Evaluation of a new experimental model to study bone healing after ridge expansion with simultaneous implant placement – a pilot study in minipigs

Dental implant therapy is currently considered the best surgical treatment for partially edentulous patients. To achieve long-term optimal functional and esthetic results, sufficient width and height of bone must be present at the recipient site. In many situations, however, there is a bone deficiency (Braut et al. 2011) and a procedure is indicated that predictably leads to sufficient new bone formation. Horizontal augmentation using the principle of guided bone regeneration (GBR), onlay bone block grafts, and alveolar distraction osteogenesis are among the techniques used to enhance the bone volume in case of a narrow alveolar ridge (Buser et al. 1999, 2002; Cordin et al. 2002; Donos et al. 2008; Chiapasco et al. 2009). While the GBR procedure can be performed with simultaneous implant placement (Wilson & Buser 1994; Zittmann et al. 2001; Aghaloo & Moy 2007; Jung et al. 2009), onlay grafting implies a reconsolidation time of at least three months to enable implant placement in a staged procedure (Buser et al. 1996; Chiapasco et al. 1999, McAllister & Highgate 2007). Many studies have demonstrated the success of these well-documented surgical approaches, but donor site morbidity, unexpected bone resorption and infection are among the drawbacks of these conventional techniques (Machtei et al. 2001, Chiapasco et al. 2006, Funaki et al. 2009).

Another therapy for treating a narrow bone ridge is the bone-splitting/ridge expansion technique introduced by Simon and coworkers in 1992. According to this procedure, the

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**Abstract**

**Objective:** To evaluate the suitability of a minipig model for the study of bone healing and osseointegration of dental implants following bone splitting and expansion of narrow ridges.

**Material and Methods:** In four minipigs, the mandibular premolars and first molars were extracted and used for a pilot study in minipigs.

**Results:** In all groups, no bone fractures occurred, no implants were lost, all 24 implants were osseointegrated, and the gap created by bone splitting was filled with new bone, irrespective of whether BCP or a barrier membrane was used. Slight exposure of five implants was observed, but did not lead to implant loss. The level of the most coronal bone-to-implant contact varied without being dependent on the use of BCP or a barrier membrane. In all groups, the BCP particles were not present deep in the bone-filled gap. However, BCP particles were seen at the crestal bone margin, where they were partly integrated in the new bone.

**Conclusions:** This new minipig model holds great promise for studying experimental ridge splitting/expansion. However, efforts must be undertaken to reduce implant exposure and buccal bone resorption.
compromised alveolar ridge is crestally opened and subsequently split with special osteotomes (Lustmann & Lewinstein 1995). The implant is then inserted into the expanded space between the medial and buccal bone walls and allowed to heal in a submerged position [Koo et al. 2008; Funaki et al. 2009]. The bone-splitting/ridge expansion procedure presents the advantage of simultaneous implant placement and avoiding bone graft harvesting from secondary donor sites [Scipioni et al. 1994, 1999; Sethi & Kaus 2000; Ferrigno & Laureti 2005; Blus & Szmukler-Moncler 2006; Chiapasco et al. 2006; Bravi et al. 2007; Elian et al. 2008; Han et al. 2011]. After four months of submerged healing, implant uncovering and loading can be performed [Basà et al. 2004].

One disadvantage of the bone-splitting/ridge expansion technique, which can be quite severe, may be the risk of bone resorption due to malnutrition of the laterally out displaced buccal bone wall [Scipioni et al. 1997; Strietzel et al. 1999; Funaki et al. 2009]. Although some approaches were proposed to reduce bone resorption [Sethi & Kaus 2000; Ferrigno & Laureti 2005; Coatoam & Mariotti 2003; Basà et al. 2004; Enslidis et al. 2006; Koo et al. 2008], evidence for their efficacy is still lacking. There are only three animal studies, all performed in dogs, available in the literature, where the ridge splitting/expansion technique was studied [Scipioni et al. 1997; Funaki et al. 2009; Han et al. 2011]. Marginal bone loss [Han et al. 2011], particularly at the buccal bone wall [Scipioni et al. 1997; Funaki et al. 2009], and exposure of implant threads in 60% and implant mobility in 40% of all implants [Funaki et al. 2009] were observed. Thus, further research is needed to optimize the outcome of ridge expansion on implant osseointegration. Because there are increasing ethical concerns about dogs being used as experimental animals, we aimed, for the first time, to use the minipig as a model for the study of bone healing after ridge expansion.

