ORIGINAL RESEARCH

International Journal of Surgery 12 (2014) 60–66

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.journal-surgery.net

Original research

Experimental findings on customized mandibular implants in Göttingen minipigs – A pilot study

J. Markwardt [a,*], P. Sembdner [b], R. Lesche [c], R. Jung [d], K. Spekl [d], R. Mai [a], M.C. Schulz [a], B. Reitemeier [e]

[a] Department of Oral and Maxillofacial Surgery, University Hospital "Carl Gustav Carus", Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany
[b] Department of Design Engineering/CAD, Technische Universität Dresden, Mommsenstraße 17, 01069 Dresden, Germany
[c] Firma Hofmann und Engel, Produktentwicklung GmbH, Kunzer Marktweg 13, 01468 Moritzburg, Germany
[d] Experimental Center, Medical Faculty "Carl Gustav Carus", Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany
[e] Department of Prosthetic Dentistry, University Hospital "Carl Gustav Carus", Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany

ARTICLE INFO

Article history:
Received 3 July 2013
Received in revised form
13 October 2013
Accepted 24 October 2013
Available online 2 November 2013

Keywords:
Customized mandibular implant
Mandibular continuity resection
LaserCUSING®
Göttingen mini pigs
Polychrome sequence labelling

ABSTRACT

Reconstructing continuity defects of the mandible is still challenging for surgeons. The currently applied conventional titanium bridging plates have considerable rates of complications. Now, a new technology enables an individual shape-identical creation of a mandibular implant in a form-board design by the method of LaserCUSING® using pure titanium. This technology has been successfully performed in previous examinations to individually reconstruct mandibular continuity defects. This pilot study evaluated the surgical procedure in 10 female Göttingen mini pigs. First, a computed tomography scan from a mini pig cranium was performed. A three-dimensional model of the mandible was designed by data conversion. Based on the data, a customized mandibular implant resembling the natural shape was virtually created and manufactured. Then, a continuity defect of the left mandible was created in a standardized way. The implants were inserted into the defect and the wounds were allowed to heal for 21, 35, 56 and 180 days. During the healing period, no signs of inflammation or infection were observed. After the sacrifice of the minipigs the mandibles were resected. Histological microsections using Donath's sawing and grinding technique were manufactured and stained with Masson Goldner trichrome staining. The histomorphological results showed a pronounced ossification at the outer and inner surface of the implants. This animal study describes a promising approach to optimize customized implants for the application in humans.

1. Introduction

Currently, the reconstruction of continuity defects of the mandible is still challenging for reconstructive surgery. Mainly, continuity defects of the mandible are the result of ablative surgery due to squamous cell carcinomas of the tongue, the floor of the mouth and the mandibular alveolar process.[1,2] Furthermore, the treatment of extended benign lesions and inflammation can cause these defects.[2] The resection of parts of the mandible severely comprises speech, mastication and deglutition.[3] The remaining parts of the mandible are irregularly moved by the masticatory and hyoid bone muscles. Furthermore, the condyles are dislocated from the temporo-mandibular joint socket. Thus, a regular occlusion is not possible. Additionally to the functional limitations, considerable aesthetic impairment is caused by the loss of the anatomical mandibular shape.[3–5] The primary mandibular reconstruction is necessary to improve the function and aesthetic. An immediate reconstruction using autologous bone transplants e.g. from the iliac crest or the fibula is desirable.[6] If an immediate reconstruction with autologous grafts is not possible, the defects are currently bridged by metallic reconstruction plates in order to assure ingestion, speech and patency of the upper airways after surgery. Diverse complications, e.g. plate fracture, loose hardware or plate exposure, have been described using reconstructions plates.[2] By modifying plate shapes and fixture systems, the plate stability could be slightly improved.[7] However, the rate of complications was not reduced significantly.[1,8–11] In order to avoid the above mentioned complications one promising approach seems to be the application of shape identical, functionally stable titanium implants to reconstruct continuity defects of the mandible. For the customized

* Corresponding author. Tel.: +49 351 458 3761; fax: +49 351 458 5348.
E-mail address: jutta.markwardt@uniklinikum-dresden.de (J. Markwardt).

1743-9191/$ – see front matter © 2013 Published by Elsevier Ltd on behalf of Surgical Associates Ltd.
http://dx.doi.org/10.1016/j.ijsu.2013.10.011

reconstruction of the resected mandibular part, a method being able to produce such a highly complex individual shape is required. The LaserCUSING®, a laser sintering technology using pure titanium has demonstrated its ability.[12,13] In a previous cadaver study, continuity defects in a domestic pig mandible have been reconstructed successfully with customized titanium implants produced by LaserCUSING® (data not published). In this cadaver study, the implants consisting of two parts showed a higher stability compared to those consisting of one part (data not published).

The aim of this pilot study was to examine the hypothesis that a stable fixation between the mandibular stumps and the newly developed customized titanium implant consisting of two parts could be achieved by osseointegration. Therefore, the model of a continuity defect in the mandible of Göttingen miniature pigs was used.

