

## ORAL PRESENTATION: REGENERATE MEETING JUNE 2004

### A Novel Biopolymer Matrix Induces BMP-2 Production and Stimulates Bone Repair in Critical Size Ulnar Defects

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Non-union fractures of bone are a challenging clinical problem requiring a regenerative healing approach and unique biomaterials to ensure healing with sufficient strength in the repaired bone. Current therapies using autologous bone fragments and/or exogenous growth factors are often ineffective, and new approaches are needed. E-Matrix™ is a copolymer of denatured collagen and high molecular weight dextran designed to mimic early fetal connective tissue. Pre-clinical studies have demonstrated increased vascularization and reduced scar formation following treatment of cutaneous wounds with E-Matrix in several animal models. Dramatic effects on healing of diabetic foot ulcers in human subjects have also been obtained in a 6 patient clinical safety feasibility trial<sup>1</sup>. This study tested whether E-Matrix efficacy for bone repair *in vivo* correlates with the stimulation BMP-2 production *in vitro*. Quantitative PCR methodology was used to assess gene expression changes in human osteosarcoma cells following E-Matrix exposure, and expression of BMP-2 protein was measured using a sandwich ELISA method (R&D Systems). SAOS-2 cells were treated 40 minutes with E-Matrix at 37°C, refed with medium, and sampled for nucleic acid extraction after 3, 9, 18, and 24 hr; medium treated cultures were used as controls. RNA from each sample was reverse transcribed to cDNA using an oligo dT primer, and PCR reactions were performed targeting BMP-2 along with GAPDH as a reference gene. BMP-2 mRNA was increased 44-fold two hours after E-Matrix treatment and remained elevated for up to 5 hr. BMP-2 protein production was increased over controls by 24 hours and was 3-fold higher by 72 hr. A rabbit ulnar model was employed to assess the ability of E-Matrix to stimulate the healing of critical size bone defects *in vivo*. A bilateral ulnar defect was created by resecting a 15 mm section of ulna; the resulting defect was filled with E-Matrix alone (7 total) or a collagen sponge infiltrated with E-Matrix (7 total). Overlying soft tissue was closed with sutures, and splints were applied for approximately two weeks. Radiographic examinations were performed 2, 4, 6, 8 and 10 weeks after surgery. The animals were euthanized at 10 weeks. The forelimbs were then examined using micro-CT and the implant areas processed for histological examination. New bone was noted within all defects 2 to 4 weeks after treatment. Moderate or extensive bone growth were detected in 6 of 7 defects treated with E-Matrix infiltrated sponges and in 5 of 7 defects treated with E-Matrix alone. Micro-CT scans and histological examination confirmed the new bone growth. These studies conclusively demonstrate that E-Matrix stimulation of BMP-2 *in vitro* in human osteoblasts is linked to enhanced healing of critical size bone defects *in vivo*.

#### References:

1. Marston, W., Usala, A., Hill, R.S., Mendes, R. and Minsley, M-A. Initial report of the use of injectable porcine collagen matrix to stimulate healing of diabetic foot wounds in humans. Symposium on Advanced Wound Care Proceedings, C113, 2002.