Furthermore, the minipig represents nowadays the animal of choice for research activities in the biomedical field [McAnulty et al. 2011], including studies on bone physiology, healing, and regeneration [Dard 2012a]. Consequently, the aim of this pilot study was to evaluate the suitability of the minipig as a model to examine bone healing after ridge splitting and expansion of narrow ridges with simultaneous implant placement. The minipig is considered as an appropriate model for bone research because of its close similarity to human bone in terms of structure (Moskilde et al. 1987), bone mineral density (Aerssens et al. 1998), healing pattern [Hönig & Merten 1993; Moskilde et al. 1993], and tissue regeneration rates [Ma et al. 2009]. It is therefore not surprising that successful use of this animal model has been demonstrated for fracture healing [Pearce et al. 2007], bone-grafting materials [Buser et al. 1998a; Jensen et al. 2006, 2007, 2009], and osseointegration of dental implants [Buser et al. 1998b, 2004; Jensen et al. 2011; Broginni et al. 2012; Gottlow et al. 2012; Freilich et al. 2012; Salmac et al. 2012].

Material and methods

Study design

On each side of the mandible, ridge expansion was performed and three titanium implants were placed in the created bone gap. According to the randomization schedule, the gap between the expanded bone was then filled with a bone substitute material in half of the sides [test group], while the other sides were filled with blood only [control group]. At four sides, additional lateral augmentation of the buccal bone plate with the same bone substitute material and a barrier membrane was performed.

Animal model and management

Study approval was obtained from the Ethical Committee of the University of Lund-Malmö, Sweden. The study design was in accordance with internationally accepted guidelines for animal trials testing of biomaterials and the related evaluation of their efficacy as stated by Dard [2012b].

In this pilot study, four adult female Göttingen minipigs™ (Ellegaard, Denmark) with an average body weight of 40 kg were housed in standard cages and were fed on a soft diet for minipigs [Special Diet Service, UK]. Prior to both surgical procedures, all animals were fasted overnight to prevent vomiting. On the day of surgery, all minipigs were pre-mediated with an intramuscular injection of atropine (Atropinum sulfuricum, 0.05 mg/kg IM). All surgical procedures were performed under general anesthesia and aseptic conditions in a dedicated animal surgical clinic (Malmö, Sweden). All animals were anesthetized according to the following procedure: 10 ml of ketamine (Ketalar Vet, Pfizer AB, Sol lentuna, Sweden, 50 mg/ml) was mixed with 3 ml midazolam (Dormicum® 5 mg/ml; Roche, Basel, Switzerland). During surgery, 10 ml of ketamine had been injected when needed. All minipigs received 10 ml of ketamine every 30 min and, if needed, 1.5 ml of midazolam. An additional local anesthesia (Xylocain Dental adrenalin 20 mg/ml + 12.5 mg/ml, Astra AB, Södertälje, Sweden) was given to reduce the dosage of the systemic anesthetic as well as to reduce the bleeding during surgery and to alleviate pain after surgery. Post-surgical treatment with systemic antibiotics (Streptocillin vet.®, Boehringer Ingelheim, Denmark) was given for seven days to avoid infections. Within the first days after surgery, all animals were monitored routinely and further analgesia was given if necessary. The whole study was accompanied and monitored by a veterinarian and researchers with extensive experience performed all surgical procedures.