2. Methods

2.1. Animal model

The protocol of the study was approved by the Commission for Animal Studies at the District Government Office, Dresden, Germany (file number 24-9168.11-1/2010-25).

The experiments were performed by using 10 adult female Göttingen mini pigs with an average age of 2.5 ± 1 years and a weight from 34.0 to 52.4 kg. The animals were obtained from a certified breeding company (Ellegard Göttingen Minipigs A/S, Dalmose, Denmark). The mini pigs were kept in the Experimental Center of the Medical Faculty and were allowed to adapt to the environment one week prior to surgeries. At the beginning of the study, all animals underwent a physical examination by a veterinarian and were found to be healthy. During the study period, the mini pigs were weighed if abnormalities in food intake were observed. The identification of the animals was enabled by an implanted identification chip. The mini pigs were randomly distributed into 4 groups according to the different healing periods; each group was placed in appropriate boxes with straw bedding. Fresh water was available *ad libitum*. Prior to surgical interventions, the pigs were fed a pellet diet (Special Diet Services, Essex, UK). For the postoperative healing period until sacrifice, the animals were fed mashed bran (Otto Räde Futtermittel, Dresden, Germany).

2.2. Anaesthesia

All interventions were performed in general anaesthesia under surveillance of a veterinarian. Anaesthesia was induced by intramuscular injection of 1 mg per kg body weight midazolam (ratiopharm GmbH, Ulm, Germany) and 10 mg per kg body weight ketamine (Riemser Arzneimittel AG, Greifswald, Germany). To reduce salivation, 0.05 mg per kg body weight atropine (Eifelfango Chem.-Pharm.-Werke, Bad Neuenahr-Ahrweiler, Germany) were administered. Maintenance of the anaesthesia was achieved by administering half of the initial dose intramuscularly. For infection prophylaxis, 15 mg per kg body weight of amoxicillin (Fort Dodge Veterinär GmbH, Würselen, Germany) were injected intramuscularly. The analgesia was performed by administration of carprofen 4 mg per kg body weight (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) subcutaneously.

2.3. Surgical interventions

One week prior to surgery, a computed tomography scan (CT) of the mandible of each animal was performed. Next, a three-dimensional model of the mandible was created. The cutting planes were defined in the region distal of the first premolar and mesial of the third molar in the left mandible. Thus, a fragment of the left mandible containing the second and third premolar as well as the first and second molar was planned to be resected. Based on this model, the templates for the osteotomy and the mandibular implant were created individually for each animal (Fig. 1a). Subsequently, the templates and the customized two part mandibular implant were produced from pure titanium by the manufacturer (Hofmann und Engel Produktentwicklung GmbH, Moritzburg, Germany). In order to produce a shape-identical titanium implant consisting of two parts resembling the removed part of the mandible the LaserCUSING® technique was chosen.

Following the induction of anaesthesia, a transoral incision of the papillary margin from the canine tooth to the third molar was performed. Subsequently, mucoperiostal flaps on the lingual and vestibular site were elevated. After a submandibular cross-section and a submandibular skin incision on the left side, the corpus of the mandible was prepared and exposed. The individually prepared templates were fixed on the mandible. Then, the part of the mandible between the first premolar and the last molar was resected (Fig. 1b). By using a standardized bone bur a bone step was created on both mandibular stump endings (Fig. 2). The customized implant was inserted from the oral and vestibular side. The two parts were fit together and fixed by 6 screws each in the vestibular and lingual cortical bone (Fig. 3). The neck lesion was sutured in layers and a liquid wound dressing was applied.

Finally, four cubes of a nanocrystalline hydroxyapatite (ARTOSS, Rostock, Germany) were inserted into the individual implant via an aperture on the vestibular site. The remaining space was filled with a granulate of the nanocrystalline hydroxyapatite (ARTOSS,

a)

b)

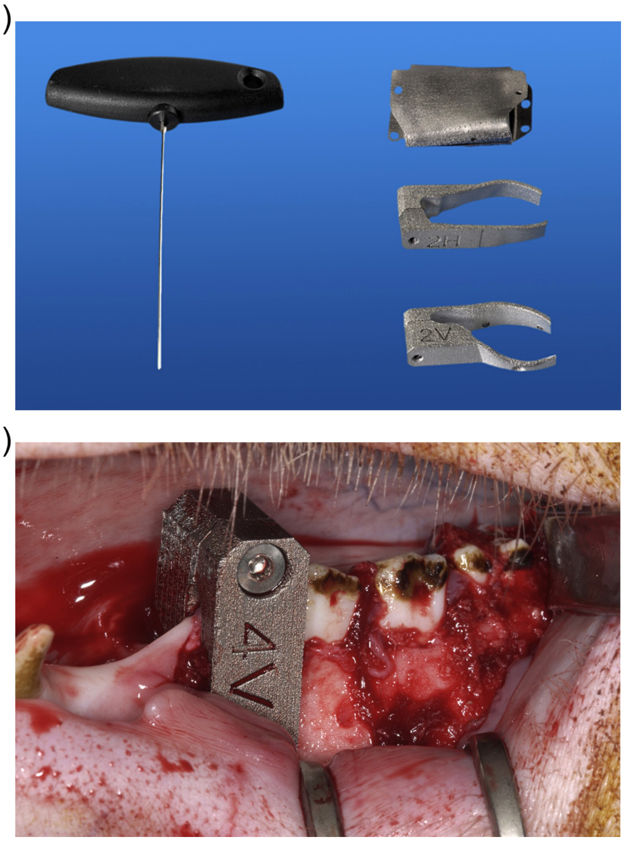

Fig. 1. Templates and instruments necessary to determine the resections planes. a) Individual mandibular implant (right at the top) with templates for both resection edges (right middle and bottom) and screwdriver (left). b) Application of the resection template *in situ*.

