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Research Paper
Bone Biology

In vivo tissue engineered bone versus autologous bone: stability and structure

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Abstract. This pilot study investigated the biomechanical properties of prefabricated, vascularized bioartificial bone grafts, which may provide an alternative bone source for the restoration of segmental osseous defects. Vascularized bioartificial bone grafts comprise an artificial customized scaffold made of beta-tricalcium phosphate. Bone formation along the prefabricated scaffold is induced by autogenous cancellous bone. Vascularization of the bone graft is provided by the host's vascular system. Within 6 months, a mammalian bioreactor (sheep were used in the present study) creates heterotopic vascularized bioartificial bone grafts of a predetermined anatomical shape, which can be harvested for reconstructing osseous defects. The bioartificial bone grafts in this study contained up to 25% bone tissue, as shown by histomorphometric analysis and computed tomography. Moreover, unconfined compression tests revealed that the constructs had mechanical characteristics similar to those of ovine cancellous bone. Therefore, this method could be applied to generate vascularized prefabricated bone substitutes for critical-size defects.

Key words: bioartificial bone; axial vascularization; bone engineering; custom-made transplants; heterotopic bone growth; flap prefabrication; maxillofacial reconstruction; vascularized bone transplants; biomechanical tests; Young's modulus; elastic modulus; micro-CT.

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The restoration of critical-size bone defects that are not compensated by endogenous bone regeneration remains a challenge in regenerative medicine, particularly in the craniomaxillofacial region.[1] A critical-size defect is classified as the smallest size of defect that will not heal completely during the natural lifetime of an animal.[2,3]

To date, autologous bone grafts, which are transferred as either vascularized or non-vascularized tissue, are the most effective method for reconstruction.[4,5] In particular, when grafted as avascularized tissue or in combination with synthetic scaffolds, the incorporated autogenous osteogenic cells and growth factors provide the main source of ossification. Hence, regeneration occurs along the natural or synthetic scaffold with osteoinductive and osteoconductive properties. Although this approach yields high rates of incorporation and initial revascularization, the disadvantages include donor site morbidity, limited availability, and plasticity.[6,7]

In contrast to orthopaedic medicine, reconstruction for facial skeleton defects poses two major challenges. First, because rehabilitation with dental implants is the principle purpose of osseous reconstruction of the maxilla and mandible, the grafted bone will inevitably be exposed to the paranasal sinus and the oral cavity and its microflora, albeit indirectly.[8] Second, the complexity of the three-dimensional (3D) shape of the facial skeleton, maxillo-mandibular unit, and masticatory tasks require bone grafts with high plasticity and mechanical strength. In craniomaxillofacial reconstruction, these defects

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are primarily treated using either vascularized or non-vascularized flaps from the fibula, scapula, or iliac crest.[9–11] However, the bone quality, structure, and 3D contour do not fully meet the requirements for a functional and aesthetic outcome true to the original.[12] Thus, secondary augmentations and soft tissue corrections are often necessary, particularly in cases where dental implants are placed. Computer-assisted surgery has recently revolutionized the field of reconstructive craniomaxillofacial surgery, and allogeneic patient-specific implants have already emerged as a standard procedure in many institutions.[13,14]

Novel synthetic materials and surface coatings have recently been developed for bony reconstruction.[15,16] In particular, synthetic calcium phosphate bone substitutes such as hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) have received increasing attention due to the similarity of their chemical composition to bone and their ability to become resorbed whilst regenerating new bone.[17–19] To date, the production of scaffolds that fully comply with all requirements remains challenging, and most *in vitro* attempts using synthetic materials have failed to engineer large viable and vascularized bone grafts. In particular, the mechanical properties of novel bone substitutes provide challenges for clinical application. Thus, innovative approaches are essential to generate viable individualized alternatives that meet the requirements for functional and aesthetic reconstruction of the facial skeleton.

In contrast to the classical *in vitro* tissue engineering approach, this study employed *in vivo* tissue engineering. Briefly, a mammalian bioreactor generates bioartificial bone grafts for osseous reconstruction, providing oxygen and nutrition through vascularization.[20] Combining the principles of autogenous bone graft reconstruction with *in vitro* tissue engineering techniques, this approach uses an osteogenic cocktail provided by bone marrow aspirates to vitalize artificial scaffolds. Customized, prefabricated bone grafts, first described in pigs and humans by Terheyden et al., have been modified and developed further and are now reproducibly available in complex shapes.[21–23] However, their biomechanical properties require evaluation before they can be applied routinely in humans. This study aimed to investigate the biomechanical characteristics of prefabricated, axially vascularized bioartificial bone grafts generated in a large mammalian bioreactor system.

