PURPOSE: The aim of this study is to describe the application of a tet-off inducible system for intervertebral disc gene therapy.
STUDY DESIGN/SETTING: Testing an adenoviral vector that expresses Fas Ligand (FasL) and Green Fluorescent Protein (GFP) under the control of a tetracycline-regulated gene expression system on nucleus pulposus cells.
PATIENT SAMPLE: Human nucleus pulposus cells (NPC) isolated from patients undergoing disc surgery.
OUTCOME MEASURES: Detection of GFP by imaging with a fluorescent microscope; evaluation of FasL expression by immunofluorescence; quantification of apoptosis using fluorescence activated cell sorting (FACS).
METHODS: Human NPC were plated in 6-well plates and transduced with Ad/FasL-GFPtet, at 0, 50,100, and 200 MOI. After 1 day, cells were cultured in the presence (1, 10, 100 mg/L) or absence of tetracycline for 2 days and then cultured again without tetracycline. Cells were imaged at day 1, 3, and 6 after transduction. The FasL expression was also documented by immunofluorescence. Apoptosis was assessed and quantified by FACS 24 hours after transduction and again after tetracycline administration.
RESULTS: Human NPC expression of GFP 1 day after transduction was proportional to the MOI used. GFP expression was not observed after 2 days of tetracycline administration at all concentrations used, either at the fluoro-microscope or by FACS. The cells again expressed GFP 3 days after removal of tetracycline. The fraction of apoptotic cells decreased after FasL transgene expression was stopped.
CONCLUSIONS: The transgene expression of FasL-GFP was efficiently regulated by inclusion of tetracycline in human NPC culture media. The presence of tetracycline turns off the protein expression and subsequent absence turns it on again. Therefore, we propose a tet-off inducible system as an efficient tool for modulating the transgene expression either for optimizing the therapeutic efficacy of the exogenous growth factors or for avoiding the toxicity that could derive from a misdirected injection.
FDA DEVICE/DRUG STATUS: This abstract does not discuss or include any applicable devices or drugs.
CONFLICT OF INTEREST: No conflicts.

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4:28

98. Chondrocyte Based Gene Therapy for the Degenerating Intervertebral Disc in the Rabbit Disc Organ Culture System

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BACKGROUND CONTEXT: Although the etiology of back pain is multi-factorial, intervertebral disc (IVD) degeneration is associated, perhaps causally, with back pain. Degenerative discs are characterized by altered matrix composition and reduced cell number. Biological treatments that restore disc matrix and cellular elements are promising alternatives to the surgical removal of the diseased disc.
PURPOSE: This is a feasibility study to demonstrate that cell based gene therapy may be a viable alternative to the direct injection of growth factors or vectors expressing growth factors into the IVD. This approach takes advantage of the ability of chondrocytes to populate the disc and sustain the release of growth factors that promote matrix formation. Autologous chondrocytes are readily available and are phenotypically similar to disc cells and, therefore, represent an appealing cell-type for transplantation.
STUDY DESIGN/SETTING: In vitro experiment.
PATIENT SAMPLE: N/A.
OUTCOME MEASURES: Survival of injected chondrocytes was confirmed with fluorescence microscopy at the end of the culture period. Proteoglycans accumulated by the IVD explants were assessed with the DMMB method.
METHODS: Whole IVD explants with end plates on both sides, and knee articular chondrocytes were isolated from young adult New Zealand White rabbits. These were cultured in DMEM/F12 supplemented with 20% FBS and ascorbic acid for 2 months. Chondrocytes transduced with adenovirus expressing human bone morphogenetic protein-7 (Ad-hBMP-7), and green fluorescence protein (AdGFP, as control) were injected into cultured IVD explants.
RESULTS: The disc retained its integrity without morphologic evidence of deterioration during the 2-month culture period. Rabbit articular chondrocytes transduced with Ad-hBMP-7 survive for at least 2 months after injection into the IVD explants, and appear to have successfully integrated into the host tissue. Graft cells were stained with Mito-tracker Red, which stains live cells. Furthermore, rabbit IVD explants injected with articular chondrocytes expressing hBMP-7 accumulated 49.2% more proteoglycans in the nucleus pulposus (NP) than those injected with articular chondrocytes transduced with AdGFP (control), 1 month after injection.
CONCLUSIONS: We have successfully cultured entire disc with intact end plates in culture for up to 2 months. We have shown, for the first time, that rabbit articular chondrocytes transduced with Ad-hBMP-7 can survive in the rabbit IVD explant where it stimulates matrix production in the NP. Chondrocytes are widely available, and can be harvested from nonspinal sites with relatively low morbidity. There also exists substantial clinical experience with chondrocyte transplantation for treatment of articular defects, making these an attractive cell population for promoting disc repair. Our encouraging findings suggest that chondrocyte transplantation may represent a promising strategy for the treatment of disc degeneration.
FDA DEVICE/DRUG STATUS: This abstract does not discuss or include any applicable devices or drugs.
CONFLICT OF INTEREST: No conflicts.

