



Growth and transplantation of a custom vascularised bone graft in a man

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P H Warnke, I N G Springer, J Wiltfang, Y Acil, H Eufinger, M Wehmöller, P A J Russo, H Bolte, E Sherry, E Behrens, H Terheyden

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Department of Oral and Maxillofacial Surgery, University of Kiel, Arnold-Heller-Str 16, 24105 Kiel, Germany (P H Warnke MD, I N G Springer MD, Prof J Wiltfang MD, Prof Y Acil PhD, E Behrens DMD, Prof H Terheyden MD); Department of Oral and Maxillofacial Surgery, Knappschafts Krankenhaus Recklinghausen, Recklinghausen, Germany (Prof H Eufinger MD, M Wehmöller PhD); Department of Rheumatology, Australian National University, Canberra, Australia (P A J Russo MD); Department of Diagnostic Radiology, University of Kiel, Kiel, Germany (H Bolte MD); and Department of Orthopaedic Surgery, University of Sydney, Penrith, Australia (E Sherry MD)

Correspondence to: Dr Patrick H Warnke warnke@mkg.uni-kiel.de

Summary

Background A major goal of research in bone transplantation is the ability to avoid creation of secondary bone defects. We aimed to repair an extended mandibular discontinuity defect by growth of a custom bone transplant inside the latissimus dorsi muscle of an adult male patient.

Methods Three-dimensional computed tomography (CT) scanning and computer-aided design techniques were used to produce an ideal virtual replacement for the mandibular defect. These data were used to create a titanium mesh cage that was filled with bone mineral blocks and infiltrated with 7 mg recombinant human bone morphogenetic protein 7 and 20 mL of the patient's bone marrow. Thus prepared, the transplant was implanted into the latissimus dorsi muscle and 7 weeks later transplanted as a free bone-muscle flap to repair the mandibular defect.

Findings In-vivo skeletal scintigraphy showed bone remodelling and mineralisation inside the mandibular transplant both before and after transplantation. CT provided radiological evidence of new bone formation. Postoperatively, the patient had an improved degree of mastication and was satisfied with the aesthetic outcome of the procedure.

Interpretation Heterotopic bone induction to form a mandibular replacement inside the latissimus dorsi muscle in a human being is possible. This technique allows for a lower operative burden compared with conventional techniques by avoiding creation of a secondary bone defect. It also provides a good three-dimensional outcome.

Introduction

Ever since the Vacanti research group revealed the mouse with a human ear on its back in 1997,¹ world-wide interest in tissue engineering and prefabrication techniques has grown. This technology might eventually allow us to produce substitute organs or body parts inside human bodies.^{2,3} Success could make the search for organ donors, and the well-described difficulties associated with allogenic organ transplants, redundant.

Research by us has focused on new approaches to find replacements for bone defects, especially for important size defects of the mandible.^{4–7} Today, a mandible with a major discontinuity defect of more than 5 cm can be repaired with an autologous vascularised fibula, scapula, iliac crest, or rib transplant, which is sometimes necessary after ablative tumour surgery. These bone transplantation techniques are clinically approved and are being successfully undertaken in cancer surgery centres worldwide. However, a major disadvantage of this technique is that the process of harvesting these bone grafts always creates another skeletal defect, which itself is associated with serious morbidity. In 2001, we showed a flap prefabrication technique in a minipig-model^{4–6} that circumvented the need for creation of a second skeletal defect through bone harvesting.

BMP7 is an osteoinductive factor that initiates conversion of undifferentiated precursor stem cells into

osteoprogenitor cells, which produce mature bone.^{5–9} With Therapeutic Goods Administration approval (Department of Health and Ageing, Australia) of recombinant human BMP7 for human use in 2001,^{9,10} prefabrication of bone grafts for reconstruction after tumour surgery has become a possibility.

A 56-year-old man, who had received ablative tumour surgery 8 years previously in the form of a subtotal mandibulectomy, asked us to reconstruct his mandible, which had been resected from paramedian left region to the retromolar right region. This important size defect of more than 7 cm had been bridged with a titanium reconstruction plate since initial surgery (figure 1). His head and neck region had been further compromised by radiation treatment given at the time (total dose 66 Gy). Because he had been given warfarin for an aortic valve replacement we had to keep bony defects to a minimum to avoid major postoperative bleeding. To prevent creation of a donor-site bone defect, the patient was selected for the bone-muscle-flap prefabrication technique. The aim was to grow a subtotal replacement mandible inside the latissimus muscle with full bony continuity and an adequate vessel pedicle to allow for subsequent transplantation of a viable graft into the defect. Furthermore, we aimed to ensure that the replacement should be individually shaped to fit the defect perfectly, thus improving the chances of adequate postoperative function and a satisfactory aesthetic result.

