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Onlay bone grafting of the mandible after periosteal expansion with an osmotic tissue expander: an experimental study in rabbits

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Abstract

Objectives: To evaluate the space-maintaining capacity of a titanium mesh or a bioresorbable mesh after periosteal expansion and to assess bone formation under a titanium mesh or a bioresorbable mesh on the lateral border of the mandible by qualitative and quantitative histological analysis.

Material and methods: In 13 rabbits, a self-inflatable soft tissue expander was placed intraorally, bilaterally under the mandibular periosteum via an extra oral approach. After 2 weeks, the expanders were removed and a particulated onlay bone graft was placed and covered by a titanium mesh or a bioresorbable mesh. After 3 months, the animals were sacrificed and specimens were collected for histology.

Results: The osmotic soft tissue expander created a subperiosteal pocket and a ridge of new bone had formed at the edges of the expanded periosteum in all sites. After the healing period of 3 months, soft tissue dehiscence was recorded in two of the sites with bioresorbable meshes. The mean bone fill was 65% under the titanium mesh and 85% under the bioresorbable mesh ($P < 0.05$). There was no significant difference between the titanium mesh and the bioresorbable mesh regarding the height of the meshes, mesh area and mineralized bone area. Scanning electron microscopy shows that new bone is growing in direct contact with the resorbable mesh and the titanium mesh.

Conclusion: This study confirms that an osmotic soft tissue expander creates a surplus of periosteum and soft tissue and that new bone can be generated under a titanium mesh or bioresorbable mesh.

Insufficient regeneration of missing bone and soft tissue may present aesthetic or functional problems in patients indicated for dental implant surgery. Several techniques such as bone grafts, bone substitutes and/or guided tissue regeneration (GTR) have been described to rebuild a compromised alveolar ridge (Wang & Boyapati 2006). To obtain a successful GTR, it is important to have good primary wound closure, space creation/maintenance and stability of both the initial blood clot and implant fixture. Autogenous

bone grafting is considered to be the gold standard (Slotte et al. 2003).

Guided bone regeneration (GBR) has been used for bone regrowth beyond the original skeletal envelope (Lundgren et al. 1995; Van Steenberghe et al. 2003; Yamada et al. 2008). In the GBR technique, it is important that the barrier is biocompatible, adaptable and maintains the created space. Titanium mesh is used in combination with bone grafts (Boyne et al. 1985; von Arx & Kurt 1999) and/or bone substitutes

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(Maiorana et al. 2001). Because titanium mesh requires removal at a second operation, a resorbable mesh could be preferable. Titanium meshes and resorbable meshes have been used to protect corticocancellous tibia bone grafting to the mandible of hound dogs (Gutta et al. 2009). This study found that macroporous titanium membranes facilitated more bone regeneration compared with microporous and resorbable meshes. A layer of soft tissue was noted under all the meshes.

Adequate soft tissue coverage of grafted bone and titanium mesh is important to avoid exposure, which may result in loss of the bone graft (von Arx & Kurt 1999). The contour of the alveolar process is often altered following infection, tumour surgery or other traumatic loss of alveolar bone, soft tissue and teeth. The resultant deficit in the alveolar process limits the possibility to reconstruct the area with dental implants without bone grafting. In this situation, the need for more soft tissue is important to reconstruct and cover a local bone graft. This is in general managed by cutting the periosteum to allow the flap to be lengthened. In plastic surgery an increase of the soft tissue area is often accomplished by expansion. Soft tissue expansion is a physiological process developed to allow surgeons to cover defects using local skin of appropriate colour, texture and function (Wieslander 1991). This technique has also been used to create a soft tissue surplus to cover a bone graft (Argenta & VanderKolk 1987). An osmotic tissue expander with a silicone shell provides a predictable localized donor tissue and could reduce the rate of complications compared with expanders without a silicone shell (Anwander et al. 2007). Expansion of the oral mucosa has been reported in few cases (Zeiter et al. 1998; von See et al. 2010). In a recent rabbit experiment from the present research group, an osmotic soft tissue expander surrounded by a silicone shell was used to create a surplus of periosteum (Abrahamsson et al. 2009).