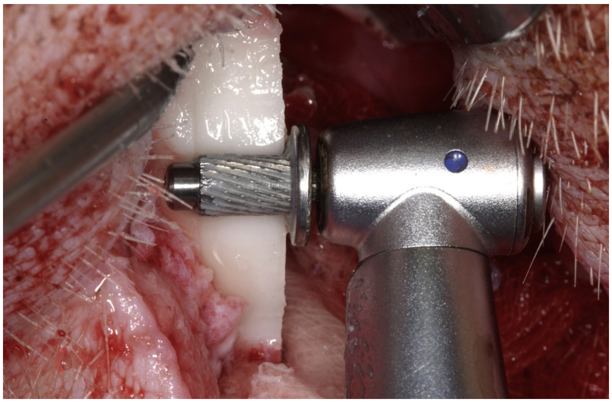

Fig. 2. Preparation of the step at the distal mandibular stump. A special bone bur with step arrester for the preparation of the bone implant interface (Fa. Busch, Engelskirchen, Germany, CE 0124, WST mandibular implant bur 21 × L06) is used.

Rostock, Germany) mixed with the pig's own blood in order to stimulate bone ingrowths into the implant.[14,15] The gingiva was re-adapted and fixed by a double suture above the implant to avoid a potential fistula between the extraoral and intraoral wounds.

To evaluate the dynamics of the bone healing, fluorescence labelling was performed. Therefore, different fluorescence dyes were administered intravenously at different points of time. Tetracyclin (Fluka Chemie, Neu-Ulm, Germany) was administered on the day of surgery (12 mg/kg body weight). Calcein blue (Fluka Chemie, Neu-Ulm, Germany) was applied two weeks post-operatively and alizarine complexone (Fluka Chemie, Neu-Ulm, Germany) three weeks postoperatively, both at a dosage of 30 mg/kg body weight. Therefore, a short anaesthesia as described previously was required. During this time, the implants were examined regarding their clinical stability and signs of inflammation e.g. suppuration, swelling or exposure of the implant.

According to the study protocol, the animals were sacrificed after healing times of 21 days (2 animals), 35 days (2 animals), 56 days (2 animals) and 180 day (4 animals) by an overdose of embutramid, mebenzonium iodide and tetracaine (Veterinaria AG, Pfäffikon, Switzerland). A CT scan of the pig‘s cranium was created in order to evaluate the interface between the mandibular stumps and the implant, including the implant position.

The mandibles were dissected and fixed in 4% formaldehyde for 48 h. After dehydration in a graded series of alcohol, the samples were embedded in methylmethacrylate (Heraeus Kulzer, Wehrheim, Germany). Cross sections of the mandible–implant interface with a thickness of 100 μm were produced using Donath's sawing and grinding technique.[16] After evaluation of the fluorescence labelling, sections were ground to 40 μm and polished. The samples were stained with Masson Goldner trichrome staining. Subsequently, the specimens were analysed using light microscopy (Olympus BX 61, Olympus Deutschland GmbH, Hamburg, Germany).

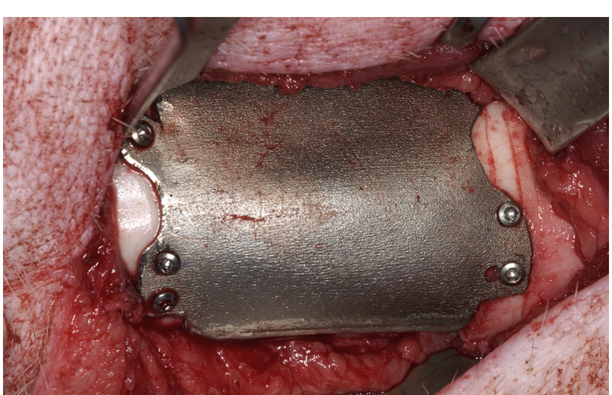

Fig. 3. Customized mandibular implant *in situ* shown from a vestibular view. The implant is distally fixed with three mono-cortical screws and mesially with two mono-cortical screws.

3. Results

3.1. Clinical follow up

The postoperative wound healing showed no abnormalities. Signs of inflammation or infection could not be observed. After a few days without analgesia the food ingestion of the animals was similar to the preoperative period. No loss of body weight could be observed during the healing times. All animals completed the study and were available for evaluation.