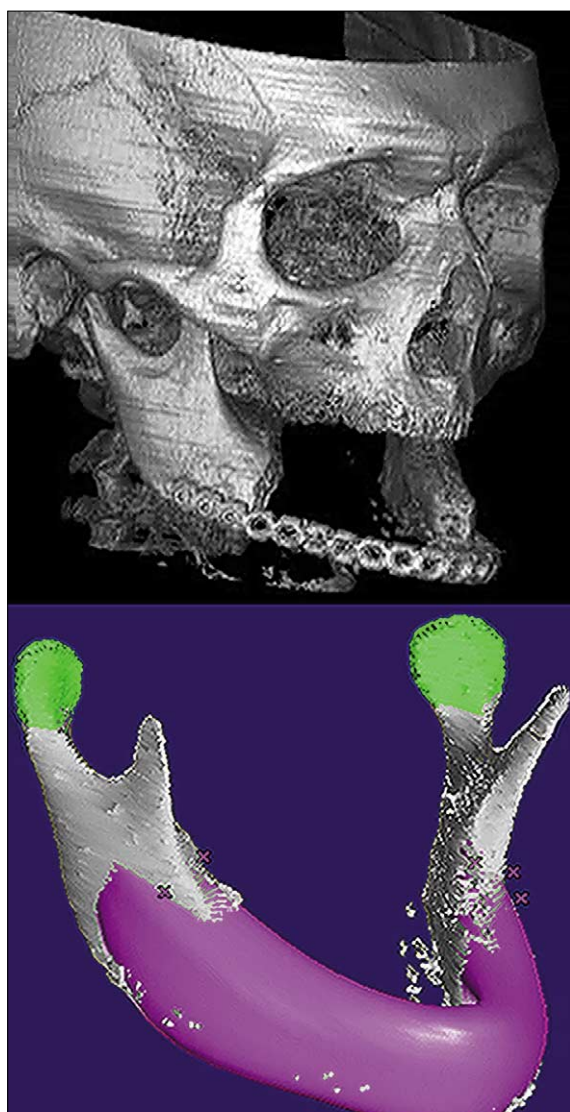


Figure 1: Three-dimensional CT scan of size defect (upper) and CAD plan of ideal mandibular transplant (lower)

Methods

We obtained ethics approval from the University of Kiel, Germany. The patient gave written consent. We did three-dimensional computed tomography (CT) of the patient's head and designed an ideal virtual replacement of the missing parts of the mandible with computer-aided design (CAD; figure 1). Data were directed to a CAD-operated three-axes milling machine, and a teflon model was created that matched the virtual mandible exactly (webfigure 1; <http://image.thelancet.com/extras/04art7155webfigure1.pdf>). A titanium mesh scaffold (MARTIN, Micromesh, Tuttlingen, Germany) was then formed onto the model, which was subsequently removed and the remaining titanium mesh cage was filled with ten bone mineral blocks as carriers (BioOss-Blocks; Geistlich Biomaterials, Wolhusen, Switzerland), which were coated