The aims of the present study in rabbits were (1) to evaluate the space-maintaining capacity of a titanium mesh or a bioresorbable mesh after periosteal expansion and (2) to assess bone formation under a titanium mesh or a bioresorbable mesh on the lateral border of the mandible by qualitative and quantitative histological analysis.

Material and methods

Animals

Thirteen adult female Swedish loop rabbits were used in this study. Their average weight was 4.75 kg (4.2–5.5 kg). Intake of water and solid food was recorded daily and the rabbits weights were monitored. This study was approved by the Malmö/Lund ethical committee for animal research.

Surgical procedures

The rabbits were anaesthetized with ketamin/medetomidin hydrochloride (Ketalar[®] [Pfizer AB, Sollentuna, Sweden]/Domitor[®] [Orion Corporation, Esbo, Finland]) 2.5 + 0.5 mg/kg by an intramuscular injection. To reverse the Domitor[®] effect, Antisedan[®] (Orion Corporation) (atipamezole hydrochloride) was used. Local anaesthesia, 1 ml of Xylocain[®] Dental Adrenalin (Dentsply Ltd, Skarpnäck, Sweden) (lidocaine 20 mg/ml + adrenalin 12.5 µg/ml), was infiltrated over the lateral border of the mandibular body.

The surgery was performed bilaterally under aseptic conditions. The rabbit skin was shaved over the operative sites and a 15 mm skin incision was made over the lower border of the mandible followed by blunt dissection to the periosteum. The periosteum was incised parallel to the lower border of the mandible. A periosteal flap was raised and then elevated towards the alveolar crest. A self-inflatable soft tissue expander (Osmed, Ilmenau, Germany) was placed under the periosteum (for a detailed description of the device see below). The expander was fixed to the lateral border of the mandible with a self-tapping titanium mini screw. The perios-

teum, muscle, fascia and skin were sutured in layers with 4-0 Vicryl.

After 2 weeks of expansion, the rabbits were anaesthetized and prepared again as above. A similar skin-periosteal flap was raised on each side at the lower border of the mandible. The expander was identified and removed. The titanium screw stabilizing the expander was left as a marker after removal of the expanders. On the right side, a pre-cut and shaped titanium mesh (Fig. 1) was fixed to the mandible with two 1.5 mm self-tapping titanium mini screws. The titanium mesh (1/1.5 mm system plate 203 × 127 mm titanium sheet 0.2 mm thick from W. Lorenz, Biomet Microfixation, Jacksonville, FL, USA) was fashioned to make a form 10 mm long and 5 mm high and 5 mm wide to fit under the expanded periosteum. On the left side, a bioresorbable mesh (Lactosorb[®] [Biomet Microfixation, Jacksonville, FL, USA] 1.5 system 100 mm × 100 mm PLLA/PGA), similarly pre-cut and shaped (Fig. 1), was fixed to the mandible with the same type of screws. The meshes were contoured to the final size of the soft tissue expander and had a close adaptation at the periphery to the underlying bone. Bone particles were harvested from the lateral border of the mandible with a bone scraper (Safe scraper[®], Biomet 3i, Warsaw, IN, USA) and packed under the titanium or resorbable meshes. The wounds were closed in layers with 4-0 Vicryl. After 3 months, the animals were sacrificed by the induction of tiopentalnatrium (Pentothal[®], Hospira Enterprises B.V, Almere, the Netherlands) 2.5 g in a 2.5% solution followed by an overdose of pentobarbital 100 mg/ml intravenously.



Fig. 1. Pre-bent bioresorbable mesh and titanium mesh.

Soft tissue expander

The self-inflating osmotic expander consisted of an osmotic active hydrogel, vinyl pyrrolidone and methylmethacrylate, surrounded by a perforated silicone envelope with a flat end for fixation. The initial size of the hydrogel was 2.5 mm wide, 7.5 mm long and 3 mm thick. The expander has the ability to increase up to six times its original volume (Fig. 2).