Surgical phase

The two surgical procedures were performed under aseptic conditions in an animal operating theater under general anesthesia. In the first procedure, three mandibular premolars and the first mandibular molars were carefully extracted followed by the removal of the buccal bone wall by a round bur. X-ray control with single-tooth film was carried out to control the complete extraction of all remaining fractured roots. After 3 months of healing, the second intervention consisted of the bone-splitting procedure. At the test and the control sides of each animal, the crestal incision was followed by reflection of the musculoperiosteal flap to expose the bone. The narrow ridge was cut crestally 30 millimeter in the mesiodistal direction and six millimeters deep with a piezo instrument (Piezosurgery®, Mectron s.p.a., Carasco, Italy). Mesially and distally, a buccal release cut of 6 mm was performed and the mobilized buccal bone plate was displaced in the buccal direction with the help of a special osteotome (Ergoplant® Aesculap AG, Tuttingen, Germany). Thereafter, the expanded gap was kept open with retraction inserts (Ergoplant®) and three implants (Straumann® Bone Level Implant, NC Ø 3.3 mm/8 mm, Institut Straumann, Basel, Switzerland), which achieved primary stability in the deeper portion of the non-fractured gap, were installed per quadrant in such a way that the implant shoulder was flush with the level of the lingual and buccal bone crest [Fig. 1a]. According to the randomization schedule of the study design, the gap between the expanded bone was then filled with a bone substitute material in half of the sides [test group], while the other sides were filled with blood only [control group], while at four sides, additional lateral augmentation of the
buccal bone plate with the same bone substitute material and a barrier membrane was performed. On one mandibular side, the expanded bone gap was filled with granules consisting of an alloplastic biphasic calcium phosphate (BCP) biomaterial [Straumann® BoneCeramic, 400–700 μm, Institut Straumann] mixed with blood (Fig. 1b), while on the contralateral side, the gap was filled with blood only. In half of the sides, a GBR procedure consisting of a lateral augmentation of the buccal bone plate with the BCP granules mixed with blood and a barrier membrane was applied. At two of these four sides, a xenogenic collagen membrane (BioGide®, Geistlich, Wolhusen, Switzerland) was used (Fig. 1c), while the other two sides were covered with a synthetic hydrogel membrane based on polyethylene glycol technology [Straumann® MembraGel®, Institut Straumann]. Multiple periosteal release incisions were performed for tension-free soft tissue closure followed by interruptive suturing of the flaps with a resorbable material [Vicryl 4-0, Ethicon Norderstedt, Germany]. Thereafter, an X-ray picture was taken from each side (Fig. 1d).

Terminal procedure
The four animals were sacrificed 6 weeks after implant installation. The termination was conducted by inducing cardiac arrest with an intracardiac injection of a 20% solution of pentobarbital (Pentobarbitalnatrum, Apoteket AB, Stockholm, Sweden, 60 mg/ml).

Block resections of the implant sites were performed using an oscillating autopsy saw to keep the soft tissue intact. The removed block sections were fixed by immersion in 4% buffered formalin for 2 weeks.

Histology
The specimens were left undecalcified and dehydrated in an ethanol series and embedded in methylmethacrylate. Serial sections of ~500 microns in thickness were cut in a buccal-lingual direction using a low-speed diamond saw with coolant [Varicut® VC-50, Leco, Munich, Germany]. Sites to be analyzed included all dental implants and the gap regions between the implants, as well as mesial to the anterior implant and distal to the posterior implant. After mounting the sections onto acrylic glass slabs, they were ground and polished to a final thickness of about 100 μm (Knuth-Rotor-3, Strauers, Rodovre/Copenhagen, Denmark). The sections were stained with toluidine blue and basic fuchsin, and the two most central ground sections per implant and per interimplant region were used for qualitative analysis. Digital photography was performed using a ProgRes® C5 digital camera [Jenoptik Laser, Optik Systeme GmbH, Jena, Germany] connected to a Zeiss Axioplan microscope [Carl Zeiss, Göttingen, Germany]. Position and osseointegration of the implants, new bone formation in the bone gap, and soft tissue condition were assessed directly in the microscope. Due to the pilot nature of this study and thus the limited number of samples per treatment group, we refrained from performing a histomorphometric analysis, because this would require a much higher number of samples per group to perform a statistical analysis. With regard to the small sample size, only descriptive statistical methods could be applied.