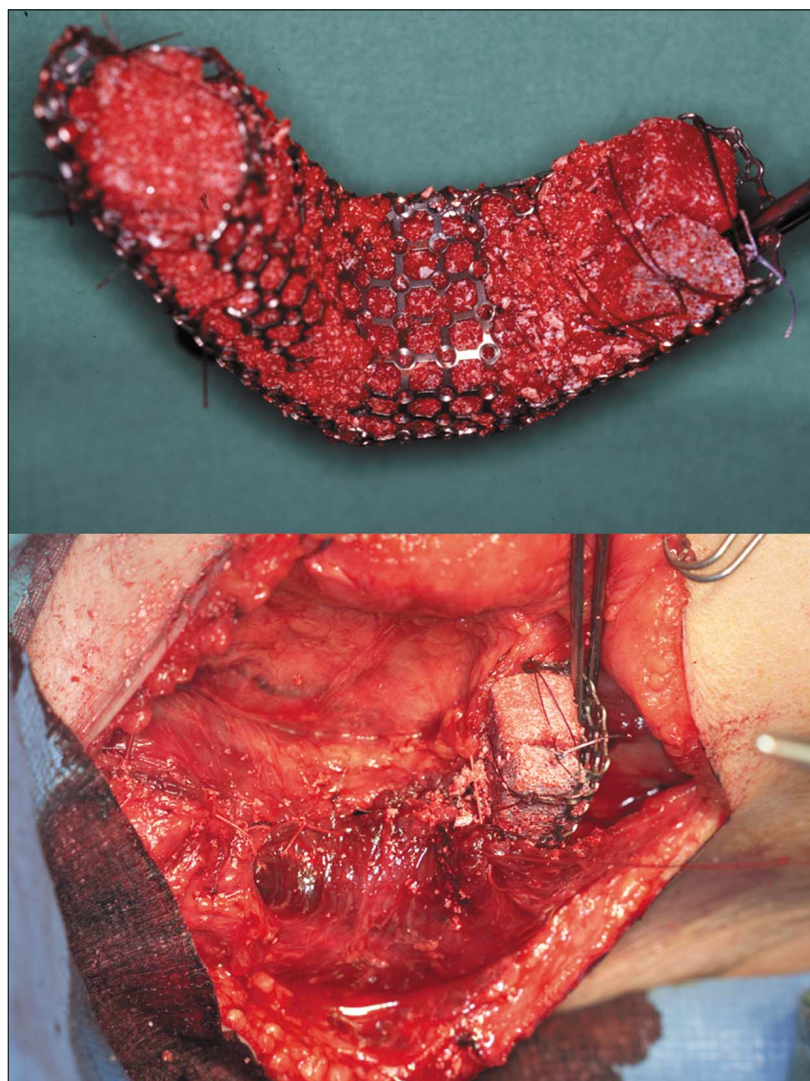


Figure 2: Titanium mesh cage filled with bone mineral blocks infiltrated with recombinant human BMP7 and bone-marrow mixture (upper) and implantation into right latissimus dorsi muscle (lower)

with 7 mg recombinant human BMP7 embedded in 1 g bovine collagen type 1 (OP-1 implant, Stryker Biotech, Hopkinton, USA; figure 2). Finally, 20 mL bone marrow was aspirated from the right iliac crest to provide undifferentiated precursor cells as a target for recombinant human BMP7. We did not undertake flow cytometry to ascertain the presence of stem cells in the aspirate. Bone marrow was mixed with 5 g natural bone mineral of bovine origin (particle size 0.5–1.0 mm; BioOss-Spongiosa granules; Geistlich Biomaterials) and this mixture was used to fill the gaps between the blocks inside the cage. The titanium mesh cage was then implanted into a pouch of the patient's right latissimus dorsi muscle (figures 2 and 3) under general anaesthesia. He developed a haematoma postoperatively that was easily drained on the second postoperative day. We gave prophylactic antibiotics, with 1.5 g ampicillin/sulbactam



Figure 3: Dorsal view of mandibular replacement 3 weeks after implantation
Arrows show area of implantation inside latissimus dorsi muscle.

three times a day for 14 days. We saw no signs of infection. The patient did not complain of pain or sleep disturbance and relayed only minor concerns about the range of motion of his right arm.

Results

4 weeks postoperatively, we did skeletal scintigraphy by intravenous injection of 600 MBq technetium-99m-oxydronate tracer. Bone remodelling with vital osteoblasts was detected inside the implant, verified by a tracer enhancement, which was the first sign of

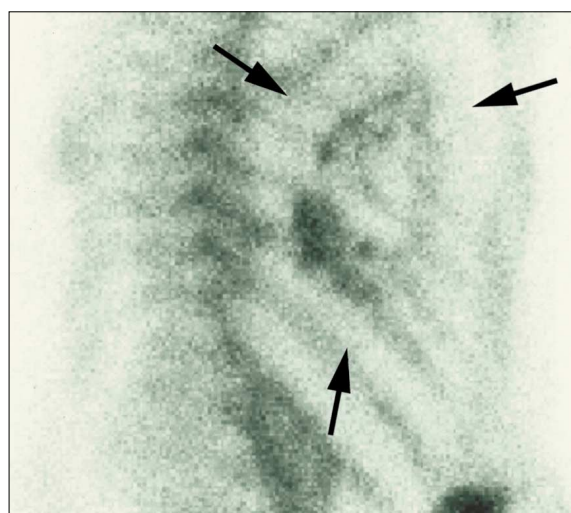


Figure 4: Skeletal scintigraphy of implant
Arrows show U-shaped mandibular replacement (scintigraphy 3 h post-tracer injection).

successful bone induction (figure 4). Furthermore, CT of the thorax gave radiographic evidence for bone formation around the implant site (webfigure 2; <http://image.thelancet.com/extras/04art7155webfigure2.pdf>).

7 weeks postoperatively, the patient again underwent general anaesthesia for transplantation of the mandibular replacement. The replacement was harvested along with an adjoining part of the latissimus dorsi muscle containing the thoracodorsal artery and vein that had supplied blood for the entire transplant. This pedicled bone-muscle flap was then transplanted into the defect site via an extraoral approach. Minor bone overgrowth on the ends of the replacement was curetted to fit the transplant easily into the defect. No further correction of the shape or form of the graft was needed.