3.2. Radiological results

The radiological evaluation focused on the localization of the implant with regard to the mandibular defect. The CT scans of the animals sacrificed after 21 day showed a correct position at the anterior bone implant interface (Fig. 4a). In the distal contact area a slight vestibular dislocation was obvious. Furthermore, a cortical fracture at the distal step could be found in one animal. Inside, the bone substitute blocks were visible. After 35 days, the implants in both animals were found to be in a correct position regarding the anterior and posterior stump. The bone substitute blocks could be differentiated from soft tissue inside the implant (Fig. 4b). The CT scans after 56 days showed a dislocation of the whole mandible in one animal. A caudal bone bridge between the stumps could be observed in one animal. The bone substitute blocks were not clearly distinguishable from the soft tissue (Fig. 4c). After 180 days, in two animals the implants were dislocated vestibularly. A caudal bone bridge could be observed in these animals (Fig. 4d). The bone substitute was hardly distinguishable from the soft tissue. In the two remaining animal the implants were not found to be *in situ*. Here, a compact bone bridge between the stumps was present (Fig. 4e).

3.3. Gross examination of the explanted mandibles

The details of the macroscopic findings are presented in Table 1. After 21 days, the bone implant interfaces were not found to be stable and screws were lost. No signs of inflammation could be observed in the peri-implant soft tissue. Examination after 35 days showed a stable bone implant interface in both animals. Only one screw was lost in one animal. The peri-implant soft tissue showed no signs of inflammation. Regarding the animals sacrificed after 56 days, a bone formation at the inferior margin of the implant could be observed. In one animal, a caudal bone bridge was obvious. The bone implant connection was found to be stable. After 180 days, two implants were stable *in situ*. A caudal bone bridge could be found. In two animals, the implants were lost. Here, a compact stable bone bridge was present.

3.4. Histological results

After 21 days, resorptions at the bone implant interface were visible. Some of the screws were not found to be *in situ* and the remaining screws were covered by soft tissue. Inside the implants, no new bone formation was detectable. The blocks of bone substitute were clearly distinguishable from the adjacent soft tissue (Fig. 5a). Histological results after 35 days showed signs of resorptions at the bone implant interface, likewise. In the posterior

