Healing of titanium implants in onlay bone grafts: an experimental rabbit model

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An experimental rabbit bone graft model for the study of bone formation and remodeling around titanium implants is described. A 2.5-cm long radius bone segment served as an onlay graft. Two commercially pure (c.p.) titanium implants were inserted into the bone graft prior to fixation to the inferior border of the mandibular base with osteosynthesis titanium screws. Each animal was operated twice, allowing follow-up periods of 6 weeks on one side and 6 months on the contralateral side. In order to study bone remodeling by means of fluoroscopy the animals received single injections of tetracyclin and alizarine complexone 2 weeks and 1 week, respectively, prior to sacrifice by perfusion fixation with glutaraldehyde. The bone and implants were excized *en bloc*, postfixed and embedded in plastic resin. Stained and unstained thin ground sections as well as microradiographed thick sections were produced for light microscopic morphometry and fluoroscopy. After 6 weeks, osteoclastic/osteoblastic activity was primarily observed in the graft-recipient contact area and in the intracortical compartment of the graft bone. New bone formation observed on the implant surface originated from the recipient site. The bone formation was evident also in the implant-graft interface. At 6 weeks the average bone fill of the implant threads was 28.4% which increased to 36.4% after 6 months as measured by morphometry. An average of 17.6% bony contact was measured after 6 weeks which increased to 29.7% 6 months after surgery. The graft bone had reduced in size from an average of 39.5% after 6 weeks down to 24.8% after 6 months ($P < 0.05$).

It is concluded that the described experimental model can serve as a useful method for the study of implant healing in onlay grafts.

1. Introduction

Lack of adequate jaw bone volume may preclude the use of oral implants in the rehabilitation of the edentulous patient. In such situations bone grafting may be one alternative for augmentation of the jaw prior to or in conjunction with implant placement. Today, free autologous corticocancellous grafts from the ilium and immediate insertion of screw-shaped commercially pure (c.p.) titanium implants clearly dominate in the literature [1–8]. Implant survival rates of about 75 to 90% after 3–5 years have been reported, which is lower than the survival rates reported for non-graft cases [1, 2, 9]. Any single factor alone being responsible for implant failure in the graft bone has not been identified, although factors relating to the surgical technique, the volume of the bone at the recipient site, stability and integration of the implant in the graft bone and the integrity of the soft tissue during healing have been pointed out to be critical [1, 5, 6, 10, 11].

It is not known if the process of bone formation around implants inserted in bone grafts occurs in the same manner as described for titanium implants in normal cortical bone [12, 13]. There is also a lack of information with regard to both morphological and microbiological characteristics in adjacent soft tissues. It is therefore hard to determine the mechanisms of failure for implants inserted in grafted bone. Adell and co-workers [1] reported that most lost implants failed during the first year and were the result of non-osseointegration along their surface and not because of rapid loss of marginal bone height. This indicates the existence of a non-optimal reparative environment around the implants during the first year which, as suggested by experimental data [14] and clinical studies [1, 5] might be due to a slow and insufficient revascularization and blood supply. This hypothesis is supported by the findings of Nyström *et al.* [15] who presented histology of one patient that had died 4 months after an onlay grafting procedure and immediated placement of titanium implants. Resorption of the graft and bone formation on the surface of graft

trabecular bone was observed. However, the major part of the implant interface consisted of soft tissue and bone condensation to the implant was only evident in the recipient bone. On the other hand, experimental data suggest that osseointegration of titanium implants may occur rapidly also in grafted bone. For instance, Neukam and co-workers [7] demonstrated integration of titanium implants in onlay grafts taken from the ilium and inserted in mandibular defects of 10 minipigs. The authors observed new bone formation and a direct bone-implant contact in both the recipient site and graft after 3 and 5 months. The similar results were obtained by Lew *et al.* [16] using a canine model where the integration of titanium implants in block grafts and particulate grafts were observed after 1–3 months. It may be speculated that the experiments in the studies by Neukam *et al.* [7] and Lew *et al.* [16] were performed under favorable conditions since the bone grafts were placed in fresh bone defects within the skeletal border. Still, several unresolved questions indicate the importance of a comprehensive analysis of the early phase of bone formation around implants in graft bone placed beyond the skeletal contour.

The purpose of the present study was to establish an experimental animal model for the study of titanium implants in autologous on-lay grafts.

