INTRODUCTION
Recombinant osteoinductive growth factors with new osteoconductive matrices represent alternatives to autologous bone for grafting indications.

Growth and differentiation factor-5 (GDF-5) is a member of a divergent subgroup of the bone morphogenetic protein (BMP) family. Members within this subgroup share 40-50% protein sequence homology with the BMP-2 and BMP-7 subgroups. Expression of GDF-5 is required for proper skeletal patterning and joint development in the vertebrate limb, and null mutations result in brachydactyly syndromes in both animal and man.

Type I collagen and hydroxyapatite represent the predominant organic and inorganic components of natural bone. The osteoconductive nature of graft materials fabricated from either component alone, or in combination, is well documented. These components were shaped into a three-dimensional matrix comparable in composition and architecture to natural bone (HEALOS®). When combined with bone marrow aspirate as a source of osteogenic components, this mineralized collagen matrix was shown to perform similar to autograft in spinal fusion.

In previous animal studies, rhGDF-5 combined with HEALOS was osteoinductive and promoted new bone formation in various bony defects and spine fusion models. In this study, two doses of rhGDF-5 combined with HEALOS were tested in a rabbit posterolateral spine fusion model and fusion was evaluated at 2 different time points: at 4- and 8-weeks. Previous research indicated that fusions were mostly achieved by 5 weeks; the 4-week time point therefore gave an insight into premature fusion sites while the 8-week time point provided final fusion rates.

MATERIALS AND METHODS
Study design: All procedures were performed under IACUC approved protocols, at the Animal Resources Center at the University of Utah. Sixteen mature female New Zealand white rabbits were randomized into 4 groups. All rabbits underwent bilateral uninstrumented posterolateral lumbar fusion between L4 and L5 with either 0.25mg/cc or 0.50mg/cc rhGDF-5. For each dose, both 4- and 8-weeks post-operative time points were evaluated.

Implant preparation: The rhGDF-5 implants were prepared as following: rhGDF-5 was lyophilized onto hydroxyapatite coated collagen type I matrices (HEALOS®), which were hydrated in saline immediately prior to implantation. Each site was implanted with 1.5cc of rhGDF-5 matrix samples measuring 1x3x0.5cm.

Surgical procedure: The rabbits were shaved, positioned and prepped in a standard surgical fashion. The L4 and L5 transverse processes were identified using a fluoroscope and exposed through two para-median fascial incisions. After irrigation, the transverse processes were carefully decorticated. Attention was given to decorticate only the extremities of the transverse processes, such that fusion would occur far laterally, away from the vertebral body. The entire thickness of the transverse processes was decorticated until cancellous bone was visible. One 1.5cc strip was placed flat in-between the transverse processes such that both ends of the strip lay on top of decorticated bone tissues.

Analyses: X-rays were taken 0, 4 and 8 weeks post-operatively. At the 4- or 8-week end points, spine segments were harvested and tested for fusion by manual palpation. Fusions – defined as a continuum of bone tissue from transverse process to transverse process – was further confirmed by micro-CT at a 46μm resolution. Spine segments were then prepared for histology. Sagittal sections of PMMA-embedded, non-decalcified H&E stained fusion sites were analyzed for new soft and hard tissue formation.

RESULTS
All animals tolerated the procedure well. No animals were excluded from the study. After 8 weeks, the fusion rate of the 0.25mg/cc rhGDF-5 reached 87% while the 0.5mg/cc rhGDF-5 group achieved 100% fusion.

While special attention was given to place grafts as far lateral as possible to avoid fusion against the vertebral bodies, some fusion sites were observed spanning most of the width of the transverse process and/or touching the vertebral bodies (Figures 3 and 4).

Remodeling of the fusion mass against the bone of the transverse processes was observed as early as 4-weeks, in some of the 0.25mg/cc and 0.5mg/cc rhGDF-5 fusion sites. In all cases of non-fusion, significant bone masses were visible and palpable, but thorough, section-by-section examinations of 46μm CT analyses were required to reveal lack of bony continuum.

DISCUSSION
This study utilized a model previously described by others, but in a particularly challenging setting, as decortication was limited to the far end of the processes and did not extend to the curve of the transverse processes against the vertebral bodies. In addition, grafts were placed as laterally as possible to avoid fusion against the vertebral bodies. In some cases, fusion masses were therefore found lateral with little or no contact against the vertebral bodies, while in other cases, migration of the grafts against the vertebral bodies was observed.

While overall fusion rates were higher at 0.5mg/cc than at 0.25mg/cc rhGDF-5 (fusion rates of 100% vs. 87%, respectively), both 0.5mg/cc and 0.25mg/cc produced large bone volumes across the transverse processes, indicative of the osteoinductivity of rhGDF-5 at low concentrations.

These results indicate that rhGDF-5 delivered in a HEALOS matrix is an effective osteoinductive bone grafting material for spine fusion indications.

REFERENCES:

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