Sample preparation

The titanium- and bioresorbable meshes, adjacent bone and surrounding tissues were removed en bloc and soaked in 10% buffered formalin. The specimens were then dehydrated in a graded series of ethanol and embedded in methyl methacrylate. A diamond-coated saw band was used to cut sections of the specimens at right angles to the mandibular cortex. After cutting, the sections were ground to a thickness of approximately 10 µm (according to the EXAKT system procedure) to obtain samples of undecalcified bone (Cano-Sanchez et al. 2005). Goldner staining was used (Horn & Garrett 2004). The scanning electron microscopy (SEM) samples were rounded and diamond polished with fine a cloth of 9, 3 and finally 1 µm. After this process, the samples were coated with a gold layer of approximately 20 nm.

Light microscopy and histomorphometry

All sections were examined under a light microscope with an integrated camera (resolution three megapixels) (Leica DMD 108 [Leica Microsystems CMS GmbH, Wetzlar, Germany] designed for research applications). The microscope was connected to a PC with an image analyse program for Leica DMD 108. In this program, both length and freehand areas can be measured. Morphometric measurements were performed on each cross-sectional area to quantify new bone formation under the titanium and the bioresorbable meshes. Measurements were made to assess:

- (1) the total cross-sectional area limited by the mesh and a line projected from the edges of the titanium plate following the cortical layer of the mandible (Fig. 3),
- (2) the cross-sectional area of the newly formed bone,
- (3) the cross-sectional area of the total amount of mineralized bone,

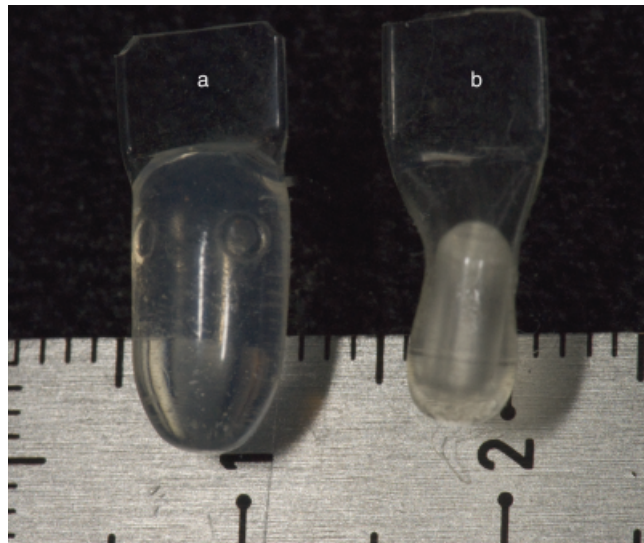


Fig. 2. Osmotic soft tissue expander (a) after expansion (b) before expansion.

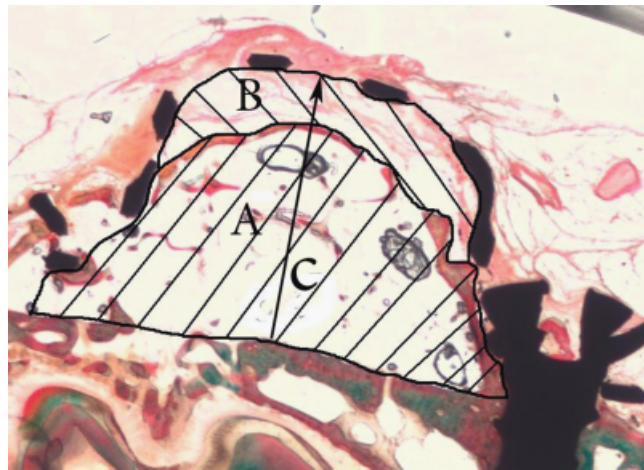


Fig. 3. (A) Marks the total bone area under the mesh and (A)+(B) marks the total mesh area. (C) The width of the mesh. (Goldner, original magnification $\times 4$).

- (4) the width of the meshes perpendicular from the projected line to the highest point of the mesh (Fig. 3).