2. Materials and methods

2.1. Animals and anaesthesia

Six adult New Zealand white female rabbits, weighing 3.5–4 kg and fed *ad libitum* were used in this study. Prior to surgery, the animals were anaesthetized by intramuscular (i.m.) injections of fluanizole (Hypnorm®, Janssen, Brussels, Belgium; $0.7\,mg\,kg^{-1}$ body weight) and intraperitoneal (i.p.) injection of diazepam (Stesolid, Dumex, Copenhagen, Denmark; $1.5\,mg\,kg^{-1}$ body weight). Additional fluanizole was given when needed during surgery.

2.2. Implants

Screw-shaped implants (ϕ 2.5 mm; length 2.5 mm) and fixation screws, used for stabilization of the graft, (ϕ 2 mm; length 8 mm) were manufactured from commercially pure titanium (c.p. titanium, grade 1). The implants were cleaned in ultrasonic baths in trichlorethylene, acetone and absolute alcohol (10 min in each solution), dried and sterilized by autoclaving.

2.3. Surgery

Surgery was performed under sterile conditions. The right radius was exposed via a skin incision and a fascial–periosteal flap reflected. A bone segment (length 2.5 cm) was osteotomized from the distal part of the radius by means of a diamond drill under generous irrigation with saline. Two holes, 7 mm apart, were drilled in the graft bone during profuse irrigation with saline. The holes were then tapped and two implants were inserted level with the surface of the contralateral cortex. The bone graft was preserved in a moist gauze while preparing the recipient site. The periosteum, the fascia and the skin over the donor site were sutured in separate layers (resorbable Vicryl® 5-0 and Supramid®).

The edge of the right basis mandibulae was exposed via a skin incision. A fascial–periosteal flap was carefully reflected. The graft was placed on the prepared recipient area and stabilized by two fixation screws (Fig. 1). The periosteum, muscle fascia and the skin were sutured in separate layers.

During 3 days postoperatively, the animals were given bensylpenicillin (Intencillin®, Leo, Helsingborg, Sweden; 2 250 000 IE/5 ml, $0.1\,ml\,kg^{-1}$ body weight) and analgesics (buprenorphine, Temgesic®, Reckitt and Colman, USA, $0.05\,mg\,kg^{-1}$ body weight) as single i.m. injections.

After 19 weeks, again the animals were anaesthetized and their left radius and mandibulae underwent the same procedure as described above. In this way the same animal represented both observation periods (the right radius–mandible = 6 month observation period and the left radius–mandible = 6 week observation period, respectively).

For bone labeling purposes oxytetracyclin ($25\,mg\,kg^{-1}$ body weight i.m.) and alizarin complexone ($50\,mg\,kg^{-1}$ body weight, i.m.) were administered 2 weeks and 1 week, respectively before sacrifice.

2.4. Specimen processing and analysis

After 6 months the animals were sacrificed by an intravenous overdose of pentobarbital (Mebumal®, ACO Läkemedel AB, Solna, Sweden) and fixed by perfusion with 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4 via the left heart ventricle for 5 min. The graft part of the mandibles and surrounding tissues were removed *en bloc*, radiographed, immersed in glutaraldehyde for 24 h and postfixed in 2% osmium tetroxide for 1 h. After dehydration in a graded series of ethanol the specimens were embedded in plastic resin (LR White, The White, The London Resin Co. Ltd, UK) and divided crosswise through the axis of each implant as well as in between the implants by sawing (Exakt cutting and grinding equipment, Exakt Apparatebau, Norderstedt, Germany). One half of each specimen was used to prepare 10 μm thick cross-sections according to the

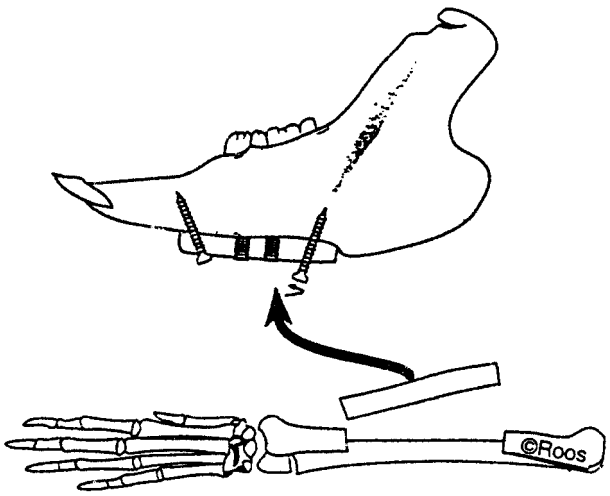


Figure 1 Schematic drawing showing the grafting procedure.

technique described by Donath and Breuner [17]. These sections were viewed unstained or after staining with 1% toluidine blue. The other half of the specimens were used to prepare 150 μm thick ground sections for microradiography. The sections were microradiographed using Kodak High Resolution Plates, type 1A and a Machlett OEG-50 X-ray tube. The plates were exposed to radiation of 17.5 kV, 20 mA and 20 min and processed in Kodak D-19 developer for 5 min at 20 °C.

