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 (Hynpnom®, 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 trichloroethylene, 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 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, Sollna, 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.