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4:34

99. Biologic Collagen PMMA Injection (Artefill) Repairs Mid-Annular Concentric Defects in the Ovine Model

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BACKGROUND CONTEXT: Annular injury in the animal model will often lead to degenerative disc disease (DDD). The natural repair of these defects often includes ingrowth of nerve or blood vessels beyond their traditional location in the outer third of the annulus. The ability to aid in this reparative process without increasing the inflammatory response would offer early treatment options in DDD.
PURPOSE: A percutaneous treatment using PMMA microspheres and bovine collagen agent is used in this study. This can be implanted into the disc space through direct injection of the nucleus or local treatment of the exposed annulus. In choosing this agent, it is our feeling that the reparative process available in the annulus can be enhanced and sealing of annular tears may allow for the maintenance of normal disc cytoarchitecture. The ability to intervene in degenerative disc disease and internal disc derangement in a reparative process and to maintain disc height as opposed to a destructive process is the basis for our diagnostic and treatment approach.
STUDY DESIGN/SETTING: An animal model for degenerative disc disease was first described by Osti and then followed by Melrose. This commonly used model, which has been replicated recently by Fazzalari et al., is an excellent model for study of degenerative changes in the disc space. The lumbar sheep spine is biomechanically similar to the human spine and its lumbar intervertebral discs are structurally very similar to humans.
PATIENT SAMPLE: We used six separate disc levels in Merino wethers of middle age. After exposure of the lumbar discs via left retroperitoneal approach, a concentric injury was made in the mid annulus of the left outer quadrant of the disc. The control levels were left without treatment after

the injury, and the experimental group were injected with the Collagen/PMMA into the created concentric tear.
OUTCOME MEASURES: The animals were sacrificed at 3 and 6 months. The discs were preserved in formalin and decalcified for histology. Axial and coronal slices were prepared using hematoxylin-eosin or Masson's Trichrome.
METHODS: The histologic samples were evaluated by an independent pathologist to identify inflammatory response, incorporation, new collagen formation, migration and ingrowth of nerve or blood vessels.
RESULTS: Histologic hematoxylin-eosin-stained sections revealed maintainence of the microspheres without migration to outer or inner annulus. Encapsulation of the injected material occurred early, within the first 6 weeks. Healing of the annular defect occurred with formation on new collagen and without intense inflammatory response (Fig. 1). There was no evidence for accelerated ingrowth of nerve fibers or blood vessels within the disc space. Control discs revealed progressive degenerative changes in the annulus surrounding the created defect. No adverse events were noted due to the treatment arm.
CONCLUSIONS: In conjunction with histology and MRI findings, outer annular healing occurred in the treatment levels. We conclude that this is a safe product that holds future promise for treating degenerative disc disease with a biochemical approach.
FDA DEVICE/DRUG STATUS: Arteseal: Investigational/not approved.
CONFLICT OF INTEREST: Author (WT) Board Member: Creative Spine Solutions.