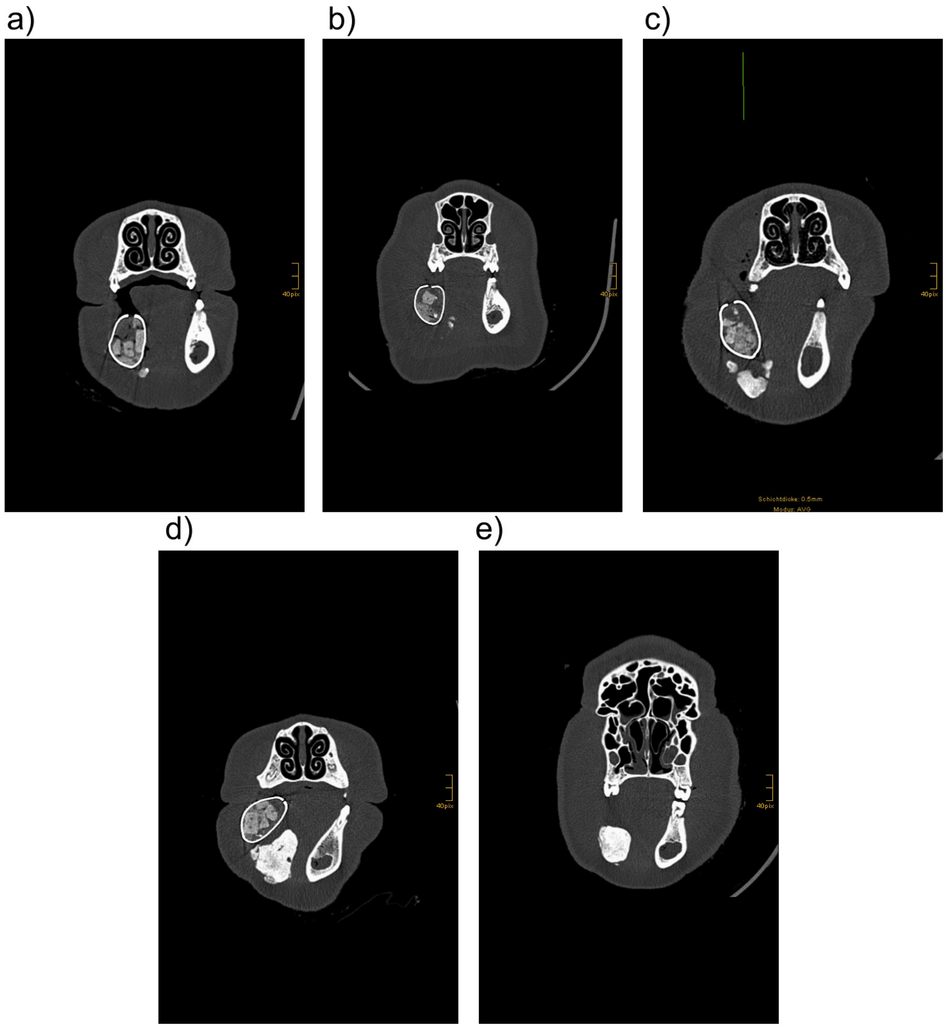

Fig. 4. CT scans showing the coronal planes of the pigs' head created at the time of sacrifice. a) 21 days: The implant is located in a correct position regarding the position of the contra lateral part of the mandible. The bone substitute blocks inside the implant are clearly visible. b) 35 days: The implant is located in a correct position regarding the position of the contra lateral part of the mandible. The bone substitute blocks inside the implant are clearly visible. c) 56 days: The mandible is laterally dislocated (to the right). The bone bridge caudal of the implant is clearly visible. Soft tissue is dividing the implant and the caudal bone bridge. d) 180 days: The implant is dislocated to the vestibular direction. A distinctive bone bridge is visible caudal of the implant. e) 180 days: A compact bone bridge between is visible replacing the right part of the mandible. At this time, the implant is not *in situ* anymore.

connection area one screw each was lost, respectively. The bone substitute blocks showed no signs of resorption (Fig. 5b). New bone formation started from the mandibular stumps. After 56 days of healing, no signs of loosened screws were observed. A tight contact was obvious at the bone implant interface. The bone formation was proceeding in the direction of the implant center. The blocks of bone substitute showed a roughened surface indicating resorptions processes. Around the inferior margin of one implant bone

Table 1
Summary of the results of the macroscopic examination. The details for each animal are presented after the corresponding healing period. N/A — not applicable.

Healing period in days	Animal	Bone implant interface	Implant position		Loss of screws		Peri-implant soft tissue	Comments
			Anterior	Posterior	Anterior	Posterior		
21	1	Not stable	Correct	Vestibular	1/3	0/3	No inflammation	–
	2	Not stable	Correct	Cranial	1/2	1/3	No inflammation	–
35	3	Stable	Correct	Correct	0/3	1/3	No inflammation	–
	4	Stable	Correct	Correct	0/2	1/3	No inflammation	–
56	5	Stable	Lateral dislocation of the mandible	1/2	3/3	No inflammation	Caudal bone bridge	
	6	Stable	Correct	Correct	0/3	0/3	No inflammation	Bone formation vestibular of the implant
180	7	Stable	Correct	Lateral dislocation of the stump	1/3	3/3	No inflammation	Caudal bone bridge
	8	Stable	Vestibular	Vestibular	2/3	3/3	No inflammation	Caudal bone bridge
	9	N/A	N/A	N/A	N/A	N/A	N/A	Compact bone bridge
	10	N/A	N/A	N/A	N/A	N/A	N/A	Compact bone bridge

