the Rabbit

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ABSTRACT

Background: A delayed approach is recommended for reconstruction of the jaws with autogenous bone grafts and dental implants. Experimental studies have shown stronger bone tissue responses to surface modified implants than to nonmodified ones.

Aim: The aim of the study was to evaluate bone integration and stability of surface modified and fluoridated implants when placed with fresh or healed autogenous bone grafts.

Material and Methods: Six rabbits were used in this study. Each right rabbit tibia (control) received an autogenous bone graft, harvested from the calvarium. Eight weeks later, a second graft was harvested from the other side of the calvarium and placed on the left tibia (test) with an implant. Another implant was installed in the healed graft of the right tibia. TiO_2-blasted and fluoridated OsseoSpeed™ implants (Astra Tech AB, Mölndal, Sweden), 3.5 mm in diameter and 9 mm long, were used. After additional 8 weeks, the rabbits were sacrificed and the implants were removed *en bloc* for light-microscopic analysis. Bone-to-implant contact (BIC) was registered as well as the amount of bone filling a rectangle indicating a region of interest (ROI). Resonance frequency analysis (RFA) was conducted both at the time of surgery and at the end of the experiment.

Results: There were no statistically significant differences either in BIC or ROI between the test and control sides. RFA showed higher implant stability for the control side at the time of the surgery, but the difference had leveled out at the time of the sacrifice.

Conclusion: The present study showed similar bone tissue responses and stability for surface modified and fluoridated implants after 8 weeks of healing in fresh or healed autogenous bone grafts.

KEY WORDS: autogenous bone graft, histological analysis, implant, implant surface

INTRODUCTION

Reconstruction of the edentulous and resorbed alveolar crest may require bone augmentation to enable placement and integration of dental implants. Autogenous bone grafts are still considered gold standard even though the use of bone substitutes has increased in recent years. In severely resorbed jaws, block bone is frequently used and the implants may be installed either simultaneously with the graft or after some months of graft healing. In a series of studies on block bone grafts, Rasmusson and coworkers suggested that a staged approach is preferable as a better integration and stability of the implants was shown both histologically and

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by resonance frequency analysis resonance frequency analysis (RFA).[1–3] If particulated bone is to be used, a staged procedure is most commonly used because no mechanical support from the graft can be appreciated. In cases of severe resorption when more bone is needed than can be harvested from the maxillofacial region, common donor sites are the iliac crest and cranial bone. Cortico-cancellous block grafts can be harvested and shaped into preferable size. In cases with severe maxillary resorption but with normal sagittal relation between the jaws, the graft can be used as a block or particulate for lateral onlay augmentation and for sinus lift procedures. If severe sagittal discrepancy between the jaws due to maxillary resorption is evident, the bone graft is usually placed as interpositional blocks in the nasal and sinus floors after a Le Fort I osteotomy. The latter technique allows for correction of the sagittal relations as the whole maxilla is mobilized.

The long-term implant survival rate for implants installed in grafted bone is generally not as good as for the nongrafted maxilla.[4–6] There are several explanations for this. Resorption of the graft is common and severity is unpredictable.[7,8] The healing situation is complex because both successful healing of the graft and integration of the implants are required. Another reason for increased implant failure rates in bone grafts could be the slow remodeling and revitalization of cortical block grafts.[3]

The marginal bone level alteration during initial healing and loading is usually assessed using intraoral radiographs. Another way is RFA.[9,10] This technique implies a resonance frequency measurement of a transducer connected to the implant fixture or abutment. The value, implant stability quotient (ISQ), reflects the stability of the implant as a function of interface stiffness and is influenced by the distance from the transducer to the first contact of supportive marginal bone. RFA is sensitive to changes in the marginal bone level and is usually used as a complement to intraoral radiographs. The technique has, for example, been used to compare stability of implants installed in autogenous bone grafts with different surgical protocols.[11]

The development of so-called bioactive implant surfaces has resulted in shortened healing periods, at least experimentally. Using an experimental model in dog, Berglundh and colleagues[12] investigated different phases of wound healing using implants with a moderately rough surface configuration. It was reported that after 2 weeks of wound healing, new bone formation appeared in most compartments of the inner threads of the implants. Using a rabbit model, Ellingsen[13] showed that titanium implants that were pretreated with sodium fluoride solutions had a four times increased retention in rabbits ulna after 4 and 8 weeks of healing periods as measured by a push out technique.

