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Prefabrication of vascularized bioartificial bone grafts *in vivo* for segmental mandibular reconstruction: experimental pilot study in sheep and first clinical application

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Abstract. The key elements for bioartificial bone formation in 3D matrices are large numbers of osteogenic cells and supplies of oxygen and nutrition. Vascularization becomes more important with the increasing size and complexity of seeded scaffolds required for clinical application in reconstructive craniomaxillofacial surgery. Prefabrication of vascularized bioartificial bone grafts *in vivo* might be an alternative to *in vitro* tissue engineering techniques. Two cylindrical β-TCP-scaffolds (25 mm long) were intraoperatively filled with autogenous bone marrow from the iliac crest for cell loading and implanted into the latissimus dorsi muscle in 12 sheep. To determine the effect of axial perfusion, one scaffold in each sheep was surgically supplied with a central vascular bundle. Sheep were killed 3 months after surgery. Histomorphometric analysis showed autogenous bone marrow from the iliac crest was an effective source of osteogenic cells and growth factors, inducing considerable ectopic bone growth in all implanted scaffolds. Bone growth, ceramic resorption and angiogenesis increased significantly with axial perfusion. The results encourage the application of prefabricated bioartificial bone for segmental mandibular reconstruction in man. In clinical practice, vascularized bioartificial bone grafts could change the principles of bone transplantation with minimal donor site morbidity and no shape or volume limitations.

Key words: mandibular reconstruction; bone substitute; flap prefabrication; tissue engineering.

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Osseous defects resulting from tumor resection, trauma or infection remain a challenging problem in reconstructive craniomaxillofacial surgery[9,19,31]. Autogenous bone grafts represent the gold standard and are frequently used clinically for osseus reconstruction[10]. After transplantation, the cells and matrix compounds of autogenous bone grafts provide high osteogenic potential, which leads to complete substitution by newly formed bone within a gradual remodeling process. In situations with compromised perfusion due to radiation or infection, microsurgical bone transfer is preferred for osseous reconstruction, inducing a healing process from the transplanted graft comparable to bone fracture healing[21]. Bone harvesting is associated with considerable donor site morbidity, which increases with the amount of harvested bone[24]. The amount of autogenous bone for transplantation is limited and the volume and shape of autogenous bone grafts may compromise prosthetic rehabilitation especially after microsurgical bone transfer[12]. Processed allogenic and xenogenic bone grafts are alternatives but are used with reservation because of the risk of disease transmission. Higher complication rates after allogenic and xenogenic bone transplantation indicate a lower osteogenic potential compared with autografts, even when osteoinductive factors are preserved during processing[3].

The generation of bioartificial bone grafts using tissue engineering techniques is an attempt to overcome the limitations of the methods mentioned above. Until now, tissue engineering techniques with cultivation of osteogenic cells and seeding on scaffolds *in vitro* did not seem effective enough to generate large bioartificial bone grafts for clinical use[25]. The key elements for bioartificial bone formation in three-dimensional (3D) matrices in any tissue engineering concept are a high number of osteogenic cells and supplies of oxygen and nutrition. Vascularization becomes more important with the increasing size and complexity of the seeded scaffolds required for clinical application in reconstructive craniomaxillofacial surgery[17].

Various vascularization concepts for 3D matrices are the focus of experimental research[15,16]. Combining tissue engineering approaches with flap prefabrication techniques may allow the application of vascularized bioartificial bone grafts grown *in vivo* with the advantage of minimal donor site morbidity compared with conventional bone grafts. In this study, osteogenic material from the iliac crest was obtained intraoperatively and used directly for cell loading 3D matrices in a surgical seeding procedure. The latissimus dorsi muscle served as a natural bioreactor to overcome the limitations of extracorporal tissue engineering techniques with cell expansion *in vitro*. The goal of this study was to determine the effect of this seeding procedure on bone formation within large bioartificial scaffolds, especially in combination with axial perfusion and whether this technique leads to sufficient bone growth for clinical use.

Materials and methods

Cylinders of 25 mm length and 14 mm diameter consisting of pure β-tricalcium phosphate with a porosity of 60–80% and a pore size of 100–500 μm were used as carriers (ChronOs®, Synthes, West Chester, USA). The cylinders were modified with a central passage of 7 mm diameter and sterilized again by gamma radiation. Animal experiments were conducted under a protocol approved by the ethics committee in accordance with German federal animal welfare legislation. Twelve healthy adult female German blackheaded sheep with an average weight of 72.5 ± 7.4 kg were included in the study. After intravenous induction (1 ml midazolam, 5 mg/kg propofol) anesthesia was maintained with isoflurane delivered in 100% oxygen (1 l/min). Additionally, buprenorphine (10 μg/kg i.m.), carprofen (4 mg/kg half i.v., half s.c.) and fentanyl (0.005 mg/kg i.v.) were applied for analgesia. Systolic, diastolic and mean blood pressure, electrocardiogram, rectal temperature and hemoglobin oxygen saturation were continuously monitored during surgery. Three bone biopsies (5 mm diameter) were harvested from the iliac crest and morselized with an electric bone mill. After mixing with amorphous bone marrow aspirated from the depth of the biopsy areas, the osteo-

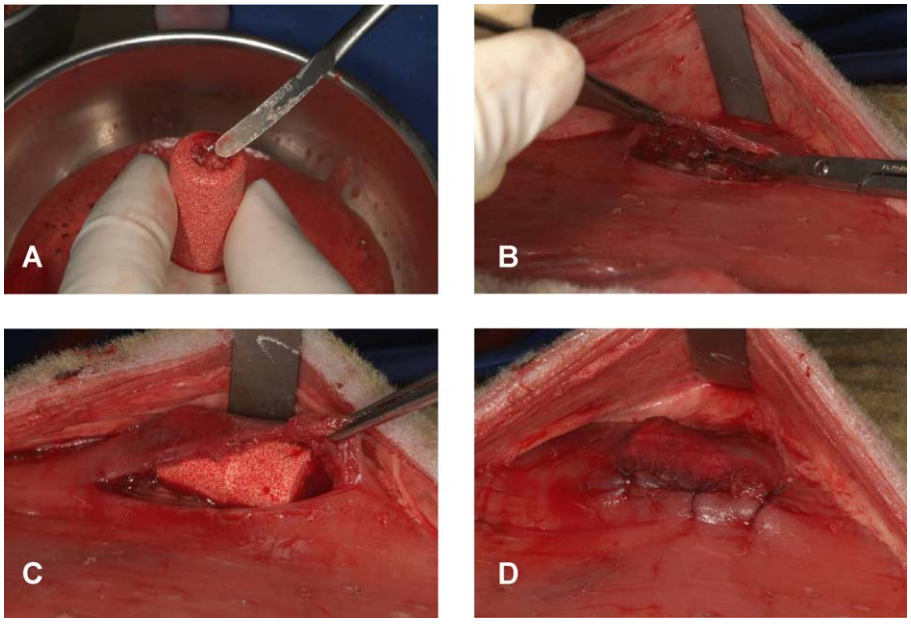

Fig. 1. Prefabrication of scaffolds for group 1: intraoperative loading of the predrilled β-TCP-cylinders with osteogenic material from the iliac crest (A), preparation of a muscle pouch in the latissimus dorsi muscle (B), implantation of the loaded cylinders directly into the muscle pouch (C and D).