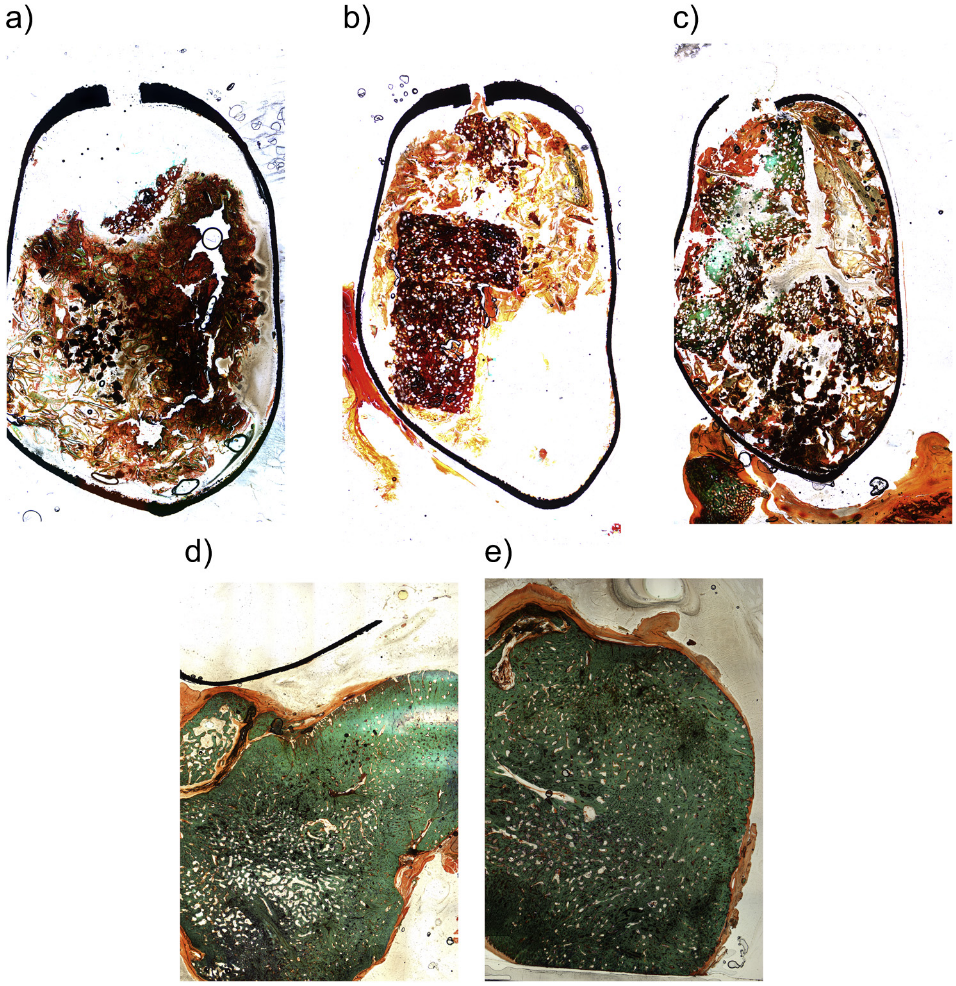

Fig. 5. Exemplary histological images showing the middle sections of the implants. a) 21 days: Inside the implant, no bone is visible at this time point. The blocks of bone substitute are clearly distinguishable from the soft tissue. b) 35 days: The blocks of bone substitute show no signs of resorption at this time point. c) 56 days: At the surface of the bone substitute blocks lacunae are obvious indicating resorption areas. At the inferior left border of the implant a spongious bone bridge is obvious. d) 180 days: Bone bridge at the inferior margin of the implant. The bone structure resembles the mandible bone consisting of a cortical layer and a spongious center part. Implant and bone are divided by a layer of fibrous tissue. e) 180 days: Compact bone bridge covered by a layer of soft tissue. The implant is not *in situ* (magnification 2 × 10; Masson Goldner staining).

formation was visible outside the implant. Caudal of the other implant a spongious bone bridge connecting both mandibular stumps could be found (Fig. 5c). In histological sections after 180 days of healing a compact bone bridge between both stumps consisting of a cortical layer and a spongious center could be observed. Compared to 56 days the bone bridge was more extended. The implants showed a lateral dislocation. A layer of fibrous tissue was dividing the implant from the bone bridge (Fig. 5d). In two animals, the implants were not *in situ* after 180 days. The bone bridge was covered by a layer of fibrous tissue (Fig. 5e).

4. Discussion

In the present study, the healing properties of a customized two part mandibular implant reconstructing continuity defects were examined in a mini pig model. Therefore, the implants were evaluated after different healing times.

For rehabilitation of continuity defects of the mandible autologous grafts are recommended.[6,17] If an autologous rehabilitation is not possible defect reconstruction can be achieved by using reconstruction plates.[18] By using bridging or reconstruction plates complications like plate fractures, screw loosening and extra- or intraoral exposure of the plates are described frequently.[1,2] Furthermore, the applied reconstructions plates are not shape identical. This might lead to a dead space between the plate and the covering soft tissue. The mentioned lack of tissue might cause a higher tension of the soft tissue increasing the risk of plate exposure.[16] The customized implant resembling the shape of the mandible might be able to reduce the risk of plate exposure. Furthermore, computer assisted reconstruction in the oral and maxillofacial area have suggested to increase the accuracy.[19] In the present study, the customized implant showed a sufficient stability under load bearing conditions. No fractures of the titanium implant could be observed within the study period. This finding is in line with the results of a cadaver study performed by our group where mandibular implants consisting of two pieces showed a higher fracture stability compared to those consisting of one piece (own data not published). The loosening of screws was observed in the present study. One reason for this might be the mobility of the mini pig mandibles during the healing period. While patients could be immobilized after surgery and fed with liquid nutrition via a nasogastric tube this is not possible in pigs. The pigs would try to remove the tube which includes the risk of injuries. Thus, in our study the mini pig were fed mashed bran. This was one limit of the study. However, the mini pig seems to be a suitable model for studying osseointegration as far as no computed or *in vitro* setting is able to simulate the complex healing process under loaded conditions. It has been successfully used in different animal studies examining the osseointegration of dental titanium implants.[20,21] The bone turnover rate of miniature pigs is similar to that in humans.[22–24] Furthermore, pigs are omnivores, likewise. Thus, the masticatory loading in the present study was comparable to the situation in humans.

The clinical and histological findings of the implants showed no signs of inflammation or foreign body reaction. Titanium is considered to be a material with the best biocompatibility and corrosion resistance.[25] The current results are supported by an *in vitro* study where primary human osteoblasts were cultivated on different titanium surfaces.[26] The findings of this *in vitro* study suggest that the initial adhesion and colonization was enhanced on untreated titanium surfaces produced by LaserCUSING®.[26] These results support the applicability of the material in organisms. Furthermore, its elasticity modulus is similar to that of bone. In addition with the applied layer strength of 0.3 mm it seems to be an optimal material to reconstruct maxillofacial bone defects. In the current study, the plates used are shape-identical resembling the removed part of the mandible. To fix these shape-identical implants in the technical most practicable way the implant was divided in two parts. Thus, it was possible to insert one part from the vestibular and the other part from the lingual side. In this way, the implant could be fixed on the mandibular stumps in a mode of form boards.