The manufacturer also claims improved integration in grafted bone. Previous studies have shown that delayed approach is a more favorable choice of treatment due to greater primary implant stability and a better osseointegration with a higher degree of bone-implant contact and more bone filling the implant threads.[1,3] The prolonged osseointegration process in the grafted area has been demonstrated for turned implants installed simultaneously with the bone graft but this has not been verified for a bioactive surface like OsseoSpeed™ (Astra Tech AB, Mölndal, Sweden).

The aim of this experimental study was to determine if there are any differences in stability and osseointegration of implants with bioactive surface installed in autogenous bone grafts using a simultaneous or delayed approach as test and control groups, respectively.

MATERIALS AND METHODS

Animals and Anesthesia

Six adult (8–9 months) female New Zealand white rabbits weighing 2 to 3 kg were used in this study. The animals were kept in a specially designed room and fed water and a standard diet ad libitum. Sedation by means of an intraperitoneal injection of Stesolid® (Dumex, Copenhagen, Denmark) 1.5 mg/kg was used and general anesthesia was administered by intramuscular injection of 0.2 mL/kg Hypnorm® (Janssen Pharmaceutical, Brussels, Belgium). Furthermore, local anesthesia, 0.8 mL (2% lidocaine/epinephrine 1:80,000, Astra AB, Södertälje, Sweden), was used. Postoperatively, single intramuscular injections of antibiotics (Intencillin® 2,250,000 IE/5 mL, 0.1 mL/kg body weight, Leo, Helsingborg, Sweden) and analgesic (Temgesic® 0.05 mg/kg body weight, Reckitt & Coleman, Hull, UK) were given. The local ethic committee for animal research approved the study (Dnr: 128–2007).

Surgical Procedure

Surgery was performed under aseptic conditions. The proximal tibial metaphysis on both sides were used as experimental sites. A subperiosteal full thickness flap was raised on the skull and a disc-shaped bone graft measuring 8 mm in diameter and 2 mm thick was harvested from the lateral aspect of the sagittal suture of the calvarium. In order to harvest an equally large volume of bone graft from all animals, a trephine bur was used with care not to penetrate the dura. Another fascial-periosteal flap was raised on the right proximal tibial metaphysis. The bone graft was fixed to the tibial bone with a mini screw 5 mm long and 2 mm in diameter. The flaps were then closed and sutured in layers. The fascia-periosteal flaps were sutured by Vicryl 5.0 (Ethicon®, Johnson & Johnson, Livingston, Scotland) and the skin with Monocryl 3.0 (Ethicon, Johnson & Johnson).

Eight weeks later (day 0), a second bone graft from the other side of the calvarium was harvested using the same procedure as the first intervention. The graft was fixed to the tibia by one fluoridated implant 3.5 mm × 9 mm, Astra Tech AB (test).

The fixation screw on the other side was removed and after preparation to the same diameter as for the test site, another 3.5 mm × 9 mm Osseospeed implant was installed (control). This implant was consequently placed into a healed graft. All implants were leveled with the superior cortical border of the graft and cover screws were installed. The wounds were sutured in layers and the animals were thereafter followed for 8 weeks to the end point of the study when they were sacrificed by an overdose of Rompun® vet (Bayer A/S, Animal Health Division, Copenhagen, Denmark) 20 mg/mL and Ketalar® (Pfizer AB, Sollentuna, Sweden) 50 mg/mL.

Specimen Preparation

The implants and surrounding bone tissue were removed *en bloc* and fixed by formalin. After dehydration in a graded series of ethanol, they were embedded in light curing methacrylate (Technovit® 7200 VCL, Kullzer and Co, Wehrheim, Germany). In order to make ground sections approximately 10 µm thick, the specimens were prepared by using a sawing and grinding technique (Exakt® Apparatbau, Norderstedt, Germany). The sections were then stained with 1% toluidine blue and Pyronin G. The sections were viewed and analyzed in a light microscope (Nikon Eclipse 80i, Tekno Optik AB, Gothenburg, Sweden) using ×1.8 to ×100 magnification connected to a personal computer with a software for morphometrical analysis (Easy Image Measurements 2000 Tekno Optik AB).

Analysis and Calculations

Morphometrical measurements of (i) bone-to-implant contact (BIC) in grafted bone, (ii) BIC in residual bone, and (iii) bone area (BA) within a region of interest (ROI) were performed on both sides of each implant in the grafted area. In order to obtain a bone area within a region of interest, a vertical line about 1.0 mm from the outer border of each implant surface, parallel to the longitudinal axis of each implant was drawn, encompassing seven threads within the grafted area. Mean values of total BIC (residual + grafted bone), BIC in grafted bone and BA were calculated for each implant as well as average values for each group and parameter.

