Prefabrication of vascularised 3-D bone flaps with different growth factors in the great omentum in rabbits for reconstruction of large bony defects. - preliminary report -

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Objectives:

Reconstruction of bony defects can be done by free flaps. Donor site morbidity and misfit of the shape are limiting factors. Prefabrication of customized bone flaps (PCBF) in the latissimus dorsi muscle has been reported in animal or clinical investigation (1). The concept of using the patients own tissues as a bioreactor solved many problems. It has shown that PCBF could reduce the donor site morbidity significantly and that the 3D form of the prefabricated bone segments could be controlled. The disadvantages are insufficient predictability of ossification and furthermore there is still considerable donor site morbidity in the latissiums dorsi site (Fig1).

The hypothesis of the present experiment is that the great omentum is a capable new anatomical site. It can be used as a bioreactor in vivo for prefabrication tissue-engineered bone. According to our recent studies the efficacy of the BMP-2 dosage will be enhanced by addition of VEGF-165, autologous bone chips and a central vascular pedicle in a cavity of the scaffold.

Materials & Methods:

In each of 30 adult male New Zealand white rabbits (4.0kg) 2 blocks of biomaterial were implanted in the great omentum with a central vascular pedicle in a cavity of the scaffold (Fig. 2).
The following 5 groups were created according to the biomaterial:

1. Bio-Oss® Block,
2. Bio-Oss® Block + rhBMP-2,
3. Bio-Oss® Block + rhBMP-2 + VEGF-165,
4. Bio-Oss® Block + autologous bone,
5. Bio-Oss® Block + autologous bone + rhBMP-2.

CT examinations were performed after the implantation and 2, 4, 6 and 10 weeks later to define the bone density. The animals were sacrificed at 10 weeks postsurgery after intraperitoneal injection of fluorochromes and the specimens were collected for histological, immunohistochemical and histomorphometric analysis.

Results:

First analyses could show that the great omentum has advantages with a long vascular pedicle, less postoperative functional impairment and more flexibility (Fig. 2). The central blood vessel in the center of the scaffold resembles the natural anatomy of the mandible and other bones with a marrow cavity and enhances the ossification.

The bone density increases particularly with the use of BMP-2 and VEGF-165 and has the highest values with additional use of autologous bone (HU: average between 483 (BioOss-Block alone) and 1054 (+ autologous bone with BMP-2) (Fig 3)

The floureszenz staining shows ossification also with BMP alone (Fig4). Also the toluidine blue and HE staining shows ossification and development of new vessels in the scaffold (Fig 5).

There are indications that the efficacy of the dosage could be enhanced by addition of VEGF-165. The histological, immunohistochemical and histomorphometric analysis will be completed shortly.

Conclusions:

We could demonstrate for the first time that the great omentum is a capable new anatomical site for prefabrication. It can be used as a bioreactor in vivo for prefabrication tissue-engineered bone. Prefabrication in the great omentum may go from bench to bedside and will show effectiveness in mandibular reconstruction.

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Ethical approval in accordance with the national law by the Minister of Nature, Environment and Forestry of Schleswig-Holstein (73-6/11).

LITERATURE:
WARNKE, P. H., SPRINGER, I. N., WILTFANG, J., ACIL, Y., EUFINGER, H., WEHMOLLER, M., RUSSO, P. A., BOLTE, H., SHERRY, E., BEHRENS, E. & TERHEYDEN, H. 2004. Growth and transplantation of a custom vascularised bone graft in a man. Lancet, 364, 766-70.