Furthermore, the implant might be filled with a bone substitute and differentiated cells to promote bone formation inside the implant.[27] In an animal study defects filled with beta-TCP and bone marrow stromal cells were reunited after 32 weeks.[28] In the present study, bone formation inside the implant was observed from the mandibular stumps. The bone substitute itself served as a space holder. A bony reunion was not achieved inside the implant during the study period. In animals sacrificed after 180 days a bony reunion forming a bone bridge at the inferior margin of the implant was observed. The implant seems to serve as an osteoconductive surface. This is in agreement with results of an *in vitro* study where the surface produced by LaserCUSING® was fostering the adhesion and colonization of human osteoblasts.[26] These findings would be unlikely in human beings. Furthermore, it has to be considered that the potential spontaneous regenerative osteogenesis also verifiably depends on the age of the animals besides the physical constellation.[29,30]

Postoperatively, all implants showed a primary stability. Due to the remodelling process in the bone, particularly at the bone implant interface primary stability decreased postoperatively until 35 days of healing. The lowest primary stability was observed after 21 days according to our clinical examinations. An increasing stability of the implants was obvious after 56 days. This agrees to findings of a study examining dental implants. The lowest stability was reached after approximately 30 days; then an increasing stability was observed.[31] During this phase, secondary stability increased due to ingrowths of new bone and primary stability decreased proportionately. After six months, a bone bridge connected both mandibular stumps. In two animals the implants were found to be *in situ*, in two animals the implants were removed. In comparison to a study examining primary and secondary stability no differences regarding the healing periods were found.[32] The primary stability of the customized mandibular implant decreased during the first three postoperative weeks and achieved its lowest stability between the fourth and fifth week. The secondary stability increased progressively after the fifth week. These findings are in line with other studies.[31,32]

Summarizing, this pilot animal study suggests promising results considering the optimization of customized implants for the application in humans. Within the limits of this study, it could be suggested that the examined implants offer a sufficient stability to connect the mandibular stumps for bridging mandibular defects under loaded conditions in Göttingen mini pigs.

Ethical approval

The protocol of the study was approved by the Commission for Animal Studies at the District Government Office, Dresden, Germany (file number 24-9168.11-1/2010-25).

Funding

This interdisciplinary study was funded by the European Union Grant 13423 and the Free State of Saxony Grant 2273.

Author contribution

Jutta Markwardt – surgical interventions, editing the manuscript.

Philipp Sembdner – virtually creation of customized implants, editing the manuscript.

Raoul Lesche – construction of customized implants, editing the manuscript.

Roland Jung – animal care, maintenance during surgical interventions, editing the manuscript.

Kathrin Spekl – animal care, maintenance during surgical interventions, editing the manuscript.

Ronald Mai – clinical evaluation, editing the manuscript.

Matthias Schulz – histological evaluation, editing the manuscript.

Bernd Reitemeier – planning the experiment, assistance in surgical interventions, editing the manuscript.

Conflict of interest statement

Jutta Markwardt, Philipp Sembdner, Roland Jung, Kathrin Spekl, Ronald Mai, Matthias C. Schulz and Bernd Reitemeier are employees of the Technical University Dresden (Technische Universität Dresden). Raoul Lesche is employed by Hofmann und Engel, Produktentwicklung Moritzburg, Germany. The authors declare that they have no conflict of interest.

Acknowledgements

This interdisciplinary study was funded by the European Union Grant 13423 and the Free State of Saxony Grant 2273.

References

1. Markwardt J, Pfeifer G, Eckelt U, et al. Analysis of complications after reconstruction of bone defects involving complete mandibular resection using finite element modelling. *Onkologie* 2007;**30**:121–6.
2. Coletti DP, Ord R, Liu X. Mandibular reconstruction and second generation locking reconstruction plates: outcome of 110 patients. *Int J Oral Maxillofac Surg* 2009;**38**:960–3.
3. Goh BT, Lee S, Tideman H, et al. Mandibular reconstruction in adults: a review. *Int J Oral Maxillofac Surg* 2008;**37**:597–605.
4. Spiessl B. *Internal fixation of the mandible – a manual of AO/ASIF principles*. Berlin: Springer; 1989.
5. Markwardt J, Jahn T, Schöne C, et al. Methodische Grundlagen individueller Unterkieferrekonstruktionen. In: Ofner H, Brom J, editors. *Psychosoziale Auswirkungen bei Gesichtsdefekten. Methodische Grundlagen individueller Unterkieferrekonstruktionen*. Linz: Univ.-Verlag; 2012. p. 5–10.
6. Bak M, Jacobson AS, Buchbinder D, et al. Contemporary reconstruction of the mandible. *Oral Oncol* 2010;**46**:71–6.
7. Herford AS, Ellis 3 E. Use of a locking reconstruction bone plate/screw system for mandibular surgery. *J Oral Maxillofac Surg* 1998;**56**:1261–5.
8. Hayter JP, Cawood JI. The functional case for miniplates in maxillofacial surgery. *Int J Oral Maxillofac Surg* 1993;**22**:91–6.
9. Spencer KR, Sizeland A, Taylor GI, et al. The use of titanium mandibular reconstruction plates in patients with oral cancer. *Int J Oral Maxillofac Surg* 1999;**28**:288–90.
10. Lindqvist C, Soderholm AL, Salo A, et al. A comparative study on four screw-plate locking systems in sheep: a clinical and radiological study. *Int J Oral Maxillofac Surg* 2001;**30**:160–6.

