INTRODUCTION
Recent work has shown that joint contracture severity can be decreased with the mast cell stabilizer ketotifen in association with decreased numbers of myofibroblasts and mast cells in the joint capsule of a rabbit model of post-traumatic contractures. Neuropeptides such as Substance P (SP) can induce mast cells to release growth factors. Using a gel contraction assay, we test the hypothesis that joint capsule cell-mediated contraction of a collagen gel can be enhanced with Substance P, but the effect is magnified in the presence of mast cells.

METHODS
With prior approval from our ethics board, anterior elbow joint capsules were obtained at the time of surgical release from 2 men (age 34 and 54) and 1 woman (age 40) with chronic (> 1 year) post-traumatic joint contractures. The capsules were minced, placed into culture flasks and resulting cells incubated. Upon confluence, cells were trypsinized, re-suspended at a density of 2.5 x 10^6 cells/ml, and mixed with neutralized Collagen solution composed with 58% Vitrogen 100 purified collagen. The human mast cell line, HMC-1, was obtained from J. H. Butterfield (Mayo Clinic, Rochester, MN). Substance P and the NK1 receptor antagonist RP67580 (NK1 is the SP receptor) were obtained from Sigma (Oakville, ON).

Aliquots (500 µl) of collagen gel with only HMC-1 cells (7.5 x 10^5), human capsule cells (2.5 x 10^5), or human capsule cells (2.5 x 10^5) and 7.5 x 10^5 mast cells (1:3) were then casted into wells of a 24-well tissue culture plate. In some experiments, SP (1 x 10^-5 M) +/- RP67580 (0.5 mM) were added. The gels were maintained with 0.5 ml DMEM composed with 2% BSA and incubated at 37°C for 12 h for gelation to occur. After 12 initial culture, the gels were detached from the wall and the bottom of culture plate wells, and photographed at 0h, 2h, 4h, 6h, 24h, 48h, and 72h post-release using ChemiDoc XRS (BIO-RAD Mississauga, ON). The areas of gel were measured using an image analyzer (Image J, National Institutes of Health, USA). Gel contraction studies were carried out on passage 4 and done in triplicate for each patient. The average value of each patients’ triplicate was combined to give a mean contraction at each time point.

Statistical analysis involved an ANOVA with posthoc Bonferroni correction. P < 0.001 was significant.

RESULTS
Mast cells alone or with SP were unable to contract collagen gels (Figures 1 & 2). Joint capsule cells were able to contract the collagen gels and this was enhanced in the presence of SP, although not statistically significant. Joint capsule cells combined with mast cells enhanced the gel contraction more than joint capsule cells alone or with SP (p<0.001). The addition of SP accelerated the joint capsule cell-mediated gel contraction in the presence of mast cells the greatest (p<0.001 over all other conditions). Finally, the inhibitor RP67580 completely abolished the collagen gel contraction of the joint capsule cells, even when mast cells and SP were present.

DISCUSSION
The in vitro experiment shows that joint capsule cell function, in the form of collagen gel contraction, is modified by the presence of mast cells and neuropeptides. Joint capsule cells contract collagen gels alone, but this is enhanced by SP or mast cells, more so by mast cells. The combination of Substance P and mast cells accelerates the contraction the most. This response is specific since it is completely abolished with the addition of the NK1 receptor antagonist RP67580.

Previous published work is consistent with our results. Substance P and RP67580 have been used successfully to modify in vitro properties in ligament and synovial explant and isolated cell experiments, although not collagen gel contraction assays. The ability of skin and lung fibroblasts to contract collagen gels is enhanced by the addition of mast cells. Mast cells themselves do not cause contraction of the gels. Our work is unique in that we combined mast cells and neuropeptides, and we did these experiments using human joint capsule cells.

These findings are significant as they strengthen the hypothesis that a myofibroblast – mast cell – neuropeptide fibrosis axis may be contributing to the joint capsule changes underlying the loss of motion in post-traumatic joint contractures. Several directions require further study. With regards to the collagen gel assays, characterization of the joint capsule cells is required, including whether mast cells are a part of the cell population. Specific mast cell components will be investigated in future. Finally, in vivo studies with the rabbit model of post-traumatic contractures will be performed using the compounds examined in the current study.

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REFERENCES