

## Nonviral DNA Vectors in Bone Regeneration

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**Background and Introduction:** There exists an ongoing need for technology to enhance gene transfection in vivo in an orthopedic setting, both in terms of vector design and in terms of DNA design. From a perspective of translation to clinical impact, nonviral approaches to gene delivery in orthopedics present a far simpler pathway than viral approaches. Candidate genes for induction of bone formation are genes that encode the bone morphogenetic proteins, of course, as well as transcription factors involved in mesenchymal stem cell differentiation into osteoblasts, including RUNX2.

**Rational:** As an approach to obtain sustained levels of therapeutic protein, such as of the BMPs, gene delivery presents a reasonable approach to sustained release approaches, since one transfection leads to prolonged expression. As an approach to obtain sustained levels of intracellular proteins such as transcription factors, transfection is also a logical choice, being far more straightforward than extracellular delivery of the transcription factor, e.g. with a membrane translocation tag. In the environment of an orthopedic defect, created by surgery or by trauma, an early competition for the delivered gene between macrophages and fibroblasts will exist. Presumably, transfected fibroblasts will have a much more prolonged impact in production of expressed extracellular proteins such as BMPs than will macrophages, given their longer lifespan. Thus, there exists a need to develop targeting approaches for fibroblasts in the environment of an orthopedic defect.

**Methods:** Multifunctional peptides are being explored as nonviral vectors for plasmid DNA.<sup>1</sup> Bifunctional peptides comprising a DNA-binding domain (lysine hexamers or nonamers), are flanked by cysteine residues to allow stabilization of formed nanoparticles by disulfide bonding in the extracellular environment. This DNA-binding domain is fused to a fibrin-binding domain, to retain DNA within a fibrin ingrowth matrix used as a surgical implant to localize administration of the DNA within a surgical defect.

**Results:** Preliminary results on the delivery of an angiogenesis-inducing gene, namely a mutant of the hypoxia-sensing transcription factor HIF1 $\alpha$  have been collected. The gene, condensed with the bifunctional peptide, has been delivered in dermal wounds of mice, and histomorphological indications of expression of genes downstream to this transcription factor, most notably vascular endothelial growth factor, are measured.<sup>2</sup> If effective transfection in this assay were taking place, then induction of angiogenesis within the tissue defect should be present. Such angiogenesis was indeed observed at prolonged durations.<sup>2</sup>

In order to adapt this system for gene delivery in bone, a number of changes are currently being made. Most notably, additional bifunctional peptides are being synthesized to bind to integrin receptors expressed on wound fibroblasts but not on macrophages. These peptides will be fused to DNA-binding peptides in an attempt to target the peptide-DNA nanoparticles to fibroblasts for selective uptake and transfection. Moreover, substantial engineering to the gene itself is underway. Notably, we are expressing the BMP-2 protein with a FLAG tag attached, to enable localized, quantitative determination of BMP-2 expression. This BMP fusion protein was demonstrated to be biologically active. Secondly, we are localizing the BMP-2 gene within a genetic environment that should enhance nuclear localization based on DNA sequence, rather than in earlier work based on nuclear localization sequences incorporated within the peptide-DNA nanoparticle.

**Discussion and Conclusion:** This project aims to adapt a soft tissue transfection system for use in forming new bone in bony defects, focusing on prolonged BMP-2 expression from transfected fibroblasts in the bone defect wound environment. Engineering is being conducted on the nonviral vector and on the gene itself, to optimize its uptake, nuclear localization and expression.

**References:** <sup>1</sup>D. Trentin et al. (2005) *J. Controlled Rel.* **102**:263-275; <sup>2</sup> D. Trentin et al., (2006) *Proc. Nat. Acad. Sci. USA* **103**:2506-2511

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