Results

All animals behaved well during the general anesthesia and in the time after the operations. In the first surgical intervention, all teeth could be extracted completely and the buccal bone plate could also be removed successfully. The second procedure [i.e. the bone splitting/ridge expansion] also occurred without any complication. In particular, no fracture of the mobilized buccal bone wall did happen. Postoperatively, a good soft tissue healing after the bone-splitting procedure could be demonstrated clinically in most cases. Three soft tissue dehiscences were, however, identified, which all occurred at sites where the bone-splitting procedure was performed without lateral augmentation. These soft tissue dehiscences presented in the histological sections with coronal implant portions partly exposed to the oral cavity in five of 24 implants.

After 6 weeks of healing, there were no implants lost and all implants were osseointegrated in a vertical position and revealed dense newly formed bone at their periphery [Figs 2 and 3]. The extension of bone in contact with the implant surface in a vertical direction varied from implant to implant, and in the majority of implants, the bone height was buccally lower than at lingual sites [Fig. 2]. There were no obvious differences observed between sites with or without filling of the bone gap with the bone substitute material. When the buccal bone wall was augmented and covered with a collagen membrane, both the thickness and the vertical dimension of the augmented bone were increased [Fig. 2c]. When a PEG membrane was used, this effect was less pronounced (not shown).

Regarding bone healing in the gap regions between implants, new bone filled the gap, regardless of whether the gap was filled with the bone substitute material and regardless of whether a barrier membrane was used [Fig. 4]. New bone (dark staining) was deposited directly against the cut surface of the old bone (light staining), which was created dur-
ing the bone-splitting procedure, and particles of old bone, which were severed during the bone-splitting procedure, were incorporated in the new bone (Fig. 5). Interestingly, when the gap was filled with the bone substitute material, such particles were present in the ridge region above the bone-filled gap, where they were partially integrated in the new bone (Figs 4b,d and 6b,d). Remnants of the collagen barrier membrane were still recognizable and partly embedded in the newly formed bone (Fig. 6c,f), whereas no residual material of the PEG membrane was visible.

Discussion

Therefore, the aim of this pilot study is to evaluate the suitability of the minipig as a model to examine bone healing and osseointegration after bone splitting and ridge expansion of narrow mandibular ridges with simultaneous implant placement.

The present experimental study in minipigs demonstrates that none of the 24 implants placed in the bone gap created by ridge expansion was lost, and all were osseointegrated. These findings are consistent with data from a ridge expansion study in dogs (Han et al. 2011) and even superior to data from another dog study where 60% of all implants showed exposed threads and 40% of the implants were mobile at 3 months (Funaki et al. 2009). That osseointegration of dental implants in large bone defects can occur without the use of a bone filler and a barrier membrane is in line with observations from two ridge expansion studies in dogs (Scipioni et al. 1997; Han et al. 2011). In the study by Han et al. (2011), the use of a bone filler and a collagen barrier membrane resulted in less marginal bone loss than without grafting and barrier membrane. Due to the pilot nature of the present study and thus the low number of implants, we cannot confirm this observation. However, our data showing that the vertical extension of bone on the implant surface varied from implant to implant and that, particularly at buccal sites without lateral augmentation, the height of the most coronal bone-to-implant contact was below the implant shoulder corroborate data from studies in dogs (Scipioni et al. 1997; Funaki et al. 2009; Han et al. 2011). In the study by Scipioni et al. (1997), three of 20 test implants were excluded from the analysis because of bone sequestration after suture removal. As suggested by these authors, the bone resorption may have occurred early as a consequence of mechanical manipulation (i.e. inadequate vascular supply) and post-surgical infection. In the present study, slight implant exposure accompanied by bone recession was histologically noticed in five of 24 implants. Thus, the minipig model presented here appears to be an appropriate model to study osseointegration of implants installed during a bone-splitting/ridge expansion procedure. Similar to observations made in the dog, particular attention must be paid to avoid implant
Although there was no reference line that allowed measurement of crestal bone height reduction in the interimplant region, the second possibility seems also unlikely, because the length of the gap filled with new bone after 6 weeks of healing was still very high. Studies showing that the same bone substitute material has a very slow resorption/deg-
radiation rate [Jensen et al. 2007, 2009, Mardas et al. 2010] and the consistent presence of the bone substitute material in the crestal region above the gap in the present study and of other bone substitute materials embedded in the peri-implant bone [Han et al. 2011] do not support the third possibility. In another study, however, it was shown that increased resorption of a normally slowly degrading bone substitute material can occur under special circumstances [Busenlechner et al. 2012]. From the present study in the minipig, it can be concluded that the gap created by ridge expansion was filled with new bone and that this new bone formation occurred with and without filling the gap with a bone substitute material and with or without the use of a barrier membrane.

