

***In situ* modification of progenitor cells for the expedited regeneration of skeletal tissues.**

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INTRODUCTION: Mesenchymal progenitor cells – also referred to as mesenchymal stem cells (MSCs) despite disagreement over whether they are true stem cells – exist in numerous tissues, including bone marrow, fat, muscle, synovium, ligament and tendon. They form an obvious starting point for strategies aiming to restore damaged components of the skeletal system. In addition to the scientific challenges to be addressed, any successful technology has to be practical and affordable. In this context, we are trying to develop methods that regenerate bone and cartilage without the need for *ex vivo* cell culture. Central to this strategy is the *in situ* transfer of genes encoding morphogenic proteins to MSCs. We are experimenting with bone marrow, muscle and fat as tissues that are rich in MSCs and lend themselves to genetic modification¹. Here we present data on the restoration of bone using muscle tissue modified with a recombinant adenovirus that carries cDNA encoding human bone morphogenetic protein-2 (Ad.BMP-2)

METHODS: Fischer rats weighing 450g were used. Biopsies of skeletal muscle and fat were harvested with a skin punch, providing discs approximately 5mm in diameter and 1 mm thick. These were incubated with Ad.BMP-2 at various concentrations up to 10¹³ particle/ml, and inserted into 5mm segmental femoral defects. Healing was monitored by weekly X-ray. Certain animals were euthanised for histology after 2 and 4 weeks. All animals were euthanised after 8 weeks and defects were analysed by histology, μ CT, DXA and mechanical testing. For Y-chromosome painting, genetically modified tissue from male donors was inserted into female animals and identified by fluorescence in situ hybridization (FISH) by Prof. J. Hoyland at Manchester University, GB. Pilot sheep studies were conducted at The AO Research Institute in Davos by L. Boure, M. Alini and M. Stoddart. Autologous fat and muscle were transduced with Ad.BMP-2 and placed into 1.5cm critical sized, bilateral iliac defects in sheep. One defect on each animal received fat, and the other

muscle. After 12 weeks, sheep were euthanised and healing assessed by histology and μ CT.

RESULTS: Genetically modified, syngeneic fat and, especially, muscle dramatically healed segmental femoral defects in the rat. Healing was more rapid, uniform and reliable than when recombinant human BMP-2 (Infuse®) was used. Histological examination suggested that the implanted muscle rapidly turned to cartilage, leading to accelerated, endochondral healing. Y-chromosome painting confirmed that cells from donor muscle indeed formed osteoblasts within the healed tissue. Preliminary data from sheep, suggest that muscle effectively forms new bone within the osseous iliac defect. One defect receiving fat also formed considerable bone, but the other did not.

DISCUSSION & CONCLUSIONS: Genetically modified muscle and fat that express human BMP-2 provide rapid, uniform and reliable healing of large, critical size segmental defects in rat femora. The implanted tissue appears to serve as a local source of BMP-2, osteoprogenitor cells and scaffold, and healing follows an endochondral process. Preliminary data from pilot sheep studies performed at ARI in Davos suggest that scale-up to a large animal is possible, permitting optimism concerning possible future clinical use in humans, despite the use of gene transfer.

REFERENCES: ¹ Evans et al. (2007) Tissue Eng 13: 1987 – 1993.

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