Materials and methods

Animal experiments

The study used six female black-headed German sheep with an average weight of 69.3 kg. All of the animal experiments were performed under general anaesthesia in accordance with the requirements of the institutional ethics committee and the federal animal protection law (reference number 33.9-42502-04-08/1621). Following induction with diazepam (0.2 mg/kg) and ketamine hydrochloride (3–4 mg/kg Ursotamin; Rebopharm GmbH, Bocholt, Germany), general anaesthesia was maintained using an isoflurane–oxygen mixture (2.5 vol% in oxygen mixture, Isoba; Essex Pharma GmbH, Munich, Germany). Intra- and postoperative analgesia was achieved via the administration of fentanyl (0.005 mg/kg) and carprofen (4 mg/kg) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and buprenorphine (10 mg/kg Buprenovet; Bayer Healthcare, Leverkusen, Germany). All of the animals also received perioperative antibiotics (cefquinome (2 mg/kg Cobactan 4.5%); Intervet GmbH, Unterschleißheim, Germany). The antibiotic regimen was continued for 1 week post-operation.

Prefabrication, implantation, and tissue harvesting

An arrangement of angulated cylinders was chosen in order to simulate the complexity of defects in the craniofacial skeleton, e.g. the mandibular angle. Two β-TCP cylinders (porosity 60–80%, pore size 100–500 μm), 14 mm in diameter and 25 mm in length (chronOS; Synthes, West Chester, PA, USA) and with a central perforation (7 mm in diameter), were assembled at a 30° angle and wrapped in a customized titanium cage (20 mm in diameter and 60 mm in length) formed using an autoclavable plastic template (Fig. 1A, B). The prefabricated angulated constructs were implanted into the latissimus dorsi region of the experimental animals, using axial perfusion provided by the thoracodorsal trunk, as described previously.[23,24] The space between the β-TCP cylinders and the titanium cage, as well as the central perforation, was filled with autogenous cancellous bone from the iliac crest (Fig. 1C, D).

After 6 months, the animals were sedated with diazepam (0.2 mg/kg) and euthanized with intravenous sodium pentobarbital (4500–6000 mg Release(R), WDT eG, Garbsen, Germany). The constructs were harvested along with healthy cortical and cancellous reference bone samples from the metacarpal, radial, and iliac crest regions of the sheep for comparative biomechanical analysis. Long bone samples were chosen as controls because of their similar cross-sectional area, as required for sufficient comparative compression testing. Following explantation, the titanium cage was removed and the constructs were prepared for immediate micro-computed tomography (micro-CT) (Fig. 1E, F).

Micro-computed tomography

Following sample harvesting and removal of the titanium cage, constructs, reference bone samples, and a β-TCP cylinder were scanned with a high-resolution peripheral quantitative computed tomography scanner (XtremeCT; Scanco Medical, Brüttisellen, Switzerland; 60 kVp, 901 μA, voxel size 41 μm, integration time 300 ms) for precise characterization of the samples. Initially a visual two-dimensional (2D) analysis was done using one longitudinal (middle position) and three cross-sectional (25%, 50%, 75%) slices. After contouring and determining threshold values for obviously different shaded regions (gauss sigma 0.8, gauss support 1.0; threshold values: β-TCP cylinder pre-operative −1000 to 135 Hounsfield units (HU) for pores filled with air, 136–1000 HU for scaffold; constructs and reference bone 136–1000 HU; soft tissue in constructs 41–135 HU), the analysis was performed using the true 3D evaluation software (μCT Evaluation Program v. 6.0; Scanco Medical) for 3D and local 3D calculations. Using the same thresholds for all samples, the volume, surface (inner and outer area including the pores), bone and scaffold microstructure (trabecular number, separation, and thickness), and density were calculated. As the peripheral quantitative computed tomography (pQCT) was calibrated against a phantom for bone density measurements, the density was recorded in milligrams hydroxyapatite per cubic centimetre ($mgHA/cm^3$). For an additional evaluation of β-TCP cylinder remnants inside the constructs, the cylinder was differentiated visually from the bone using the parameters structure, shape, and position and accordingly contoured accurately in each slice. Then a new calculation was performed using the previous threshold.[25] Representative 3D reconstructions of a single β-TCP cylinder pre-operation and post-explantation are shown in Fig. 2.

Sample preparation for mechanical testing

Immediately after micro-CT, constructs and reference bone samples were prepared