RFA

RFA was performed in all animals at the time of surgery and at the end of the experiment (Osstell Mentor, Osstell AB, Gothenburg, Sweden). A transducer (Smartpeg, Osstell AB) was attached and registrations were made perpendicular and longitudinal to the long-axis of the tibia for each implant. Mean values for the test and control implants were calculated respectively.

Statistics

The Wilcoxon signed-rank test was used for statistical analysis and a difference between the two groups was considered significant if p was <.05.

RESULTS

The healing after surgery was uneventful in all six animals from both protocols. At the time of reentry for implant placement at the control side, the bone graft was observed to have a smooth and mature texture and seemed well integrated in the residual bone. A typical cross-section of both control and test sides showed the tibia with an implant inserted through a roughly 2 mm thick graft and then protruding into the recipient tibial bone.

Control specimens demonstrated bone graft integration with the residual bone; however, it was still possible to distinguish between grafted and residual bone. Test specimens showed a more distinct border between the bone graft and residual outer cortex (Figures 1 and 2).

Total BIC was 42.45 ± 7.31% for the control side and 42.09 ± 7.69% for the test side (n.s.). The mean BIC in the grafted part was 70.33 ± 11.86 % for control compared with 59.65 ± 9.98% for the test side

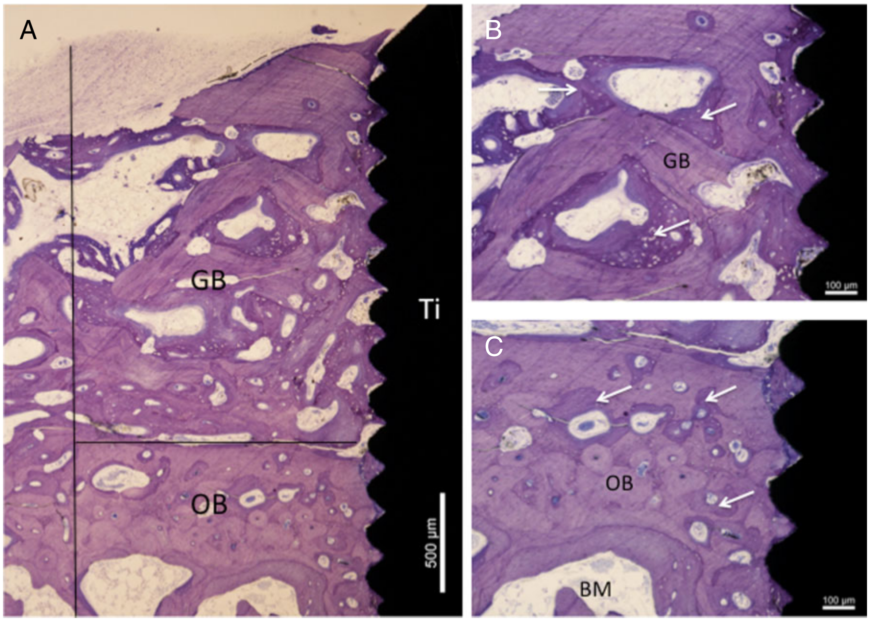

Figure 1 Light micrographs of a test specimen after 8 weeks of healing. *A*, Overview. The implant (Ti) seems to be well integrated in both grafted (GB) and original bone (OB). Bar = 500 µm. *B*, Close up of grafted area showing an admixture of GB and newly formed immature bone (arrows). Bar = 100 µm. *C*, Close up of the original cortical bone showing new secondary osteons (arrows). Bar = 100 µm.

(Figure 3). The difference was not statistically significant. The BA was calculated to 83.50 ± 3.60% for the control side and 81.80 ± 3.70% for the test side (n.s.) (Figure 3).

The RFA showed higher stability for the control side at the time of implant installation. However, the difference between the control and test sides was leveled out at the time of sacrifice (Figure 4).