Because buccal bone resorption was observed after ridge expansion [Scipioni et al. 1997; Funaki et al. 2009], reduction of resorption of the mobilized buccal bone is an issue of great clinical importance. One possible technique is a combined simultaneous ridge expansion and horizontal GBR procedure as applied in the present pilot study. Height and width of the buccal augmentation appeared to be superior in the six implants with a collagen membrane compared with the sites where a synthetic PEG membrane was used. Our observation that residual collagen membrane material was detected in all six implants, but no residual PEG material was found suggests that the barrier function of the collagen membrane may have lasted longer than that of the PEG membrane, due to a slower resorption rate of the collagen membrane. It is noteworthy that the PEG membrane has been shown to cause problems such as detachment possibly due to the presence of blood [Zambon et al. 2012]. This may explain the differences seen at the augmentation sites. However, a definitive conclusion on this cannot be drawn at the present time, because of the small numbers of augmentation sites for the two barrier membranes in this pilot study. The effect of horizontal augmentation of the buccal bone wall needs to be investigated in a future study with a larger number of animals and an appropriate number of implants per group to allow quantification and statistical analysis.

exposure during the healing period. Like with other delicate techniques, the ridge expansion technique appears to be user-sensitive, irrespective of the animal model used.

In all quadrants, the bone gap created by ridge expansion was filled with new bone, irrespective of the use of a bone substitute material to fill the gap and irrespective of the use of a barrier membrane. This implies that no soft connective tissue from the oral mucosa could expand into the deeper portions of the gap and that the stabilization of the blood coagulum was not dependent on the presence of a bone filler material. Gap filling with new bone without the use of a bone filler and without a barrier membrane was also observed in two previous studies in dogs [Scipioni et al. 1997; Han et al. 2011]. Surprising-

Fig. 4. Histological view of the interimplant gap spaces (a) without gap filling, without lateral augmentation, and without a membrane, (b) with gap filling, without lateral augmentation, and without a membrane, (c) without gap filling, with lateral augmentation, and with a membrane, (d) with gap filling, with lateral augmentation, and with a membrane.

Fig. 5. Higher magnifications of the central portion of the interimplant gap spaces. (a) without gap filling, without lateral augmentation, without membrane, (b) with gap filling, without lateral augmentation, without membrane, (c) without gap filling, with lateral augmentation, with membrane, (d) with gap filling, with lateral augmentation, with membrane.
Conclusions

In this ridge expansion model in minipigs, all implants were osseointegrated and new bone filled the created gap, irrespective of the use of a bone substitute material or a barrier membrane. Thus, this new minipig model has great potential to study various conditions that may influence the outcome of experimental ridge expansion on osseointegration and soft tissue conditions of dental implants. However, similar to studies in dogs, efforts must be undertaken to reduce implant exposure and bone resorption during healing.

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References