Fig 1: Reconstruction of the mandible with a prefabricated flap in the latissimus dorsi muscle after resection of scamous cell carcinoma (ct-scan and clinical outcome)

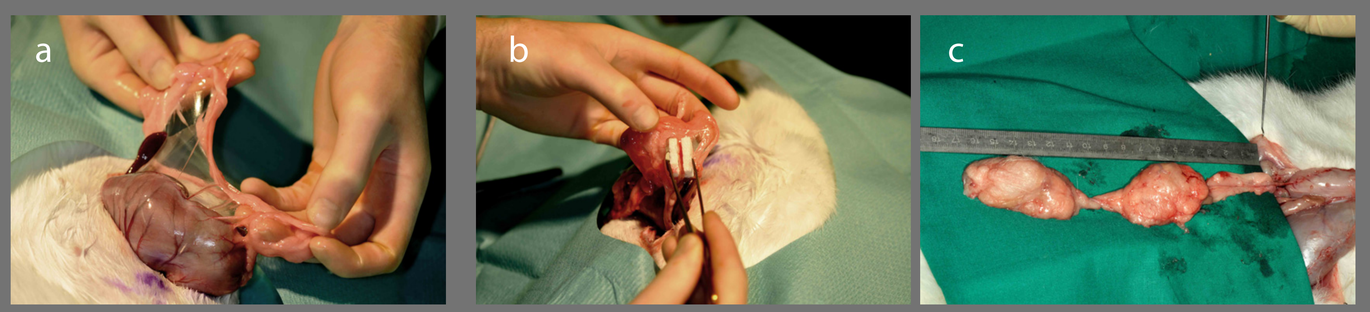

Fig 2: Surgical procedure with a) preparation of the great omentum, b) placing the central vessel and wrapping the scaffold. Preparation after sacrifice with long pedicle (16cm) and enveloping soft tissue