11. van Minnen B, Nauta JM, Vermey A, et al. Long-term functional outcome of mandibular reconstruction with stainless steel AO reconstruction plates. *Br J Oral Maxillofac Surg* 2002;**40**:144–8.
12. Gebhardt A. *Rapid prototyping*. München: Hanser; 2003.
13. Schöne P, Stelzer R, Sembdner R, et al. Individual contour adapted functional implant structures in titanium. In: Bartolo A, editor. *Innovative developments in virtual and physical prototyping*. London: Taylor & Francis Group; 2012. p. 29–34.
14. Maurer P, Holweg S, Knoll W, et al. Study by finite element method of the mechanical stress of selected biodegradable osteosynthesis screws in sagittal ramus osteotomy. *Br J Oral Maxillofac Surg* 2002;**40**:76–83.
15. Xu W, Ganz C, Weber U, et al. Evaluation of injectable silica-embedded nanohydroxyapatite bone substitute in a rat tibia defect model. *Int J Nanomedicine* 2011;**6**:1543–52.
16. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage-Schliff (sawing and grinding) technique. *J Oral Pathol* 1982;**11**:318–26.
17. Cordeiro PG, Disa JJ, Hidalgo DA, et al. Reconstruction of the mandible with osseous free flaps: a 10-year experience with 150 consecutive patients. *Plast Reconstr Surg* 1999;**104**:1314–20.
18. Ettl T, Driemel O, Dresp BV, et al. Feasibility of alloplastic mandibular reconstruction in patients following removal of oral squamous cell carcinoma. *J Craniomaxillofac Surg* 2010;**38**:350–4.
19. Essig H, Rana M, Kokemueller H, et al. Pre-operative planning for mandibular reconstruction – a full digital planning workflow resulting in a patient specific reconstruction. *Head Neck Oncol* 2011;**3**:45.
20. Stadlinger B, Pilling E, Huhle M, et al. Suitability of differently designed matrix-based implant surface coatings: an animal study on bone formation. *J Biomed Mater Res A* 2008;**87**:516–24.
21. Stadlinger B, Hintze V, Bierbaum S, et al. Biological functionalization of dental implants with collagen and glycosaminoglycans-A comparative study. *J Biomed Mater Res* 2011.
22. Hönig JF, Merten HA. Das Göttinger Miniaturschwein (GMS) als Versuchstier in der human-medizinischen osteologischen Grundlagenforschung. *Z Zahnärztl Implantol* 1993;**9**:244–54.
23. Hönig JF, Merten HA, Wiltfang J, et al. Der Stellenwert des Göttinger Miniaturschweins in der craniofacialen osteologischen Grundlagenforschung. *Osteologie* 1997:125–33.
24. Murison PJ, Jones A, Mitchard L, et al. Development of perioperative care for pigs undergoing laryngeal transplantation: a case series. *Lab Anim* 2009;**43**: 338–43.
25. Neumann A, Kevenhoerster K. Biomaterials for craniofacial reconstruction. *GMS Curr Top Otorhinolaryngol Head Neck Surg* 2009;**8**. Doc08.
26. Markwardt J, Friedrichs J, Werner C, et al. Experimental study on the behavior of primary human osteoblasts on laser-cused pure titanium surfaces. *J Biomed Mater Res A* 2013 Jun 14. http://dx.doi.org/10.1002/jbm.a.34824. [Epub ahead of print].
27. Cancedda R, Giannoni P, Mastrogiacomo M. A tissue engineering approach to bone repair in large animal models and in clinical practice. *Biomaterials* 2007;**28**:4240–50.
28. Yuan J, Cui L, Zhang WJ, et al. Repair of canine mandibular bone defects with bone marrow stromal cells and porous beta-tricalcium phosphate. *Biomaterials* 2007;**28**:1005–13.
29. Owen M. Cellular dynamics of bone. In: Bourne H, editor. *Biochemistry and physiology of bone*. 2nd ed. New York: Academic Press; 1971. p. 271–98.
30. Soost F. *Validierung des Knochenumbaus von Knochenersatzmaterialien in der Mund-, Kiefer- und Gesichtschirurgie*. Berlin; 2001.
31. Rokn A, Ghahroudi AR, Mesgarzadeh A, et al. Evaluation of stability changes in tapered and parallel wall implants: a human clinical trial. *J Dent (Tehran)* 2011;**8**:186–200.
32. Gotz W, Lenz S, Reichert C, et al. A preliminary study in osteoinduction by a nano-crystalline hydroxyapatite in the mini pig. *Folia Histochem Cytobiol* 2010;**48**:589–96.