INTRODUCTION: Spinal fusion has become a popular surgical technique used to provide segmental fixation and to maintain stability and correct deformity. As more spinal fusions are performed, problems of fusion failure or pseudoarthrosis as well as complications at the bone graft donor site are becoming more common. Ex vivo gene therapy involving mesenchymal stem cells (MSCs) and bone morphogenetic protein (BMP) genes can provide a local supply of precursor cells and a supraphysiological dose of osteoinductive molecules that may promote bone formation and lead to spinal fusion. Exogenous control and monitoring of transgene expression in vivo is of great importance for gene therapy. In such a system, transgene expression can be regulated over time at a defined level of expression, thus controlling the regeneration process. We have already shown that posterior spinal fusion is possible by injecting exogenously regulated MSCs into the paraspinal muscles (Hasharoni, et al., 2005). Nevertheless, it is well recognized that anterior spinal fusion or interbody fusion is a more favorable goal in terms of spinal stabilization and biomechanics. We hypothesized that direct injection of MSCs that overexpress the human BMP-2 (hBMP-2) gene under a tetracycline-controlled expression system (Tet-Off system) could induce regulated bone formation and lead to interbody anterior spinal fusion.

METHODS: Our institutional animal care and use committee approved all procedures, which were consistent with guidelines set forth in the Guide for the Care and Use of Laboratory Animals. We used a Tet-Off system in which genetically engineered MSCs harbor the inducible human BMP-2 expression vector (ptTATop-BMP2), which has a bidirectional promoter (TATA sequence) that uses elements of the tetracycline regulatory system. The system was regulated by the administration of Doxycycline (Dox), a tetracycline analog. To determine the exact volume that can be injected into a mouse intervertebral disc, we injected the contrast agent Telebrix into a caudal disc in a mouse. Following the establishment of the injection system, C3H/HeN immunocompetent female mice, between the ages of 6 and 7 weeks, were anesthetized with an intraperitoneal injection of ketamine and xylazine. Each animal received an injection of 10^5 genetically engineered MSCs into caudal and lumbar intervertebral discs under fluoroscopic guidance. The animals were provided with water and food ad libitum throughout the duration of the study. Animals in the negative control group were given drinking water supplemented with 0.5 mg/ml Dox. Animals in the experimental group received no Dox. A longitudinal quantitative analysis of vertebra interbody fusion was performed using noninvasive micro-computerized tomography (µCT), which enabled us to quantify bone density, volume and trabecule number and thickness at the site of the injection. In addition, engineered MSCs were also infected with retroviruses encoding for the luciferase or green fluorescent protein (GFP) marker gene. Cell survival within the disc was monitored noninvasively by using a quantitative bioluminescence imaging system and a novel in vivo fibered confocal microscopy imaging system (Cell-vizio).

RESULTS: Fluoroscopic monitoring during injection of the radiocontrast agent Telebrix into the disc confirmed that the injected material was localized in the region of the nucleus pulposus (Figure 1 A&B, arrow). Three-dimensional reconstruction of µCT images obtained in experimental and control animals at 4 weeks postinjection revealed bone formation and fusion of vertebral bodies in the area adjacent to the nucleus pulposus in all the animals in the experimental group (Figure 2A & B). We did not find any new bone formation in the control animals, in which hBMP-2 expression had been inhibited (Figure 2C & D). In vivo quantitative noninvasive imaging of the injected genetically engineered MSCs was achieved using the bioluminescence system. Longitudinal monitoring of the luciferase-labeled MSCs in vivo at 1, 2 and 4 weeks postinjection revealed a high level of expression up to 4 weeks postinjection (Figure 3A, arrow). In addition, to detect GFP labeling at the cellular level, we used a novel system of molecular imaging called Cell-vizio. This is a fluorescence-based imaging system based on a fibered confocal microscope, which is able to detect viable cells expressing the GFP marker gene in the intervertebral disc up to 4 weeks postinjection (Figure 3B).

DISCUSSION: Exogenously regulated expression of hBMP-2 enabled us to regulate interbody fusion in vivo by using the genetically engineered MSC system (Tet-Off). The effect of hBMP-2 in inducing bone formation was monitored in real time noninvasively and quantitatively. Our data demonstrate for the first time the use of an injectable, regulated, and monitored system for the induction of anterior spinal fusion. These results may pave the way toward the advancement of a biological solution for spinal fusion that will replace currently nonoptimal prosthetic implants.