**Introduction:** Studies on human and rabbit joint capsules obtained from joints with post-traumatic contractures have identified an association between elevated numbers of myofibroblasts and contracture severity.[1,2] Myofibroblasts are fibroblasts that express contractile smooth muscle proteins including α-smooth muscle actin (α-SMA) and contract collagen gels more forcefully than fibroblasts. Neuropeptides are mediators that cause mast cells to degranulate, releasing factors that up regulate myofibroblast numbers and activity.[3,4] Mast cells and neuropeptides have been associated with fibrotic conditions.[3-8] Our hypothesis is that mast cell numbers and neuropeptide containing nerve fiber numbers are significantly elevated in joint capsules exhibiting post-traumatic contractures.

**Materials and Methods:** This study was approved by our institution's ethics board and animal care committee and informed consent was obtained from the patients. Joint capsules were obtained from 6 patients (29±12 yrs old, 50:14φ) undergoing surgical release for chronic post-traumatic contractures, 6 organ donors free of elbow contractures (26±15 yrs old, 40:26φ) and 6 skeletally mature female New Zealand White rabbits with one knee having a surgical intervention that leads to a post-traumatic contracture and the contralateral knee an unoperated control. The six rabbits had an intraarticular fracture coupled with immobilization of one knee which leads to contractures.[1] The rabbits were sacrificed 4 weeks after surgery. The joint capsules were placed immediately into OCT and frozen with liquid nitrogen for immunohistochemistry evaluation. A triple labeling technique was used following protocols established in our laboratory.[1,2] Sections 8 mm thick were analyzed. Monoclonal antibodies to α-SMA coupled with a peroxidase secondary antibody were used to detect myofibroblasts; polyclonal antibodies to chymase, an enzyme unique to mast cells, coupled with an appropriate Alexa 488 secondary antibody were then applied; followed by polyclonal antibodies to the neuropeptide Calcitonin Gene-Related Peptide (CGRP) coupled with the appropriate Cy3 conjugated secondary antibody. The nuclear stain DAPI was applied to aid in cell counts. Images were captured from 5 random areas in 4 different sections of each joint capsule obtained using a microscope at 200x magnification. Image analysis software assisted in counting the total number of cells, number of α-SMA positive myofibroblasts not associated with blood vessels, mast cell numbers and numbers of neuropeptide containing nerve fibers (≥50μm). Myofibroblast, mast cell and neuropeptide numbers were expressed as percentages of total cell numbers. Statistical analysis involved t-tests for human and paired t-test for rabbit group mean comparisons. Regression analysis examined correlations between myofibroblast, last cell and neuropeptide markers. Significance was set at p < 0.05.

**Data are presented as mean ± standard deviation**

**Results:** The human studies indicated that there were statistically significant differences between the experimental contracture capsules and the control capsules for all properties sampled. Table 1: Human Capsule

<table>
<thead>
<tr>
<th>Total Cell</th>
<th>SMA</th>
<th>% SMA</th>
<th>Chym</th>
<th>% Chym</th>
<th>CGRP</th>
<th>% CGRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>43±15</td>
<td>55</td>
<td>24±15</td>
<td>33.5</td>
<td>10±1</td>
<td>6.9</td>
</tr>
<tr>
<td>Con</td>
<td>35±10</td>
<td>58</td>
<td>33±15</td>
<td>25.5</td>
<td>15±1</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Expressed as mean ± standard deviation

**Discussion:** These studies have revealed that the myofibroblast marker α-SMA, the mast cell marker chymase and the neuropeptide marker CGRP were significantly elevated in the capsules obtained from joints with contractures when compared to joints free of contractures. There were strong, statistically significant correlations between the markers. Finally, the animal model of post-traumatic contractures reflected the human condition as we have shown previously with collagen, MMP, TIMP and TGF-β.[9-11] Similar observations have been made in other fibrotic pathologies. Dupuytren's contracture of the hand has increased numbers of myofibroblasts, mast cells and neuropeptides in the pathologic palm fascia that causes this condition.[5,6] There were significant direct correlations between the mast cell numbers and neuropeptides.[5] Human burn wounds and a porcine model of hypertrophic / hypercontractile skin wound healing have exhibited increased numbers of myofibroblasts, mast cells and neuropeptides.[7,8] The consistent abnormalities in the pathologic joint capsule in post-traumatic contractures, palmar fascia in Dupuytren's contracture, and burn and hypertrophic skin scars supports the mast cell – nerve axis hypothesis of fibrosis.[12,13] A myofibroblast – mast cell – neuropeptide fibrosis axis is a potential mechanism contributing to the pathologic changes in the joint capsule in post-traumatic contractures. Strategies to interfere with this pathway such as mast cell stabilizers that prevent degranulation, inhibiting particular mast cell products (enzymes that modify proteinase-activated receptors) or preventing the neuropeptide stimulation of mast cell degranulation would be avenues to explore. Recent work in our research centre has examined one such strategy showing that a mast cell stabilizer can ameliorate a hypercontractile porcine wound healing response.[14] Future work will evaluate these strategies in post-traumatic joint contractures.


**Acknowledgements:** CIHR, AHFMR, HRF

| Exp | 10±1 | 20±4 | 30±15 | 24±15 | 10±1 | 6.9 |
| Con | 15±1 | 20±5 | 15±15 | 20±15 | 15±1 | 7.6 |

**Table 2: Rabbit Capsule**

Total cell numbers were greater on average in the contracture group but this was not statistically significant (p = 0.1). The absolute numbers were about 5x greater in the contracture capsules, and the percentages were approximately 4.5x greater in the contracture capsules (p < 0.0001). There were statistically significant correlations between α-SMA and chymase, α-SMA and CGRP and chymase and CGRP (p < 0.0001, r2 = 0.98 – 0.99).

**Table 1: Human Capsule**

**Table 2: Rabbit Capsule**

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