Examination, fluoroscopy, morphometrical measurements and photography were made in Leitz Metallux and Orthoplan microscopes equipped with a Microvid Computer System. A qualitative description was made based on light microscopy of stained and microradiographed sections in addition to fluoroscopy of unstained sections. The morphometrical measurements comprised: (i) calculation of the degree of bone–titanium contact, expressed as % bone contact; (ii) the amount of bone within the implant threads, expressed as % bone area; and (iii) calculation of the area (bone volume) at the inferior half of the grafted bone (5.5 mm^2). The reason for not having measured the entire volume of the graft was the difficulties in determining the boundary between the graft and the mandible.

2.5. Statistics

The Bonferroni method and the Student's *t*-test, respectively, were used for the statistical analysis of the bone–implant contact as well as the bone area within the threads and for the graft bone volume resorption, respectively. Data were analyzed by analysis of variance, regarding time and position as fixed effects and animal as random effect. The interaction between animals, time, and position were also included in the model.

3. Results

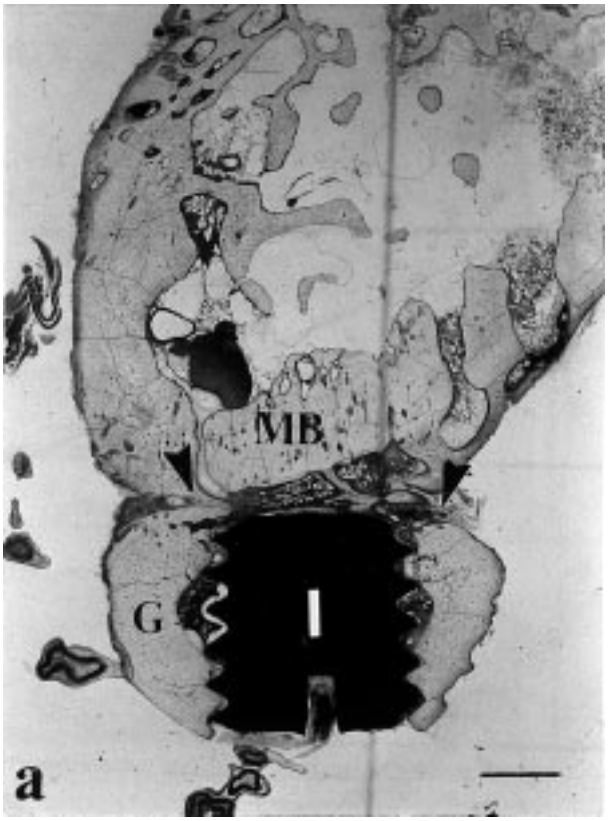
3.1. Clinical observations

All animals recovered well after surgery and no postoperative complications were noted during the 6-month observation period.

3.2. Histological observations

A typical cross-section of the experimental area comprised mandibular bone containing one root of a molar with the most apical part located approximately 2 mm from the inferior border of the mandibular base and the bone graft with an implant (Fig. 2a,b). After 6 weeks the grafts (in 9/12 specimens) were in direct contact with the mandibular bone while it was observed that only in 8/12 specimens the grafts were in direct contact with the host bone after 6 months. In the non-contact specimens a fibrous tissue occupied the area between the graft and the mandible. In general the implant appeared to be stabilized only by the inferior cortical layer of the graft. There were no signs of trabecular bone in the marrow cavity of the graft.

After 6 weeks there were signs of bone resorption and new bone formation at the recipient site in the contact area to the graft (Fig. 3). Bone mineralization units (BMUs) were primarily seen in the graft–recipient contact area (Fig. 3) but also in the intracortical compartment of the graft, while the periosteal and endosteal surfaces of the graft showed little or no fluorescence (Fig. 4). Revascularization of the graft