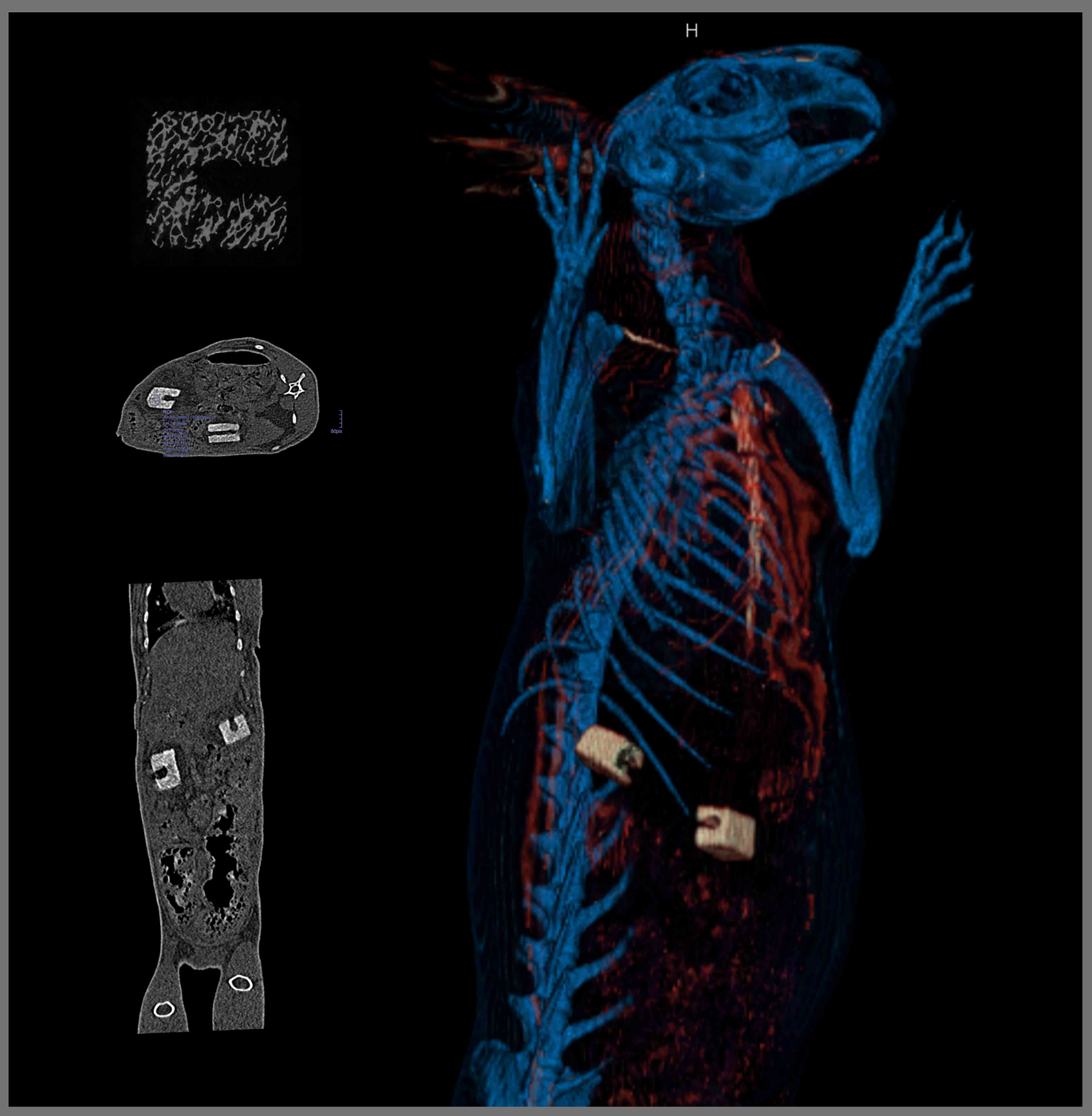

Fig 3: CT scan of the rabbit with measurement of Hounsfield Units in 3 ROI´s per scaffold

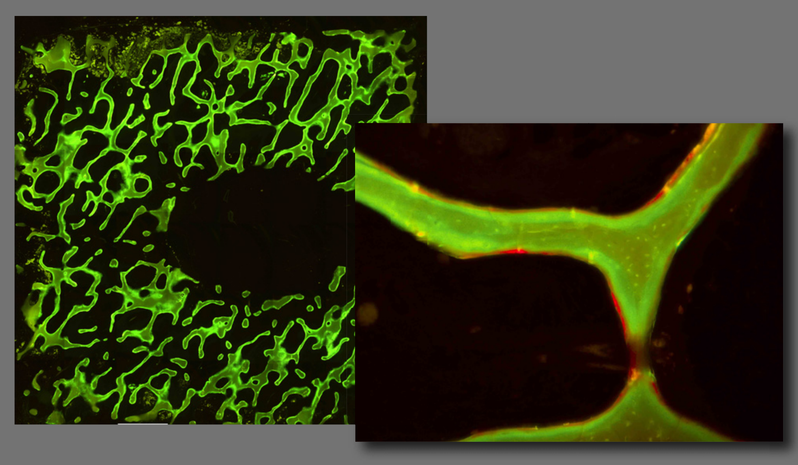

Fig 4: Fluoreszence microscopy with signs of first bone apposition already after 2-3 weeks (orange) (BioOss-with BMP-2)

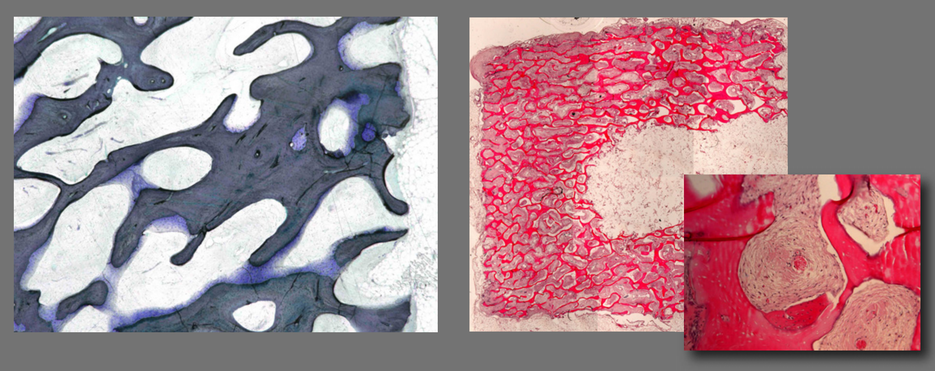

Fig 5: Toluidine blue and HE staining shows ossification and development of new vessels in the scaffold. (BioOss-with BMP-2)