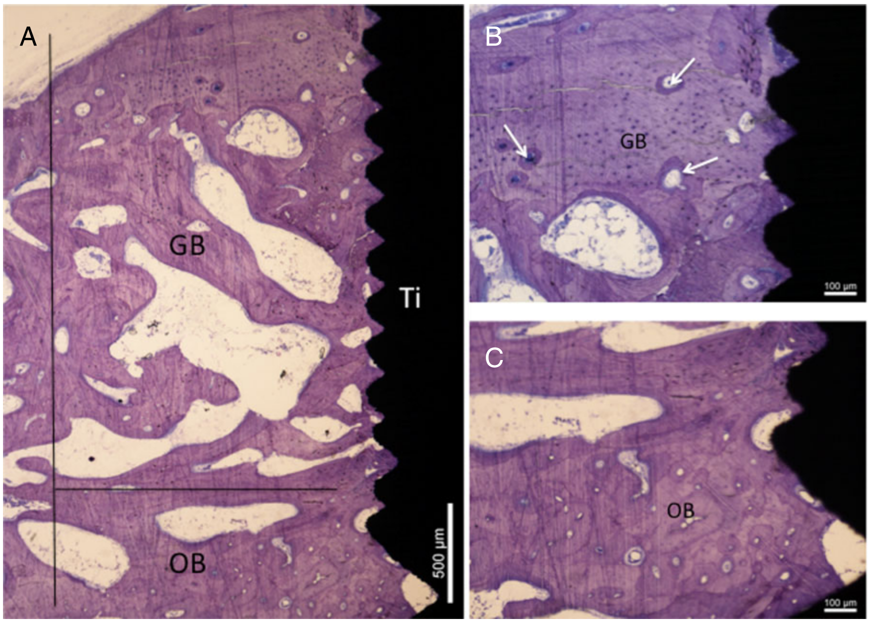

Figure 2 Light micrographs of a control specimen after 8 weeks of healing. *A*, Overview. The implant (Ti) seems to be well integrated in both grafted (GB) and original bone (OB). Bar = 500 µm. *B*, Close up of grafted area showing a more mature bone than at the test side. Arrows point to secondary osteons. Bar = 100 µm. *C*, Close up of the original cortical bone showing similar morphology as at the test side. Bar = 100 µm.

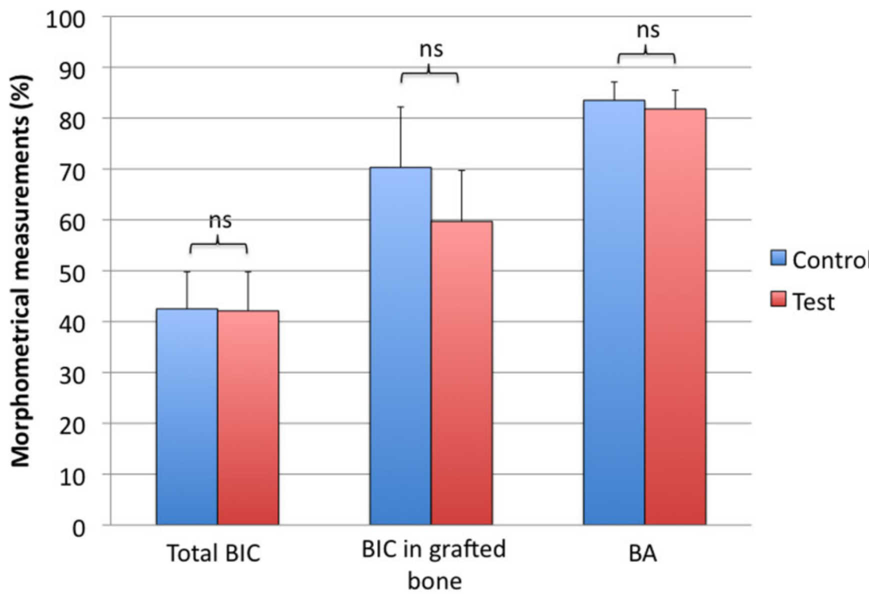

Figure 3 Histomorphometry. Percentage of bone-to-implant contact (BIC) and bone area (BA) in a region of interest. Significance level, $p < .05$.

DISCUSSION

The purpose of the present study was to evaluate whether there are any differences in osseointegration of endosseous implants when placed simultaneously with an onlay bone graft versus a delayed approach, allowing a healing period of 8 weeks before implant placement. Rasmusson and colleagues[1] and later Sjöström and colleagues[14] showed significant benefits of a delayed approach when using turned implants. They found histomorphometrical differences measuring the bone to implant contact in the grafted area only.

According to Rasmusson and colleagues,[1] the differences in the grafted areas could be due to the ability of the revascularized bone graft to respond to the surgical stimulation during implant placement in the delayed procedure. However, they concluded that with more time elapsed, the differences became less obvious.