The mesh barrier and the underlying cortical bone determined the margins for the morphometric evaluation (Fig. 3). Soft tissue was defined as all fibrous tissue in the intratrabecular space and beneath the meshes. The values obtained were expressed in millimetre square. The measurements were repeated three times for each section and expressed as mean values.

SEM examination

The specimens were also examined with SEM (JEOL JXA-8600, Tokyo, Japan). SEM was used to evaluate the newly formed bone interface to the titanium mesh and

bioresorbable mesh. In this process, the intensity response of calcium, phosphor and oxygen was recorded to compare the bone quality in the base of the rabbit mandible and the newly formed bone.

Statistics

Spearman's correlation test was used to test the accuracy between the three measurements. The correlation was significant at $P < 0.01$ (two-tailed). A κ analysis with an acceptance of 1 mm² also showed correlation between the three measurements. All data were analysed for statistical significance using independent sample *T*-test (software SPSS). The statistical significance level was set at $P < 0.05$

Results

Clinical observations

The recovery after surgery was uneventful. Within 24 h, the rabbits resumed normal dietary habits. The average weight gain was 0.31 kg (0.1–0.6 kg) during the total experimental period of 14 weeks. At the removal of the expander after 2 weeks, there were no signs of soft tissue dehiscence and all 26 expanders had successfully expanded the periosteum. New bone formation was observed at the edges of all expanders (Fig. 4).

After a healing period of 12 weeks, wound dehiscence was observed in two of the rabbits in the sites with bioresorbable meshes. No titanium meshes were exposed intraorally.

Histological and histomorphometric evaluation

In general, the surgical sites demonstrated uncomplicated healing. There was no inflammatory cell response except in the two rabbits with exposure of the resorbable meshes, where moderate inflammation could be observed. One of the exposed meshes was completely mobile without new bone formation and this case was excluded from the study.

A typical cross-section of the experimental area comprised bone, soft tissue and the bone-grafted areas with the titanium mesh or bioresorbable mesh. Both new bone formation and bone resorption were seen at the recipient site in contact with the bone graft. The newly formed bone consisted of a new cortical layer under both the titanium mesh and the bioresorbable mesh. This bone consisted of slender mineralized bone and large marrow spaces with a fat-

rich content, as in the body of the rabbit mandible (Fig. 5A). Newly formed bone was seen in close contact with the bioresorbable mesh. There was soft tissue in-growth through the perforations of the meshes, predominantly seen with the titanium meshes (Fig. 5B). New vessels penetrated the cortical bone into the area of the newly generated bone.

Histomorphometric analysis

There was no significant difference between the titanium mesh and the bioresorbable mesh regarding the height of the meshes, mesh area and mineralized bone area (Table 1).

There was a significant ($P < 0.05$) difference in the total bone area under the titanium mesh and the bioresorbable

