Introduction: During fracture repair, a number of growth factors and cytokines are present at elevated levels at the fracture site such as Transforming Growth Factor Beta (TGF-β), Fibroblast Growth Factor (FGF), Platelet Derived Growth Factor (PDGF) and Bone Morphogenetic Protein (BMP) [1]. A recent study [2] examined growth factor expression in human established non-unions. However, the presence and distribution of these growth factors during abnormal bone healing at both early and late time points have not been documented. Therefore the aims of this study were to: (1) develop and validate a clinically relevant small animal atrophic non-union model and (2) to test the hypothesis that growth factor expression is diminished in atrophic non-unions.

Methods: 28 adult female wistar rats underwent application of a novel circular frame external fixator to the right tibia under general anaesthesia (The Home Office approved this study under the Animals Scientific Procedures Act 1986). The fixator construct was standardised, with eight needles (27G Hypodermic needles) which were drilled through the skin into the proximal and distal metaphyses of the tibia. An osteotomy was performed with a 1mm burr under constant irrigation. The periosteum was removed on 14 of the 28 animals using a scalpel and the intramedullary canal was curetted. Both insults happened proximally and distally for a distance equivalent to 1 diameter of the tibia at the level of the osteotomy. A 1mm gap was introduced at the osteotomy site and the wound was closed. Once the animal had recovered it was allowed unrestricted weight bearing. Anteroposterior radiographs were performed every 2 weeks. Animals were sacrificed at 1, 3, 8 and 16 weeks.

Results: At 8 and 16 weeks post osteotomy all animals where stripping and curetting were performed went on to form an atrophic non-union. All animals where stripping and curetting were not performed went on to unite successfully. The immunohistochemical results are as follows:

1 week: Positive staining of all four growth factors was observed in the initial haematoma in both groups. New bone formation in the non-union group occurred away from the interfragmentary gap. More staining of growth factors was observed in the new bone matrix of the healing group than the non-union group.

3 weeks: Staining of the growth factors was similar in the two groups, with most tissues continuing to express growth factors.

8 weeks: The proliferating layer of the periosteum in the healing group no longer expressed TGF-β and BMP 2/4 as it began to remodel. However, the non-union group continued to express all four growth factors.

16 weeks: As the healing group remodelled staining of growth factors became weaker in all tissues and cells. The fibrous tissue in the non-union group appeared more weakly stained than at earlier time points.

Table I: A comparison of growth factor staining between healing fractures and non-unions in relation to time.

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>3</th>
<th>8</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>FGF-2</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<tr>
<td>PDGF</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<tr>
<td>BMP 2/4</td>
<td>H</td>
<td>H</td>
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<td>H</td>
</tr>
</tbody>
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Key to table: H (healing fracture); NU (non-union); 2 (positive staining tissue in gap); 1 (variable staining tissue in gap); 0 (negative staining tissue in gap); NP (gap tissue not present)

Discussion: This study has examined growth factor staining during normal and impaired bone healing in a controlled animal model at time controlled intervals. At one week after injury the osteotomy gaps were filled with haematoma which was positively stained in both groups. At three weeks there was little difference in growth factor expression between the two groups. At 8 weeks many cells and tissues ceased to stain positively for growth factors during the remodelling stages of the healing group. Strong growth factor staining persisted at 8 weeks in the non-union group. At 16 weeks the growth factor expression appeared weaker in the non-union group than at earlier time points. These results may suggest that at 16 weeks the fibrous tissue within the gap are no longer attempting chondrogenesis or osteogenesis.

Conclusion: These results may have relevance for new therapies that can be aimed at delivering growth factors to treat non-unions. At 1, 3 and 8 weeks post osteotomy the non-union group appeared to stain similarly to the healing group. At 16 weeks, however, consistent with our hypothesis there was less staining of the fibrous tissue in the non-union gap than at earlier time points. These results may suggest that at 16 weeks the fibrous tissue within the non-union gap had reached a steady state, therefore this may be a suitable time for delivery of growth factors to the non-union site.

References:

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