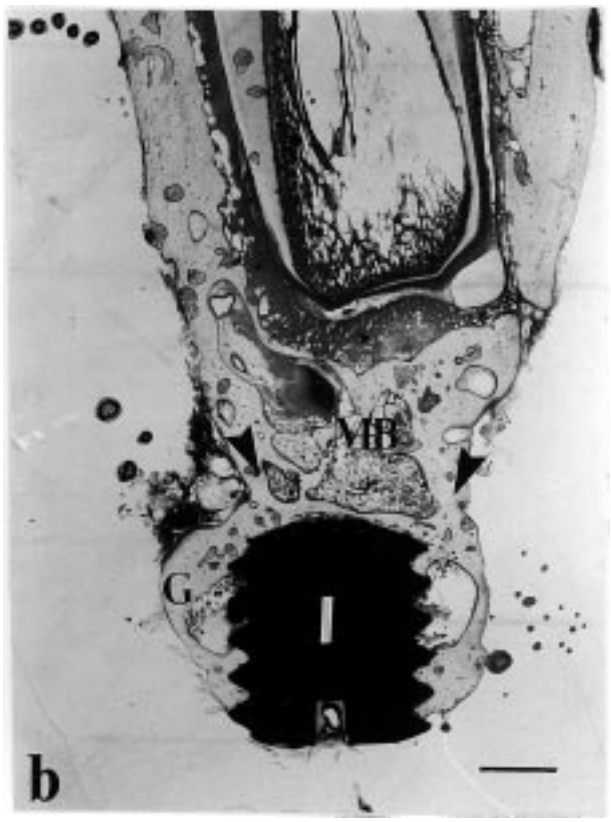


Figure 2 Light micrographs showing an overview of the specimens after 6 weeks (a) and 6 months (b). The boundary between the graft and the mandibular base (arrows) can be distinguished after 6 weeks (a) but not after 6 months. G = graft bone, I = implant, MB = mandibular base. Bar = 1000 μm.

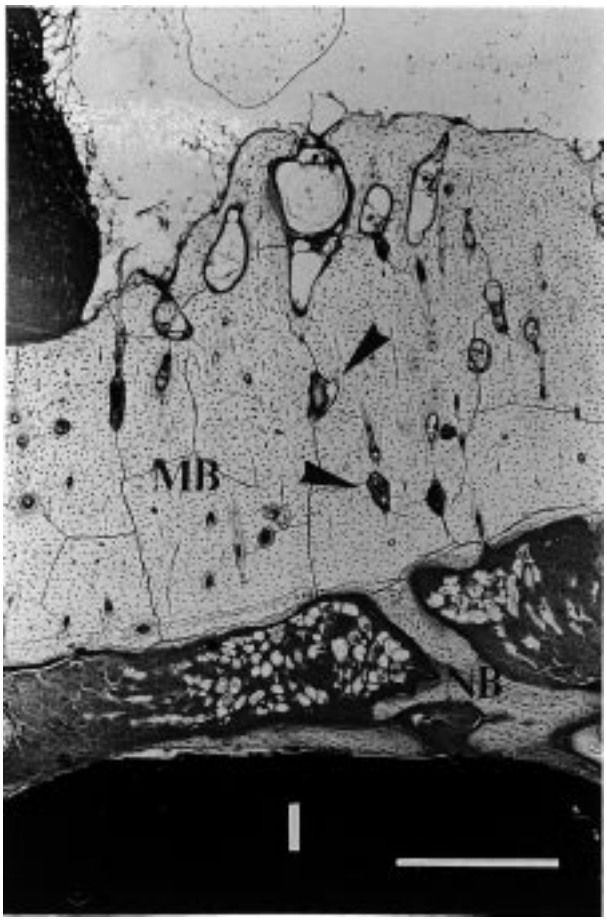


Figure 3 Light micrograph showing the contact area between the graft–implant and the mandibular base after 6 weeks. An increased osteoclastic activity is evident in the cortical bone of the recipient site (arrows). Newly formed bone (NB) is approaching the implant surface (I). MB = mandibular base. Bar = 500 μm.