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4:40

100. Neurotoxic Potential of Submicron Particles of UHMWPE

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BACKGROUND CONTEXT: Total disc replacements (TDR) for the intervertebral joint are composed of various bearing surfaces that will produce wear particles. The potential for these wear particles to have a neurotoxic effect on the central nervous system needs to be addressed.
PURPOSE: The purpose of this study was to determine the neurotoxic and osteolytic potential of ultrahigh molecular weight polyethylene (UHMWPE) particles injected into the epidural space without the surgical trauma, mimicking the long-term situation post-surgical implantation period with a normally functioning device.
STUDY DESIGN/SETTING: GUR 1050 UHMWPE particles were analyzed for average diameter and four size ranges and concentrations prepared (Table 1).

Table 1

Size	Concentration particles/ml
UHMWPE size 1 average median diameter: 0.79±0.16 μm	1.86E+09
UHMWPE size 2 average median diameter: 2.43±0.26 μm	4.13E+07
UHMWPE size 3 average median diameter: 6.62±1.04 μm	3.29E+07
UHMWPE size 4 average median diameter: 12.43±2.80 μm	1.46E+07

PATIENT SAMPLE: not applicable.
OUTCOME MEASURES: not applicable.
METHODS: Epidural injections under direct visualization were performed on 54 New Zealand White Rabbits at L7-S1. Each animal was dosed with 0.3 ml of the carrier solution, 0.9% NaCl, or with one of four UHMWPE sized particles. Animals survived for either 3 or 6 months. Daily observations were done for any clinical signs of neurotoxicity (blindness, ataxia). At necropsy, the lumbar spine was removed with spinal cord intact; brain and the heart, lung, kidney, spleen, liver, and tracheobronchial lymph nodes were retrieved and evaluated histologically.

Cycles ($x10^6$)	Mean ± Std Dev. (μm)	Max (μm)	Min (μm)
2	0.35 ± 0.13	0.92	0.16
7	0.48 ± 0.14	0.87	0.21
8	0.41 ± 0.19	1	0.14
9	0.40 ± 0.25	1.38	0.08
10	0.42 ± 0.37	2.29	0.1

Fig 1. Simulator results.

RESULTS: No clinical signs of neurotoxicity were observed. Soft-tissue histology revealed no lesions associated with the injections or the UHMWPE particles. Undecalcified histological evaluation from coronal sections from L4-L5 was evaluated for inflammation, neuronal degeneration, dural irritation, and vertebral bone loss, and residual implant material. In a 6-month submicron particle group rabbit, mild neural cell degeneration and mild bone reaction on the periosteal surface was seen. All other subjects showed no reaction (Fig. 1).
CONCLUSIONS: The potential for tissue reaction to submicron to micron sized particles of UHMWPE was evaluated after lumbosacral epidural injection. Particle size and dose used in this study was meant to mimic the size and quantity bell curve found in wear simulator studies. Submicron UHMWPE particles represent the greatest potential for neurotoxicity as these can easily be attached to cell walls or engulfed. The dose that was delivered represents the neural tissues being exposed to a larger quantity of particles at once than would be expected clinically in well functioning prostheses. This worst case neurotoxic potential was evaluated short and long term, and a mild reaction was seen in only 1 of 12 rabbits in the submicron particle group and in 0 of 36 rabbits exposed to particles larger than 2 microns.
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CONFLICT OF INTEREST: Authors (HA, CA) Employee: Synthes; Author (DD) Grant/Research Support: Synthes Spine.

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4:22–5:22 PM
Special Interest Poster Presentation 5: Fusion With New Technologies

4:22

101. Posterolateral Lumbar Spine Fusion With Infuse Bone Graft

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BACKGROUND CONTEXT: Infuse has been proven effective in conjunction with threaded cages and bone dowels for single-level ALIF.