

## The Facilitated Endogenous Repair of Bone by Gene Transfer

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### INTRODUCTION:

Tissue engineering strategies usually involve the *ex vivo* combination of progenitor cells, scaffolds and morphogenetic stimuli to generate an implantable repair structure. While feasible, such an approach is likely to be too expensive and cumbersome for widespread clinical use. Because of this, we are developing technologies that obviate the need for *ex vivo* cell culture and manufactured scaffolds. Instead, differentiation of the progenitors is stimulated *in situ* or in an expedited *ex vivo* fashion that can be accomplished intraoperatively. Because these methods provide little opportunity for prolonged *ex vivo* exposure to morphogens, these proteins are provided by transfer and expression of the appropriate cDNAs. Tissue repair and regeneration then occurs *in vivo* under the influence of the expressed transgene. We call this approach "facilitated endogenous repair"<sup>1</sup>

Two examples relevant to bone will be discussed here: the direct injection of vector and the implantation of genetically modified fat and muscle. Both are studied in a critical-sized, 5mm defect in the rat femur.

### DIRECT INJECTION OF VECTOR:

Based upon the foregoing logic, the simplest means of stimulating a regenerative response is to introduce a cDNA directly into the site of injury. Adenovirus vectors are well suited to this purpose, because they are easy to construct, grow to high titres, and infect non-dividing cells of most types very efficiently. The major drawback is that they are inflammatory and antigenic, but this does not preclude their local application to discrete anatomical locations. We have generated a first generation, serotype 5, recombinant adenovirus carrying the human BMP-2 cDNA (AdBMP-2) for bone healing studies.

When injected directly into the critical sized, rat femoral defect, AdBMP-2 promotes vigorous intra-lesion bone deposition, which is remarkable because the same virus does not stimulate osteogenesis when injected intramuscularly. Histological examination suggests that healing occurs via endochondral ossification<sup>2</sup>.

We are presently optimizing the method and note that healing is improved when the

administration of AdBMP-2 is delayed by 5 days or more presumably because, by this time, a stable clot has formed. Although it is possible to achieve 100% union rates by this method, the healed bone is poorly structured and mechanically weak. Present research addresses these issues by investigating the effect of the mechanical environment on healing.

### EXPEDITED EX VIVO DELIVERY OF GENETICALLY MODIFIED TISSUE:

Certain tissues contain osteoprogenitor cells and also provide the functions of a space-filling scaffold. They thus lend several advantages in the present context. We are exploring the use of muscle and fat.

Syngeneic rat strains enable the transfer of tissue from one individual to another as if it were autograft. Fragments of donor skeletal muscle or fat are readily transduced with AdBMP-2. When the transduced tissues are maintained in organ culture, they express BMP-2 at high levels and respond to it by expressing genes associated with osteogenesis, including alkaline phosphatase, and they deposit a mineralized matrix.

When the transduced tissues are implanted into 5mm rat femoral defects there is a dramatic and early osteogenic response that occurs in 100% of animals within 2 weeks. Complete bridging is seen within a month. Histological examination suggests that bone formation does not occur via endochondral ossification.

### CONCLUSIONS AND PERSPECTIVE:

Expedited gene transfer technologies allow the efficient repair of critical sized defects in a rat femoral model. These are being optimized as a prelude to evaluation in a large animal model. If successful, they promise to provide simple, cost effective methods for the repair and regeneration of massive bone defects.

### REFERENCES:

<sup>1</sup> C.H. Evans, G.D.Palmer, A. Pascher, et al (2007) *Tissue Eng* In Press. <sup>2</sup> O. Betz, V. Betz, A. Nazarian et al (2006) *J Bone Jt Surg Am* **88**: 355-365.

### ACKNOWLEDGEMENTS:

Supported, in part, by NIH (NIAMS RO1 050243) and the AO Foundation.