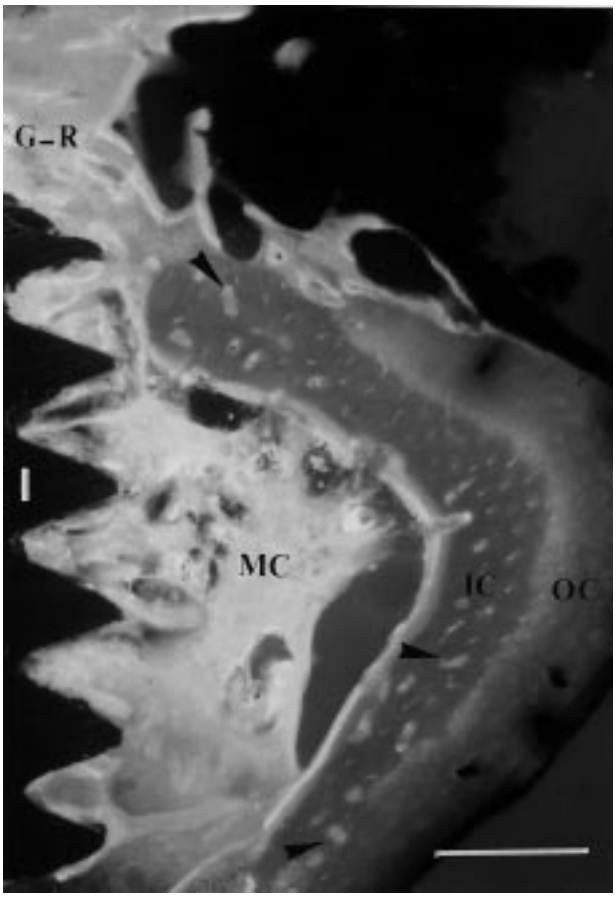


Figure 4 Fluoroscopy of a 6-week specimen. Fluorescence is seen in the graft–recipient contact area (G-R) and intracortically in the graft (IC). The confluent fluorescence in the bone marrow cavity (MC) is not due to bone formation but accumulation of dyes in the soft tissues. Arrows are pointing to secondary osteon-like bone formation. No fluorescence is observed in the outer compartment (OC) of the graft bone. I = implant. Bar = 500 μm.

seemed to occur from the bone marrow cavity of the graft from where vessels penetrated into the intracortical compartment (Fig. 5). There were few signs of bone resorption and bone formation in the implant–graft interface of implant threads located in the graft (threads no 1, 2, 4 and 5 (thread 5 being next to the host bone)) indicating low degree of bone activity or non-vital bone (Fig. 6). The newly formed bone found in the implant interface was often in continuity with the mandibular bone and appeared to originate from the recipient site (Fig. 3).

The 6-month specimens had a relatively thinner transcortical width compared with specimens obtained after 6 weeks (Fig. 7a,b), which probably was due to a periosteal and endosteal resorption, resulting in a thin cortical layer. There were no signs of any osteoclastic activity at the periosteal or endosteal surfaces at this time. The remaining graft seemed to be vital and corresponded to the part of the intracortical compartment which showed signs of bone remodeling at 6 weeks. Bone formation was evident in the implant-graft interface (Fig. 8). The newly formed bone seemed to undergo remodeling since few Haversian systems were observed in this region (Fig. 8).

3.3. Morphometrical measurements

The result of the light microscopic morphometry is presented in Fig. 9. An average of $17.62 \pm 3.62\%$ (mean $\pm$ standard error of the mean, SEM) of bony contact was measured after 6 weeks, while the average bone–implant contact was $29.7 \pm 3.95\%$, after 6 months. The average bone fill of the implant threads was $28.42 \pm 4.88\%$ after 6 weeks. The corresponding value after 6 months was 36.39 ± 3.38. Neither the bone–implant contact nor the bone area within the threads differed significantly from 6 weeks–6 months (Fig. 9a,b). However, the graft bone was significantly reduced in size between 6 weeks ($39.54 \pm 1.42\%$) and 6 months ($24.83 \pm 4.32\%$) ($P < 0.05$).

4. Discussion

The objectives of the present experimental study were: (a) to perform a qualitative as well as a quantitative evaluation of the bone healing around titanium implants in graft bone inserted in an onlay position, and (b) to make a qualitative analysis with respect to bone remodeling in graft bone in conjunction with the host bone. On the basis of the present results several advantages with the described model are apparent: (i)

