Optimized Delivery of Bone Morphogenetic Protein cDNA to Human Mesenchymal Stem Cells with Adenovirus 
Induces Efficient BMP-2 Expression and in vitro Mineralization

INTRODUCTION
Recombinant Bone Morphogenetic Protein-2 (rBMP-2) is widely used in the clinic to promote bone healing, even though it fails to stimulate the deposition of mineralized matrix by human Mesenchymal Stem Cells (hMSCs)\(^1\). As an alternative to administering rBMP-2, transfection of MSC genetically modified to overexpress a BMP transgene remains an attractive therapeutic approach as it provides both BMP and a sequestered osteoprogenitor cells\(^2\). Recombinant adenovirus (Ad.) vectors have been extensively used for MSC modification as they (i) lead to transient expression of the BMP transgene (only a few week expression of BMPs is likely to be required until the bone is healed) and (ii) do not integrate into the host genome. However, the bone forming capacity of BMP-2 engineered hMSCs is inconsistent\(^3\). Such limited osteogenic potential could result from either (i) the poor infection rate of hMSCs, limiting the successful gene expression or (ii) the poor responsiveness of hMSCs to BMP-2\(^1\). The aim of the present study was to improve the adenoviral transduction of hMSCs by centrifuging, and to optimize this method\(^4\) to obtain efficient BMP-2 expression by Ad.hBMP-2 transduced hMSCs. The osteoblastic differentiation of hMSCs in response to optimized BMP-2 expression was then evaluated.

METHODS
Optimal centrifugation parameters for efficient hMSC transduction
In accordance with an IRB-approved protocol, bone marrow-derived MSCs were isolated from the intramedullary canal of patients undergoing hip hemiarthroplasty and expanded as previously described\(^6\). First generation adenovirus serotype 5, carrying human BMP-2 cDNA (Ad.hBMP-2), or green fluorescent protein cDNA (Ad.GFP) driven by the cytomegalovirus early promoter were prepared as previously described\(^7\). After addition of the virus (5000 viral particles per cell (vp/c)), the cells were centrifuged under prescribed centrifugation forces at 37°C for prescribed periods of time. After centrifugation, the virus-containing medium was removed. BMP-2 secretion and cell proliferation were quantified to evaluate transduction efficiency.

Effect of Ad.hBMP-2 transduction on hMSC osteoblastic differentiation and BMP-2 expression
hMSC were transduced with various doses of Ad.BMP-2 or Ad.GFP at 2000g for 30 min at 37°C. Transduced cells were maintained in media supplemented with 50 ug/ml ascorbic acid and 10 mM beta-glycerophosphate for 21 days. Non-transduced dexamethasone (dex, 10 M) treated cells were used as positive controls. Osteoblastic differentiation was assessed by the induction of alkaline phosphatase (ALP) enzyme activity and the deposition of a mineralized matrix. BMP-2 production by transduced hMSCs was quantified in cell supernatants at prescribed time points by ELISA.

RESULTS
Optimal centrifugation parameters for efficient hMSC transduction
BMP-2 expression was clearly dependant on the centrifugation force and/or duration (Figure 1a). hMSC transduced with Ad.BMP-2 at 2000g for 90 min showed a 67-fold increase in BMP-2 production compared to non-centrifuged cells 3 days after treatment. Unexpectedly, centrifuging the cells at 2000g for 90 min compromised the plastic adhesion/proliferation properties of hMSCs, regardless of the presence of the virus (Figure 1b). Centrifugation at 2000g for 30 min was the optimal parameter as it resulted in high transduction efficiency, good cell proliferation and good reproducibility.

Effect of Ad.hBMP-2 transduction on hMSC osteoblastic differentiation and BMP-2 expression
Increased Ad.hBMP-2 doses resulted in increased BMP-2 expression. 100, 500 and 5000 vp/cled to 1, 18, and 440 ng secreted BMP-2/ml/48h, respectively, 5 days after transduction (Figure 2a). Regardless of the virus dose, maximal amounts of BMP-2 (ug/ml/48h) were secreted at day 14 after transduction. Interestingly, doses above 500 vp/c Ad.hBMP-2 led to concentrations of secreted BMP-2 that were capable of stimulating hMSC ALP activity to the same extent than dex (Figure 2b). Notably, Ad.hBMP-2 transduced hMSCs showed matrix mineralization to the same extent as dex treated cells (Figure 2c).

CONCLUSIONS
This study provided the evidence that (i) Ad.hBMP-2 engineered hMSCs are capable of producing high quantities of BMP-2 and (ii) hMSCs can undergo an osteoblastic differentiation pathway through ALP expression and matrix mineralization in response to BMP-2. It is interesting that BMP-2 delivered by adenoviral transduction is able to induce mineralization by BMP-2, whereas the recombinant protein cannot\(^6\). This suggests that gene delivery may be superior to protein delivery as a mean of inducing bone formation. The reasons for that are under investigation.

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REFERENCES
\(^1\)Diefendorfer et al. Connect Tissue Res. 2003.