INTRODUCTION: Although autogenous cancellous bone is currently the "gold standard" for stable spinal fusion, supply is limited and donor site complications exist. Allografts are an alternative but not desirable because of immunogenicity and the potential to transmit diseases. Bone graft substitutes are therefore being investigated extensively. Successful use of bone grafts in spinal fusion is based on the osteogenic, osteoinductive, and osteoconductive properties of the grafts. In this study, we used a cloned osteoprogenitor cell to serve as a graft material for spinal fusion.

MATERIALS AND METHODS: An osteoprogenitor cell, D1-BAG, cloned from Balb/c mice and transfected with LacZ and neomycin resistance genes, and mixed stromal cells from marrow blowouts, were used in athymic rats to establish a posterior spinal fusion. The cells were cultured for 7 to 10 days and their osteogenic properties in vitro examined by measuring osteocalcin mRNA using Northern blots and alkaline phosphatase activity. A suspension containing 20 x 10^6 cells/ml phosphate buffered solution was prepared for cell transplantation. For spinal surgery, thirty-six athymic Harlan nude rats were used in the experimental group and sixteen in the control group. The fusion bed from L3 to L5 was prepared. Two million D1-BAG cells or marrow stromal cells were suspended in a solution containing 1 mg Matrigel in a total volume of 100 µl and implanted into the fusion bed of animals in the experimental group, while Matrigel without cells was used in control animals. Lumbosacral spine x-ray and CT scans were obtained immediately after surgery and at 2, 3, 6, and 9 weeks. Tissue specimens were fixed, decalcified, stained with x-gal to identify implanted D1-BAG cells. Histological sections were counter-stained with hematoxylin and eosin and examined microscopically. New bone formation in fusion beds was quantitated in histological sections using image processing software (Image Pro).

RESULTS: D1-BAG cells are pluripotential and primarily osteogenic, as demonstrated by the expression of osteocalcin mRNA and positive staining for alkaline phosphatase. Two weeks after surgery, radiopaque tissue was seen at transplantation sites with either D1-BAG cells or marrow stromal cells plus Matrigel, but not at sites with Matrigel alone. Histology demonstrated that these tissues consist of newly formed trabecular bone. X-gal positive cells were detected in the spinal fusion mass of animals which received D1-BAG cells by transplantation. PCR of DNA extracted from the fusion mass demonstrated presence of the neomycin resistance gene indicating that newly formed bone originated from transplanted cells. Histomorphometric measurements showed that bone formation began at 2 weeks and reached the highest levels at 6 weeks (Figure 1). No cartilaginous tissue was noticed at any time point during the study in animals transplanted with D1-BAG cells, indicating that the process of ossification was not endochondral. However, endochondral bone formation was observed in animals transplanted with mixed stromal cells. Histological sections of all lumbar spine specimens (N = 52) examined morphometrically using a computerized image analysis system showed that transplantation of D1-BAG cells into the paravertebral space produced more bone and better spinal fusion (Figure 1). X-ray examination and CT scans (Figure 2) demonstrated that animals transplanted with D1-BAG cells had achieved spinal fusion by 9 weeks.

DISCUSSION: The cloned osteoprogenitor cell, D1-BAG, can form bone after transplantation into para-spatial sites, leading to spinal fusion. D1-BAG cells form bone directly without endochondral changes indicating that cells cloned from bone marrow stroma can serve as a grafting material and contribute to improved bone formation. Compared to mixed marrow stromal cells, cloned osteoprogenitor cells can produce larger amounts of mature osseous tissue at an earlier time point. In addition, this study demonstrates that gene labeling is a useful tool to study cell differentiation in vivo and that osteoprogenitor cells may serve as a carrier of genetically engineered factors in the treatment of skeletal diseases.

References:

Figure 1. Volume of newly formed bone in the spinal fusion mass. Histomorphometry was performed on five sections taken from each specimen (At each time point, 8 animals were transplanted with D1-BAG cells and 4 animals each either transplanted with mixed stromal cells or with Matrigel only). Mean volume of trabecular bone was obtained by measuring total pixels.

Figure 2. Three-dimensionally reconstructed CT of rat spine. A. from animals transplanted with Matrigel (carrier) only which served as the control, B. from animals transplanted with both Matrigel and D1-BAG cells showing marked bone formation within the spinal fusion mass.