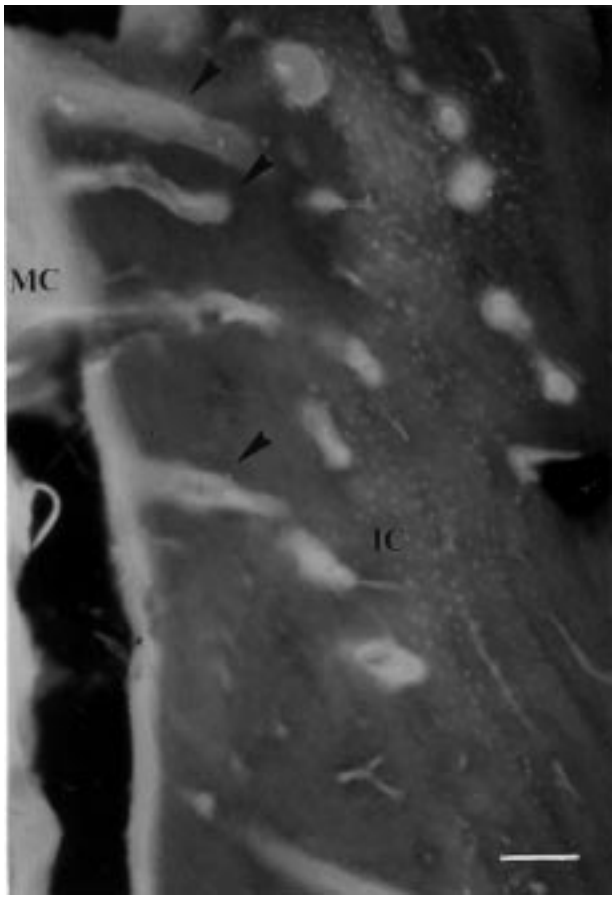


Figure 5 Fluorescent light micrograph showing penetration of vessels (arrows) from the marrow cavity (MC) into the intracortical compartment (IC) of the graft bone after 6 weeks. Some vessel channels are cut longitudinally and some crosswise. Bar = 100 μm.

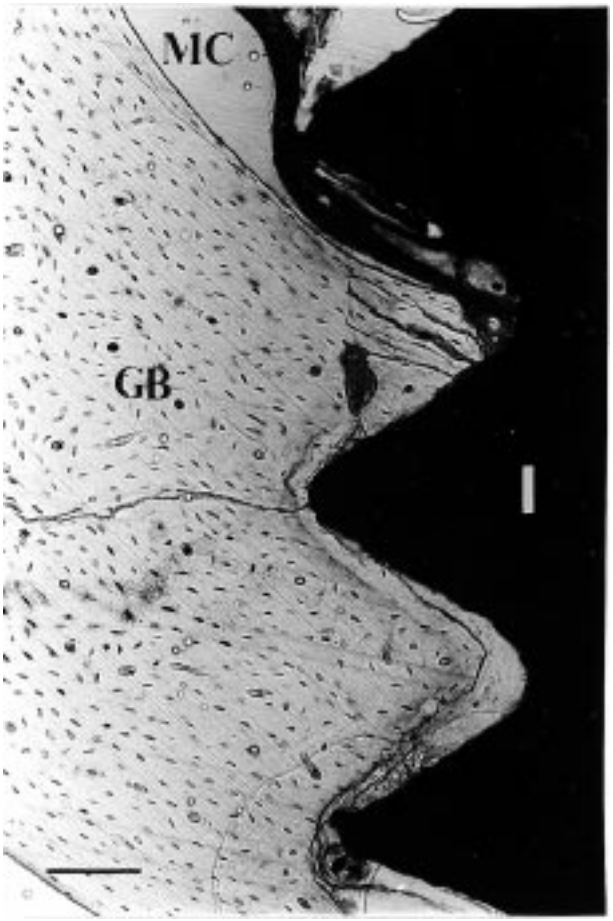
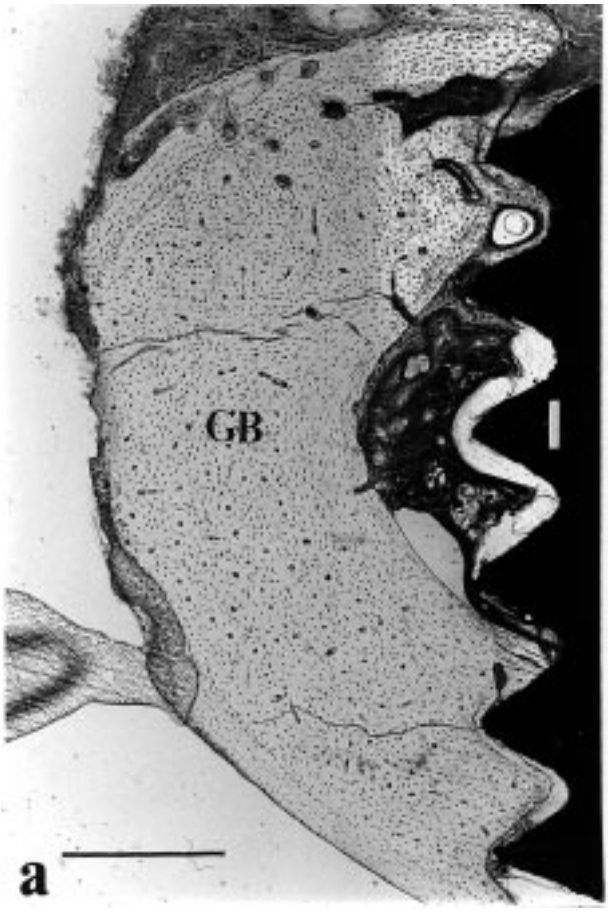