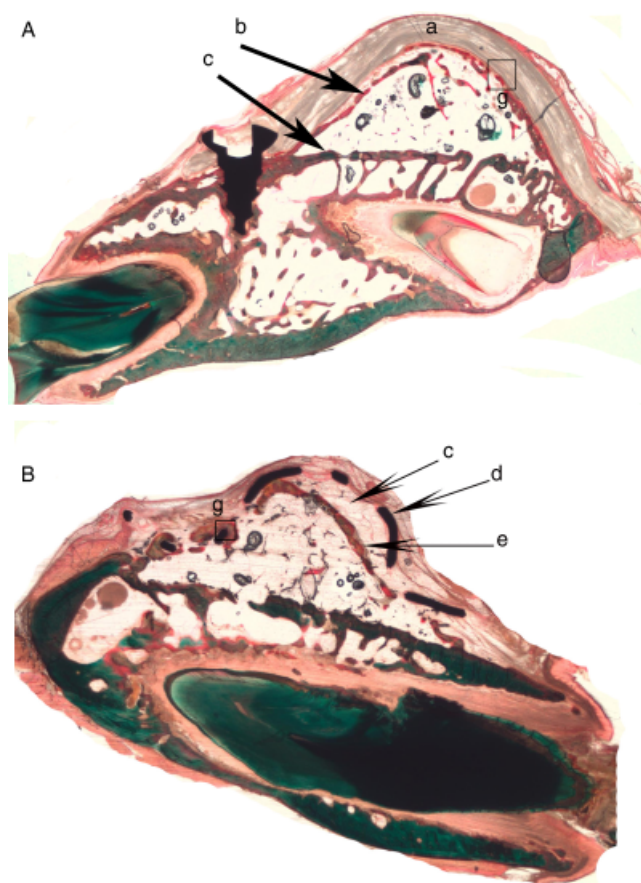


Fig. 5. (A) a, Bioresorbable mesh; b, new cortical bone layer forming in close contact with the mesh; c, old cortical layer; g, box indicating area of interest SEM (Fig. 6A) (Goldner, original magnification $\times 2$); (B) c, soft tissue in-growth; d, titanium mesh; e, new cortical bone layer; g, box indicating area of interest SEM (Fig. 6B) (Goldner, original magnification $\times 2$). SEM, scanning electron microscopy.

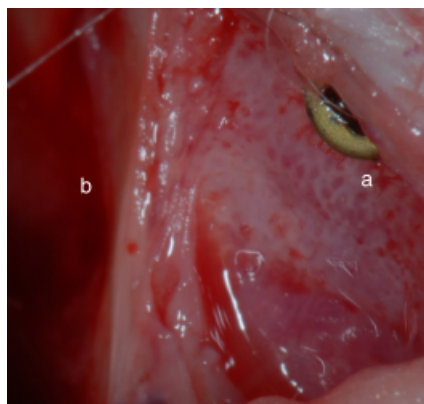


Fig. 4. a, Titanium miniscrew; b, new bone ridge formation after periosteal expansion.

Table 1. Morphometric measurements in ground sections

| Parameter (mean) | Titanium mesh | | Bioresorbable mesh | | Statistic <i>P</i> -value |
|--|---------------|--------|--------------------|--------|---------------------------|
| | n13 | SEM | n12 | SEM | |
| Mesh area (mm ²) | 23.14 | (2.13) | 21.09 | (1.20) | 0.298 |
| Total bone area (mm ²) | 14.78 | (1.28) | 18.35 | (1.92) | 0.165 |
| Mineralized bone area (mm ²) | 2.34 | (0.24) | 2.53 | (0.32) | 0.247 |
| Mesh width (mm) | 4.54 | (0.17) | 5.10 | (0.27) | 0.256 |
| Mineralized bone in the bone area (%) | 16 | (1.2) | 15 | (1.9) | 0.298 |
| Total bone area under the mesh area (%) | 65 | (2.9) | 85 | (6.4) | 0.046 |

SEM, standard error mean.

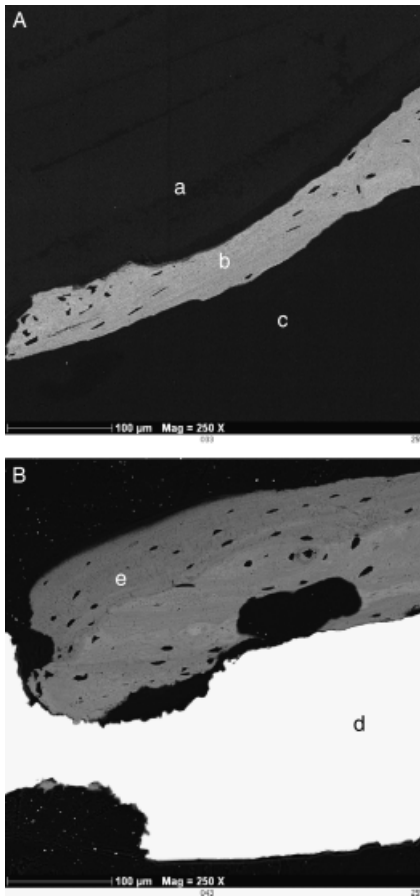


Fig. 6. (A) a, Bioresorbable mesh; b, new cortical bone layer forming in close contact with the mesh; c, soft tissue (SEM, original magnification $\times 250$); (B) d, titanium mesh; e, new cortical bone layer (SEM, original magnification $\times 250$). SEM, scanning electron microscopy.

mesh, 65% and 85%, respectively. The amount of mineralized bone was about the same in both groups, 16% under the titanium mesh and 15% under the bioresorbable mesh.

