Prefabrication of Vascularized Bone Grafts in the Latissimus Dorsi Muscle

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ABSTRACT INTRODUCTION

The treatment of large bone defects by tissue engineered implants is limited due to the lack of blood supply. There have been reports of prefabrication of tissue engineered bone grafts within the latissimus dorsi, however, a thorough experimental analysis of these implants is missing. The aim of this study was to investigate two different approaches to prefabricate bone grafts on a large scale within the latissimus dorsi of sheep and to evaluate the efficacy of these approaches in terms of scaffold degradation, bone and vessel formation.

METHODS

Cylinders of 25mm in length and 14mm diameter consisting of pure β-tricalcium phosphate with a porosity of 60-80% and a pore size of 100-500µm were used as carriers (ChronOs®, Synthes, West Chester, USA). The cylinders were customised with a central drill hole of 7mm diameter. Animal experiments were conducted under a protocol approved by the Ethics Committee in accordance with German Federal Animal Welfare Legislation. 12 healthy adult female German blackheaded sheep with an average weight of 72.5±7.4kg were used for the study. Under general anaesthesia, bone marrow aspirates were taken from the iliac crest and the scaffolds were incubated according to the guidelines of the manufacturer. A bioreactor system with a combined angiogenic-osteogenic media containing Ham’s F-12, 10%FCS, 100IE/ml penicillin/ streptomycin, 250µg/ml amphotericin B, 30ng/ml MALP-2, 10µM dexamethasone) was used for a culture period of 3 weeks in a perfusion bioreactor.

Before implantation of the scaffolds, the thoraco-dorsal vessels were exposed and branches were clipped in order to provide a free length of 10cm. Each animal received three cylinders prepared as follows: In group S, spongiosa from the iliac crest was harvested and placed within the inner 7mm of the TCP cylinder. In group C, scaffolds were taken out of the bioreactor system and implanted. Six months after implantation, all animals were sacrificed after deep sedation (80mg/kg pentobarbital i.v.). After explantation from the latissimus dorsi muscle, the cylinders were divided into two halves and fixed in 3.5% neutral buffered formalin. One half of the cylinder was embedded in methylmethacrylate (MMA) and sectioned perpendicular to the axis using a diamond saw. Undecalified slices of 8-10µm were surface stained with H&E and alizarin red for light microscopy and histomorphometric analysis. The other half of each cylinder was embedded in paraffin for immunohistochemical determination of the vascular density. Immunologic reactions in decalified slices of 3µm thickness were performed with the antibodies Factor VIII rabbit polyclonal® (Biocare Medical Inc., Concord, CA, USA), the ZyroChemPlus (HRP) Polymer Bulk Kit® (Zytochem Systems, Berlin, Germany) and the DAB High Contrast Kit®. Digital images of each slide were obtained using a Zeiss Axiosimaging MI Microscope equipped with an AxioCam MRc digital camera and AxioVision 4.5 software (Carl Zeiss, Oberkochen, Germany). The AxioVision module MosaiX was used to scan the total specimen. Total bone area and residual ceramic area were quantified using the image-analysis software Analysis 3.0 (Olympus Soft Imaging Solutions, Muenster, Germany). For vessel counts, three regions of interest measuring 2mmx2mm were defined, representing the central, intermediate and outer areas of the cylinders. Five slides of every cylinder were analyzed for histomorphometric evaluation and for vessel counts. Results were compared using a non parametric Wilcoxon Test and a p-level of 0.05 (SPSS 16.0, SPSS Inc., Chicago, IL).

RESULTS SECTION

All animals survived until the end of the study (n=6 for each group). There was one superficial wound infection that healed uneventfully after administration of systemic antibiotics. Histomorphometric analysis revealed that there were new capillaries within the scaffolds and areas of new synthesized bone along with remnants of TCP within the samples (Fig. 1).

The area of ChronOs® left after 6 months was 52.0±4.7mm² in group C compared with 34.3±4.7mm² in group S (p<0.01). The area of bone was determined with 20.4±6.4mm² in group C and 30.0±10.6mm² in group S (p<0.02). At the same time, the number of vessels was 8.6±1.6 in group C and 8.9±2.1 in group S (p<0.22; Fig. 2).

DISCUSSION

Prefabrication of vascularized bone grafts can be readily performed using central vessels surrounded by a TCP cylinder and autologous cells. In this study, the use of spongiosa within the scaffold proved to be more effective in terms of scaffold degradation and mineralized bone matrix synthesis. The angiogenic effect was similar in both groups. Future studies must focus on the use of higher cell concentrations and the application of alternate growth factors in order to obtain similar effects as autologous bone.

LITERATURE

1Kokemueller et al., Int. J Oral Maxillofac Surg, 2010

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