INTRODUCTION
Muscle-derived stem cells (MDSCs) are a potential cell source for orthopedic tissue engineering applications (1). Recent research has demonstrated sex-related differences in the abilities of male and female MDSCs to promote skeletal muscle regeneration, with female MDSCs exhibiting the higher transplantation efficiency (2). However, we have never directly compared the osteogenic and chondrogenic potentials of male MDSCs with those of female MDSCs. The objective of this study was to determine if MDSCs also display sex-related differences in their abilities to promote osteogenesis and chondrogenesis.

METHODS
Isolation and culture of MDSCs: A modified preplate technique (3) was used to isolate 4 populations of MDSCs from 3-week-old C57BL/10J mice: 2 female populations (F-MDSC1 and F-MDSC2) and 2 male populations (M-MDSC1 and M-MDSC2). Cells were cultured in phenol red-free proliferation medium (DMEM supplemented with 10% FBS, 10% HS, 0.5% chick embryo extract, and 1% penicillin/streptomycin) and maintained at less than 30% confluency.

Osteogenic differentiation: MDSCs were initially plated at a density of 1500 cells/cm² and were stimulated on the following day with proliferation medium supplemented with bone morphogenetic protein 4 (BMP4; 200 ng/ml). After 2 days of stimulation, the presence of alkaline phosphatase (ALP), an early osteogenic marker, was evaluated by cytochemical staining (AP Kit 86-C, Sigma Diagnostics).

Chondrogenic differentiation: The 4 populations of MDSCs were retrovirally transduced with the CLB2/4G vector to express human BMP4 and cultured as pellets in chondrogenic medium (DMEM supplemented with 10% FBS, 1 ng/ml BMP4, 200 ng/ml pyruvate, [100 µg/ml], and ITS+Premix [50 mg/ml; Becton Dickinson]), in the presence or absence of transforming growth factor-beta 1 (TGF-β1; 10 ng/ml). The medium was replaced every 2–3 days, and the pellets were harvested on days 14 and 21 for paraffin embedding. Chondrogenesis was evaluated histologically by staining with Alcian blue for proteoglycans and with Safranin O for proteoglycans.

Statistical analysis: All experiments were done in triplicate. Data were reported as mean±standard deviation and were analyzed by one-way ANOVA. A Tukey test was used for pairwise comparisons (SigmaStat, Jandel Corporation).

RESULTS
Osteogenic differentiation: Stimulation of MDSCs with BMP4 increased the percentage of ALP-positive cells in all tested populations. A significantly higher percentage of M-MDSCs than F-MDSCs were ALP positive (P<0.05, Figure 1).

Chondrogenic differentiation: Sex-related differences were also apparent in the pellet culture experiments. After 14 days in culture, both male populations displayed a denser extracellular matrix than the female populations and stained positive for Alcian blue (Figure 2). All pellets contained cells that resembled chondrocytes (Figure 2, arrows), but the pellets comprising male MDSCs displayed the clearest chondrocyte morphology. After 21 days in culture, the female MDSCs began to exhibit an Alcian blue-positive matrix and the pellets contained cells with round morphology similar to that of chondrocytes (data not shown). Safranin O staining revealed that only one of the M-MDSC pellets contained proteoglycans at both 14 and 21 days, whereas pellets made with female MDSCs did not (data not shown). Interestingly, MDSCs cultured in chondrogenic medium without TGF-β1 also displayed chondrocytic morphology, although the cells took longer than cells cultured with TGF-β1 to adopt that morphology (data not shown).

DISCUSSION
The results from this study indicate that male and female MDSCs can be induced towards both the osteogenic and chondrogenic lineages when cultured with BMP4; however, the rates at which they undergo such differentiation differ. Our group has previously reported that female MDSCs have a higher regeneration index in skeletal muscle than do male MDSCs (2). The study reported here, however, indicates that MDSCs isolated from male mice undergo osteogenic and chondrogenic differentiation more readily than MDSCs isolated from female mice. We are repeating these experiments with more populations of male and female MDSCs and are examining additional markers of osteogenesis and chondrogenesis to confirm any sex-related differences. Research has shown that adipose-derived stromal cells also exhibit sex-related differences: Cells from female mice differentiate into adipocytes more readily than their male counterparts (3). Those findings suggest that further investigation of the putative sex-related differences exhibited by MDSCs and other stem cells and the mechanisms underlying these differences is warranted. The findings generated by such studies could facilitate efforts to advance the use of stem cells in cell therapy and tissue engineering for bone and cartilage repair.

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