SEM

New bone is growing in direct contact with the resorbable mesh (Fig. 6A) and the titanium mesh (Fig. 6B). The phosphor and calcium level in the newly formed bone was compared with the old bone in the base of the mandible. Under the meshes in both groups, there was a similar level of mineralization of the newly formed bone compared with the base of the mandible (Fig. 7A and B)

Discussion

The present experimental study evaluated bone graft volume and bone quality in a

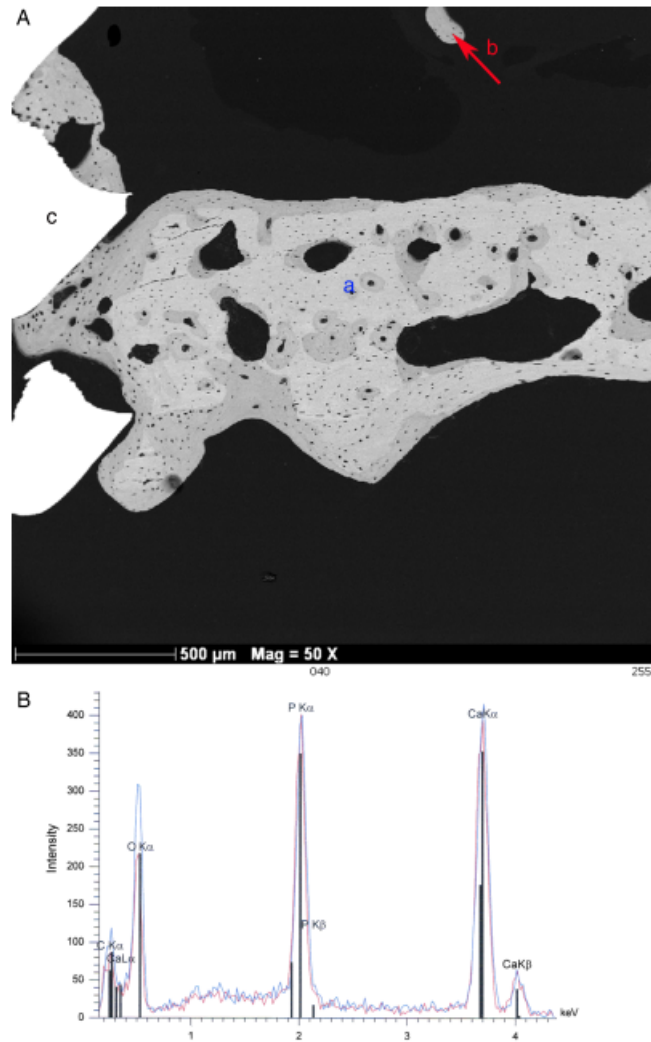


Fig. 7. (A) a, Old cortical bone layer; b, new formed bone island; c, titanium miniscrew (SEM, original magnification $\times 50$). (B) Ca, P and O level in old cortical bone layer (blue line) and in new bone island (red line). Expressed as intensity on the y-scale and keV on the x-scale. SEM, scanning electron microscopy.

created subperiosteal space. An extra oral approach was used because there is a limited working field intraorally in rabbits and thus lack of wound control.

The osmotic self-inflatable expander created a surplus of periosteum and soft tissue in accordance with our previous study (Abrahamsson et al. 2009). The slowly raised periosteum formed new bone ridges peripherally that could be seen clinically at the edges of the expanders after 2 weeks at the second stage operation. This bone formation appeared similar to the type of bone seen after periosteal distraction in minipigs (Kessler et al. 2007).