After the old titanium reconstruction plate (figure 1) was removed, the mandibular transplant was fixed onto the original mandible stumps (webfigure 3; <http://image.thelancet.com/extras/04art7155webfigure3.pdf>) with titanium micro-osteosynthesis screws, returning the contour of the patient's jaw line to roughly that present before the mandibulectomy. The vessel pedicle was then anastomosed onto the external carotid artery and cephalic vein by microsurgical techniques. The cephalic vein was taken from the upper arm and relocated into the neck since few usable local veins were available because of a previous radical neck dissection and irradiation. Because of the patient's non-elastic irradiated skin we could not close the wound completely in the submandibular region. Prophylactic antibiotic cover was again provided with 1.5 g ampicillin/sulbactam three times a day. 12 days postoperatively this small area had closed spontaneously with growth of granulated tissue. This area was then covered with a full thickness skin graft. Except for a small manageable area of necrosis at the wound margin (previously irradiated skin), healing proceeded uneventfully. Vascular supply to the flap was maintained successfully. Repeat three-dimensional CT showed that both mandible stumps were in the correct position with relation to the transplant (figure 5). We did a second skeletal scintigraphy with 600 MBq Tc99m-oxydronate tracer on the 11th postoperative day and showed continued bone remodelling and mineralisation inside the mandibular transplant, indicating undisturbed vascular perfusion and survival of the induced bone cells (figure 5).

By the 4th week post-transplantation the patient enjoyed his first dinner in 9 years (bread and sausages); before reconstruction he had only been able to eat soft food and soup. Even with his edentulous jaws, he was now able to undertake a small amount of mastication. The patient was also satisfied with the aesthetic outcome of the procedure.

Discussion

To maximise the potential for successful bone induction in this study, we used both recombinant human BMP7



Figure 5: Three-dimensional CT scan (left) after transplantation of bone replacement with enhancement of soft tissue (red) and repeat skeletal scintigraphy (right) with tracer enhancement showing continued bone remodelling and mineralisation (arrows)

and whole bone marrow. Although this method was clearly successful, we cannot conclude whether regeneration of bone tissue was attributable to the bone-marrow cells or BMP7—it is likely to have been a combination of the two. Previous research in our department with a minipig model has suggested that recombinant human BMP7 is a potent bone-inducing protein when used in the latissimus dorsi muscle, even without autologous bone marrow.^{4–6,11} Therefore, we can expect that the bone formation reported was a result of recruitment by recombinant human BMP7 of both local undifferentiated precursor cells and undifferentiated precursor cells of bone-marrow origin.

With further mineralisation of the new induced bone, we hope to remove the external titanium mesh scaffold 1 year after transplantation. If removal is possible, the repaired mandible will be subject to normal functional loading and will subsequently undergo remodelling.¹² Such a process might result in development of a histological structure that approximates that of a normal mandible. Whether or not removal of the titanium mesh is possible, insertion of dental implants will be an option to further increase masticatory function. Because of ethical concerns, we have not been able to harvest tissue from the mandibular replacement for histological analysis. If an attempt to insert dental implants is undertaken in the future, we might then have the opportunity to gain small samples of the former mandibular transplant for such analysis.

Avoidance of large bone defects in bone transplantation is a major goal. Although our procedure still involves creation of a soft-tissue donor-site defect, it might allow us to avoid completely the creation of a bone defect in patients. This possibility would greatly reduce

the morbidity associated with bone transplantation and might even improve functional and aesthetic outcomes achieved with such procedures. If conventional techniques in mandibular reconstruction had been applied in a defect of this size, transplantation of a microvascularised fibula bone would have been advisable. This approach would have created a major bone and soft tissue defect of the lower leg—the morbidity associated with this would have been great.

To the best of our knowledge, this case provides evidence that heterotopic bone induction to form a mandibular replacement inside the latissimus dorsi muscle in a human being with a prefabrication technique is possible. The prefabrication technique described, and subsequent free-flap reconstruction of the mandible, allows for a lower operative burden and a better three-dimensional outcome than conventional reconstruction techniques. We suggest that our results represent a proof of principle. Many vital questions still remain unanswered. The exciting nature of the result achieved in this patient to date has prompted our group to extend this trial to include additional patients. For us to draw firm conclusions, an extended period of follow-up is necessary. We hope to present this patient's long-term outcome and those of future patients at a later date.

Contributors

P H Warnke was head and H Terheyden senior head of the project; both these authors designed the project. P H Warnke wrote the report. I N G Springer, J Wiltfang, Y Acil, E Behrens, E Sherry, and P A J Russo were involved in project organisation, data interpretation, and helped to prepare the report. Surgical procedures were done by P H Warnke, H Terheyden, and J Wiltfang. H Eufinger and M Wehmöller did the CAD work. H Bolte did the three-dimensional CT imaging.

Conflict of interest statement

We declare we have no conflict of interest.

Acknowledgments

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