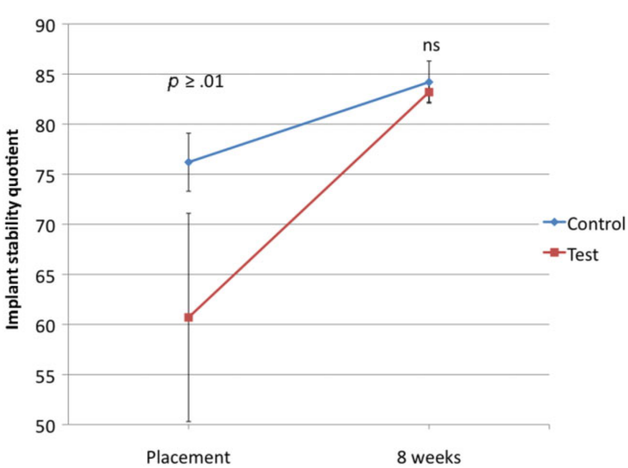

Figure 4 Implant stability by (ISQ) at placement and after 8 weeks.

The implants used in this study have a fluoride acid-etched surface and seems to induce strong and early bone tissue responses after installation.[12,15–17] Thor and colleagues,[18] in a dog experimental model, found that the TiO_2 grit-blasted and fluoridated implants exhibited more new bone formation compared with TiO_2 grit-blasted implants and also displayed a shorter distance from first bone contact to the margin of the bone envelope. In another experimental study, Abrahamsson and colleagues[19] also found a significantly larger area of osseointegration established within the defect at fluoride-modified implants than at implants with a TiOblast™ (TiO_2 grit-blasted) (TiOblast™, Astra Tech AB, Mölndal, Sweden) surface after 6 weeks of healing. Furthermore, the degree of BIC within the defect area was larger at fluoride-modified implants than at the TiOblast implants.

The present study showed a tendency of more BIC in the grafted bone area for the delayed approach; however, the difference between test and control was not statistically significant. Our results could be due to either insufficient sample size or that there was in fact no difference between test and control. If the latter is true, it shows that modern surface implants integrate effectively also when using a one-stage protocol.

Fluoride enhances the incorporation of newly formed collagen into the bone matrix and increases the rate of seeding of apatite crystals as well as increasing trabecular bone density and stimulating osteoprogenitor cells number in vitro.[20] Furthermore, fluoridated hydroxyapatite has better resistance to dissolution than hydroxyapatite.[21]

The values registered with RFA at the time of surgery appeared to be lower when using simultaneous implant placement. This could be due to the obvious fact that the grafted bone has yet not been integrated in the host bone. The subsequent healing period of 8 weeks allowed for the integration of bone grafts and implants, resulting in almost identical RFA values in both the delayed and simultaneous approach when using implants with a modified and fluoridated surface. The ROI between the implant threads among the implants with the delayed approach showed more bone filling, however without displaying a statistical significant difference from the simultaneous bone graft and implant test approach. Hence this experimental study in the rabbit tibia shows that when installing a modified and fluoridated implant in autogenous bone graft in a simultaneous procedure, the amount of bone to implant contact and implant stability does not differ significantly from the same implants placed in a delayed procedure. The background mechanisms are not fully understood, but our results could have been influenced by the small sample size. Other factors that could be relevant to our results are dimensions of the bone graft, inserted implants, and healing time. Nevertheless, an optimal surface roughness has been proposed based on experimental studies. In a series of studies by Wennerberg and colleagues[22–25] a surface roughness of Sa 1 to 1.5 µm has been concluded to be optimal. Furthermore, according to Ellingsen,[20] the benefit of increasing roughness on a micrometer scale reaches a maximum level between 1.0 and 1.5 µm and above this level no further positive response in the bone can be expected. This is also in accordance with a previous study by Wennerberg and Albrektsson[26] in which the moderately rough surfaces (Sa 1–2 µm) have shown stronger bone response than rough (Sa > 2 µm). The OsseoSpeed surface has a Sa of 1.4 µm.[27] It has been reported in the literature that fluoride ions stimulate osteoblast proliferation and activities in vitro.[28] Furthermore, Cooper and colleagues[29] examined the fluoride modification on osteoblast behavior. It was reported that fluoride ion modification of TiO_2 grit-blasted surface enhanced osteoblastic differentiation in vitro and interfacial bone formation in vivo. However, it has been documented that the OsseoSpeed fluoridated surface is less than 1 atomic %.[28] Whether or not this surface is attractive to the grafted and the residual bone due to its nanotopography is unknown; however, according to Wennerberg and Albrektsson[26] and Bjursten and colleagues[30] some indications exist that surface topography influences bone response at the nanometer level.

CONCLUSION

The present study showed similar bone tissue responses and stability for surface modified and fluoridated implants after 8 weeks of healing in fresh or healed autogenous bone grafts in a rabbit model.

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