Figure 6 Light micrograph showing the graft–implant interface after 6 weeks. There are no signs of osteoclastic or osteoblastic activity. There is not apparent tissue within the bone-implant interface. I = implant, GB = graft bone, MC = marrow cavity. Bar = 100 μm.

the incorporation of implants can be studied in enchondral bone grafts used for augmentation of intramembranous bone; (ii) the experimental graft is placed outside the oral cavity and is, thus, not subjected to mechanical trauma nor to the oral micro-organisms. This enables studies on graft performance under relatively controlled conditions; (iii) the model is challenging because the graft and implants are placed beyond the skeletal borders. However, it should be taken into consideration that when extrapolating the results from the present model to clinical conditions the drawbacks are the lack of cancellous interface against the host bone and that the bone graft was covered by skin and fascia rather than by oral mucosa.

The bone graft was significantly ($P < 0.05$) reduced in size during the 6-month observation period [18]. The size of the remaining bone graft corresponded well to the size of the area in the intracortical compartment, containing Haversian systems, which showed fluorescent lines at 6 weeks. These findings indicate that the graft in part was revascularized via the pre-existing Haversian and Volkmann's canals. This is in line with previous descriptions of revascularization of cortical bone [19]. Also, in sections where the graft was not in direct contact with the recipient site there were signs of intracortical bone resorption and formation. Since the revascularization occured via osteoclastic activity from the marrow cavity of the graft into the intracortical compartment, it is possible that the vessel sprouts met with and developed longitudinally along pre-existing Haversian canals. Our findings also indicate that the periosteal and endosteal parts that did not show any fluorescence after 6 weeks were resorbed after a 6-month period.

Our morphological observations indicated that the bone area within implant threads and the bone–implant contact measurements revealed higher values for the part of the implant which was located close to mandible. This may be attributed to the rapid response of the recipient bone to the surgical trauma, resulting in bone formation which approached the implant surface. No bone formation was observed in the marrow space of the graft. It can be speculated that bone formation might have occured at this location if cancellous bone, serving as a solid base for bone formation, had been present in the graft. Bone was also found in the threads located in the cortical passage of the graft but since there was initially little evidence of fluorescent labeling it can not be excluded that the implant–close bone had a reduced vitality after 6 weeks. On the other hand after 6 months, when revascularization and remodeling of the graft was ongoing, vital bone appeared to be present in the implant–graft interface as judged by the localization of the fluorescent markers. Our findings suggest that the initial integration of the implant occured from the recipient site, while the integration of the graft was delayed.



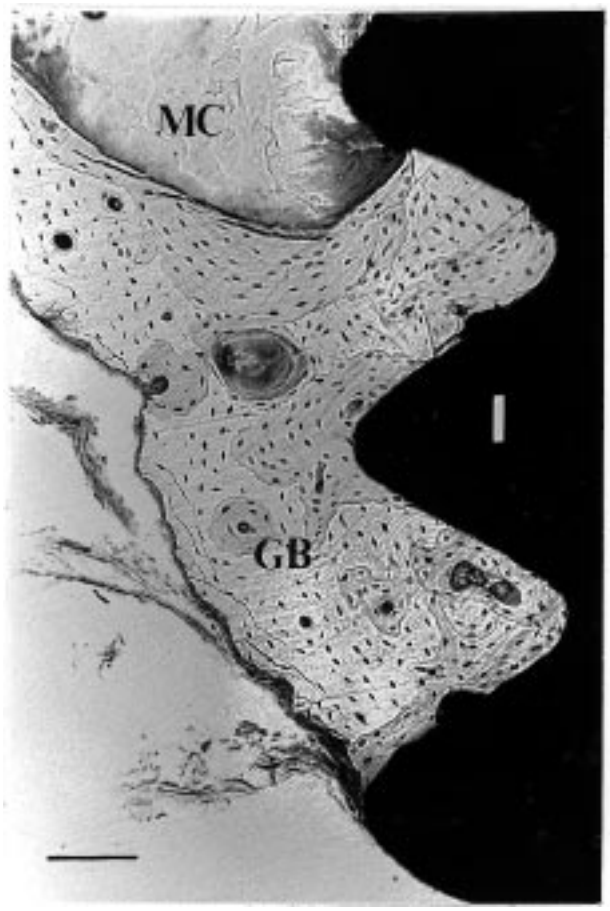


Figure 8 Light micrograph of a 6-month specimen. The implant threads are filled with grafted bone and newly formed, and remodeled bone (darker areas), which appear to be in direct contact with the implant surface. I = implant, MC = marrow cavity. Bar = 100 µm.

