Introduction: Progressive destruction and erosion of articular cartilage is pathognomonic of osteoarthritis (OA) and ultimately necessitates joint replacement surgery. An early feature of cartilage breakdown in OA is loss of aggrecan from the extracellular matrix, despite its increased synthesis by chondrocytes. Aggrecan depletion in OA is primarily due to excessive proteolysis by members of the A Disintegrin And Metalloproteinase (TACE) & Thrombospondin repeat family (ADAMTS) [1]. The decrease in aggrecan reduces the resistance of the cartilage to compressive loads and exposes the collagen fibrils to proteolysis by the collagenolytic matrix metalloproteinases (MMPs) [2]. Increased ADAMTS and MMP activity in OA cartilage may be due to both elevated expression, increased activation and/or decreased levels of Tissue Inhibitors of MetalloProteinases (TIMPs). Importantly, destruction of cartilage in OA is focal, and while it is apparent that mechanical factors play a role in this topographical variation it is unclear whether a regional difference in the expression or activation of degradative enzymes and their inhibitors contributes to this localized erosion. Studies of ADAMTS, MMP and TIMP expression in human OA have not compared different joint regions [3,4]. In this study, we utilized a model of OA in sheep where focal cartilage damage is reproducibly induced. Our hypothesis was that expression changes that were restricted to pathological cartilage rather than being more widespread, might better define the enzymes that were specifically involved in the degenerative process.

Methods: Six purebred 4 year old Merino wethers underwent bilateral lateral meniscectomy and 6 were sham operated. Animals were housed in pasture for 3 months prior to sacrifice. In one knee from each animal the dynamic elastic modulus of cartilage on the medial and lateral tibial plateau (MTP, LTP) normally covered or uncovered (cov, uncov) by the meniscus was determined prior to processing an osteochondral slab for histochemical and immunohistochemical evaluation and scoring using a modified Mankin system. In the opposite knee of each sheep, articular cartilage was harvested separately from the MTP and LTP cov and uncov regions, finely diced, snap frozen in liquid nitrogen and stored at -80°C prior to further analysis. Portions of cartilage were dried, weighed and total glycosaminoglycan (GAG) and hydroxyproline measured. A second aliquot of cartilage was extracted and proteoglycans separated by SDS-PAGE, and analysed by Western blotting with antibodies recognizing the ADAMTSs- and MMP-generated neoepitopes (EGE, PEN, respectively. DNA was extracted from a third cartilage aliquot from all regions of all sheep, and expression of ADAMTS-1, -4 & 5; MMP-1, -2, -3, -13 & -14; and TIMPs 1-4 were measured relative to the house-keeping gene GAPDH using semi-quantitative RT-PCR.

Results: Focal cartilage damage with fibration and partial thickness erosion was confined to the LTP-cov region of meniscectomised joints. Marginal osteophytosis particularly in the lateral compartment of meniscectomised knees was also evident. Changes in the mechanical properties of the articular cartilage were confined to the LTP-cov region, which showed a significant decrease in dynamic modulus consistent with a decrease in cartilage stiffness. There was a significant increase in the histological cartilage damage in both the LTP-cov and LTP-uncov regions, with the former being the most marked (scores of 4.4±1.2 to 17.7±2.7 and 3.1±1.1 to 6.7±1.9, respectively). There was no change in histopathological cartilage damage in either region of the MTP. Interestingly, despite the cartilage pathology in the LTP-cov region and evidence of focal loss of toluidine blue staining on histology, there was an overall increase (~30%) in GAG/mg dry weight, suggesting a hypertrophic or repair response in the region. There was a corresponding decrease (~20%) in hydroxyproline/mg dry weight in the LTP-cov.

Western blot analysis demonstrated more EGE in the cov compared with uncov cartilage in both the MTP and LTP of sham operated joints. In contrast, uncov cartilage from the MTP and LTP contained more PEN neopeptide than cov cartilage. There was no change in EGE or PEN by Western blot analysis in any region following meniscectomy. The EGE and PEN neopeptides were immunolocalized predominantly in the interterritorial matrix, with the former primarily in the deep of cov and the latter throughout the depth of the uncov cartilage in sham joints. Following meniscectomy, there was a focal increase in EGE in the cartilage immediately surrounding the lesion in the LTP. In sham-operated joints the expression of MMP-2, -13 & -14 and ADAMTS-5 was higher in cov compared with uncov cartilage, while there was little regional difference in expression of the other MMPs, ADAMTS or TIMPs. Following meniscectomy, MMP-1, -2, -3, -13 & -14, and ADAMTS-1 & -4 expression was increased in all four regions compared with sham-operated joints (Fig. 1). In contrast, ADAMTS-5 mRNA was only increased in the LTP cartilages following meniscectomy (Fig. 1) and there was little change in TIMP-1 mRNA in any region.

Discussion: Topographical differences in aggrecan metabolism exist in normal joints. However, EGE and PEN aggrecan metabolites accumulate over the life of the animal and so may not correlate with the expression of enzymes at any single point in time. Nevertheless, the fact that the cov cartilage consistently had elevated mRNA levels for several MMPs and yet lower levels of PEN than uncov cartilage, suggests that post-translational activation of these enzymes rather than expression levels, plays a central role in controlling their local proteolytic activity in normal joints. In contrast, the ADAMTSs are secreted in a furin-activated form although their activity can be further modulated by C-terminal truncation [5,6]. Greater EGE in cov compared with uncov cartilage coincided with elevated ADAMTS-5 mRNA levels in these regions of sham joints. Following meniscectomy cartilage degeneration was restricted to the LTP. The lack of increase in EGE or PEN fragments on Western blotting following meniscectomy is likely associated with the very focal nature of the aggrecan loss within the LTP-cov and masking by the overall increase in aggrecan within the pooled cartilage from this region. The only expression change restricted to the pathological cartilage was an increase ADAMTS-5, implicating this enzyme specifically in the focal disease process. The global increase in expression of MMPs and ADAMTS-1 & -4 suggests regulation of their expression by humoral factors such as IL-1 in the synovial fluid. Understanding the processes that control local activation of the upregulated MMPs and possibly ADAMTSs may provide targets to modulate focal cartilage damage in OA.