Evaluation of BMP-2 and Mesenchymal Stem Cells Subcutaneously and in the AV-Loop Model in the Sheep to Generate Axial Vascularized Bone Tissue

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Introduction
If the normal physiologic reaction to fracture does not occur, such as in fracture nonunion or large-scale traumatic bone injury, surgical intervention is required. Autografts and allografts represent current strategies for surgical intervention, but each possesses limitations like donor site morbidity. Synthetic bone-graft substitutes, developed in an effort to overcome the inherent limitations of autograft and allograft, represent an alternative and well established strategy but lack an intrinsic vascularisation.

The purpose of this study was to generate axial vascularised bone tissue constructs in clinical relevant dimensions in sheep, in an attempt to overcome disadvantages of existing solutions.

Materials and Methods
Sheep mesenchymal stem cells were isolated, characterised by FACS and rtPCR analyses, labelled and implanted subcutaneously and in the AV-loop model in combination with BMP-2 and β-TCP/HA granules in merino land sheep. Constructs were explanted at different time points up to 12 weeks followed by histological evaluation. Standard histology and immunohistochemistry staining were performed checking for apoptosis and proliferation of the cells, vascularisation and bone formation. Non invasive imaging of vascularisation has been performed using micro CT and MRA techniques.

Results
We were able to characterize MSC by FACS and rtPCR analyses. Sheep MSC are CD29, CD44 and CD166 positive following selection by ficoll gradient centrifugation and plastic adherence. Freshly isolated MSC express CD29 and CD166 weaker as cultured MSC and hematopoetic cells can not be totally separated by the ficoll gradient method.

Subcutaneously implanted MSC have a constant proliferation rate and a decreasing apoptosis rate over time. 60 g/ml BMP-2 or MSC are necessary for de novo bone formation subcutaneously in sheep.

Discussion and Conclusions
We were able to generate bone subcutaneously in sheep using β-TCP/HA granules in combination with BMP-2 and / or MSC within 12 weeks. Bone can be achieved using MSC with β-TCP/HA granules alone, so that BMP-2 stimulation is not further necessary. Once successfully employed in the sheep, clinical application of vascularized bone tissue engineering will hopefully be put into reach.

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