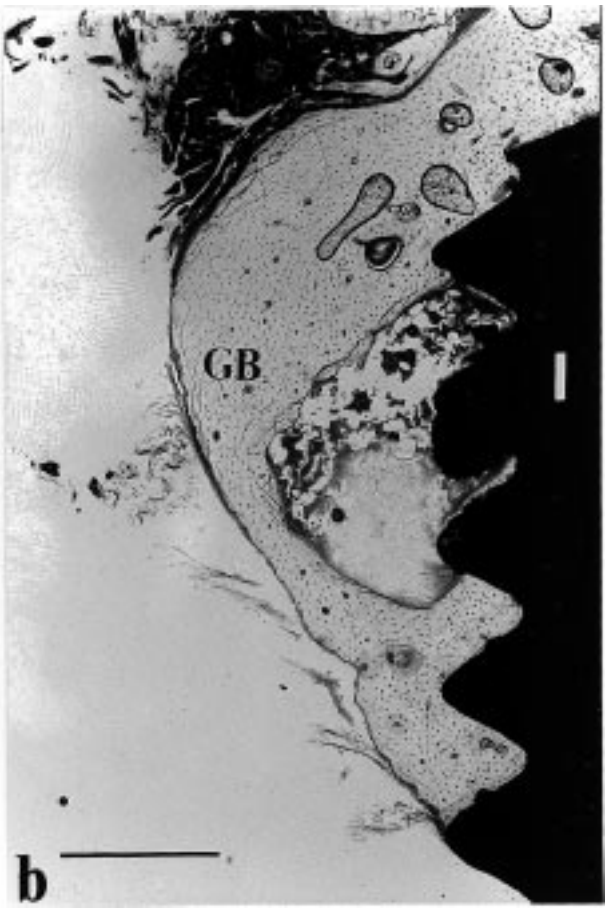


Figure 7 Light micrographs of a 6 weeks (a) and a 6 month specimen (b). Note the thinner transcortical width of (b) as compared to (a). I = implant, GB = graft bone. Bar = 500 µm.

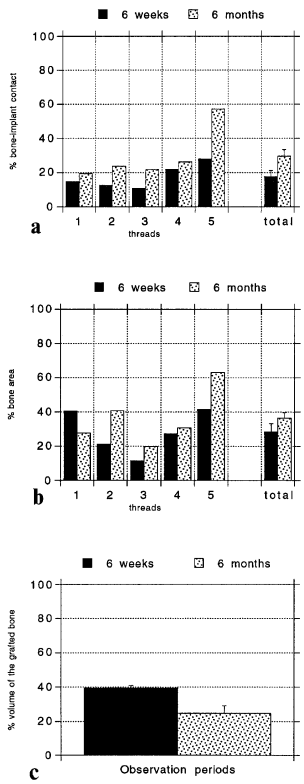


Figure 9 Results from the morphometrical measurments of (a) the bone–implant contact as well as (b) the bone area around the implants and (c) the bone graft volume after 6 weeks and 6 months.

In the present study there was a tendency towards a high degree of bone–implant contact and amount of bone within the threads after 6 months. However, due to high interindividual disparities between animals the differences in implant–bone parameters between 6 weeks and 6 months were not statistically significant. Reasons for the differences between the animals could be caused by the variations in structure of bone in the radius graft and implant installation, difficulties to prepare standardized sections of relatively small implants and inherent differences in the biological response between animals. One possibility, not further explored in the present study, is that the time for implant insertion (for instance, placement in the radius graft prior to osteotomy versus after osteotomy) could be important. The absence of sufficient blood supply (blood cells and proteins) in the graft-implant interface might jeopardize the initial bone formation close to the implants.

5. Conclusions

It is concluded that the use of radius bone graft transferred to the inferior border of the mandible may serve as a model for the study of implant healing in onlay grafts placed beyond the skeletal contour. The present results show that the initial integration of the implant occured from the recipient site, while the integration process in the graft was delayed.

Acknowledgments

Financial support from the King Gustav V 80-year Fund, the Medical Faculty and the Faculty of Odontology, Unversity of Göteborg, the Swedish National Association against Rheumatism, the National Board for Industrial and Technical Development (NUTEK) and the Swedish Medical Research Council (9289 and 9495) is gratefully acknowledged.

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Received 19 June
and accepted 21 July 1998