INTRODUCTION
Recent evidence indicates that intervertebral disc (IVD) degeneration is associated with ongoing mineral deposition [1]. The presence of calcium deposits and type X collagen (COL X) in degenerative IVDs, but not in control discs, and the level of the indicators of calcification potential (alkaline phosphatase (ALP), Ca²⁺ ions and Pi) was consistently higher in degenerative and sclerotic discs than in control discs. We also showed that in mesenchymal stem cells (MSCs), parathyroid hormone (PTH 1-34) is able to inhibit the expression of COL X while promoting the expression of COL II, thereby preventing endochondral ossification [2]. In this study, we investigated the effect of PTH on expression of COLII, COLX and ALP in human IVD cells and analyzed the potential mechanisms related to its effect.

MATERIALS AND METHODS
Isolation and culture of human IVD cells: Human lumbar IVDs from donors without spinal pathology were obtained through organ donations via Transplant Quebec within 24h after death. The procedure was approved by Institutional Research Ethics Committee. Nucleus pulposus (NP) and annulus fibrosus (AF) tissues from the IVDs were digested and the corresponding NP and AF primary cells were isolated as previously described [3]. These cells were cultured in DMEM supplemented with 10%FBS, 100U/ml pencillin and 100 μg/ml streptomycin to 80% to 90% confluence. Then the cells were incubated overnight in serum free medium followed by treatment with 100nM PTH 30 min to 48h. The cell extracts were prepared using NP40 lysis buffer. Total protein in the cell extracts was determined by Bradford assay. Protein expression was analysed by immunoblotting using specific antibodies to COL II, COL X, COL I, AKT, phospho-AKT and MAPKs (phospho-ERK, ERK, phospho-JNK, JNK, phospho-p38 and p38). Western blot images were quantified by using ImageJ (NIH) software. Alkaline Phosphatase activity was measured colorimetrically using the StemTAG kit (Cell Biolabs, San Diego, CA).

Statistical analyses were done using One-way ANOVA, post-hoc test.

RESULTS
PTH increased the expression of COL-II significantly in both AF and NP cells in a time-dependent manner, from 6 to 48h of incubation (Figure 1). Expression of COL-X was not altered in AF cells, whereas it decreased in NP cells from 6 to 48h (Data not shown). However, the activation of MAPKs (pERK, Figure 2) occurred much earlier, by 30min of incubation with PTH in both cell types. Similar results were obtained with JNK and p38 (data not shown). The activity of alkaline phosphatase was significantly decreased by PTH after 24h of incubation in NP cells. In AF cells, no significant changes were observed, even though there was a trend to increase at 48h (Figure 3).

DISCUSSION
PTH has previously been shown to promote chondrogenesis and inhibit the expression of COL-X in chondrocytes probably via the activation of MAPK signaling pathways [4]. The present results demonstrate that PTH up-regulates COL-II and down-regulates COL-X in IVD cells, indicating that PTH has the potential of being able to stimulate disc repair and improve nutrient supply in the degenerative disc. Our data also suggests that activation of MAPK pathway takes place much earlier than the alterations in COL-II or COL-X expression. Interestingly, COL-II expression inversely correlates with alkaline phosphatase activity in NP cells treated with PTH.

SIGNIFICANCE
A thorough understanding of IVD calcification would be of great value, not only for elucidation of its mechanism, but with an eye toward eventual therapeutic intervention. PTH can thus be used towards disc regeneration therapy.

REFERENCES

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