Space maintenance is one of the most critical factors in the success of bone-regenerating techniques (Lundgren et al. 1998; Stavropoulos et al. 2004). In this

study, a bioresorbable mesh or titanium mesh was used to maintain the created space between the periosteum and the bone. The periosteal expansion facilitates a tension-free closure of the mucoperiosteal flap, thereby avoiding the need to use any periosteal-releasing incisions, which could reduce the blood supply to the bone-grafted area. The periosteum plays an important role in the initial healing process in fractures. Without a periosteum, its unlikely to create an instant or a rapid substantial cortical bone layer (Li et al. 2004). In this rabbit model the newly formed bone consisted of a new cortical layer in accordance with a previous study in dogs (Strong et al. 2003). The underlying original cortical layer showed signs of resorption, and this probably contributes in part to the revascu-

larization of the grafted area through the cortical bone (Kahnberg et al. 1998).

The newly formed bone showed a high fat content as seen in the cancellous bone in the body of the rabbit mandible (Sencimen et al. 2007). This is in contrast to studies where domes have been placed on the rabbit skull and the newly formed bone is more dense with less bone marrow and fat cells (Lundgren et al. 1995; Slotte et al. 2003; Yamada et al. 2008).

Bone islands and the new cortical bone were seen developing in direct contact with both the titanium and the bioresorbable meshes. There was an in-growth of soft tissue through the perforations in both the titanium- and bioresorbable meshes. Our results confirm the observations from an experimental study in rats (Hartman et al. 2004). In this study, the bioresorbable mesh had a better bone fill compared with the titanium mesh, which might be due to the fact that the titanium mesh had more perforations than the resorbable mesh. An occlusive barrier is supposed to reduce bone healing in a grafted area and some perforations are preferable because the periosteum is allowed to play a part in the initial mineralization and remodelling of the grafted area (Elshahat et al. 2005; Zhang et al. 2008). Other studies have used membranes to cover surgically created bone defects to protect the area from soft tissue in-growth (Lundgren et al. 1998; Stavropoulos et al. 2004; Elshahat et al. 2005). A study with the purpose to identify

the optimal pore size of barrier membranes showed that microporus titanium meshes and bioresorbable meshes have more soft tissue in-growth compared with macroporus titanium meshes (Gutta et al. 2009), in contradiction to the results in the present study. One explanation to the different results might be that they used only one titanium fixation screw while we used two, which is more likely to prevent micromovement of the mesh and graft.

Producing bone outside the genetically predetermined skeletal envelope has been carried out in other experimental studies, not intraorally but on the rabbit skull using titanium domes or cylinders (Lundgren et al. 1995; Slotte et al. 2003; Yamada et al. 2008). Lateral ridge augmentation of the rabbit mandible was chosen to mimic the common clinical situation of anterior alveolar bone loss. The meshes in this study had a height of approximately 5 mm and a volume of 330 mm³. This is an adequate size for bone augmentation in humans for a single implant placement. This is also a similar size as used in a study in rabbits and humans (Van Steenberghe et al. 2003).

Two of the bioresorbable meshes were exposed to the intraoral environment. Mesh exposure has been reported in several publications, both with titanium and bioresorbable meshes (von Arx & Kurt 1999; Strong et al. 2003; Van Steenberghe et al. 2003). The technique with periosteal expansion before bone grafting was chosen to minimize the risk of mesh exposure. In

this study, there was no exposure of the titanium meshes. The difference in exposure rates is probably owing to the fact that the titanium mesh is rather easy to pre-bend and form while it is more difficult to form and smooth out the edges of the bioresorbable mesh. The problems in contouring bioresorbable meshes has also been described in other areas such as reconstruction of the orbital floor and rim (Tuncer et al. 2007).

In conclusion, this experimental study shows that the osmotic soft tissue expander creates a surplus of periosteum and soft tissue, which can be used to cover bone grafts. It also confirms that new bone can be generated under a titanium mesh or bioresorbable mesh. There was less bone generated under the titanium mesh but this mesh is easier to form and adapt and there is a lower risk for dehiscence. The periosteum plays an important role in bone healing but does not completely protect the bone-grafted area from soft tissue in-growth. Studies on the possibility to minimize the soft tissue in-growth and to use bone substitutes are in progress.

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