**Introduction:** Hyaluronan (HA) confers unique rheological properties to synovial fluid, including efficient lubrication of articular cartilage and peri-articular tissues. In osteoarthritic (OA) joints, synovial fluid HA concentration and molecular weight are decreased and the synovium is more cellular, vascularized and fibrotic than normal tissue [1]. Intra-articular therapy with Hyalgan® has been shown to provide symptomatic relief in OA and it has been suggested that it may exhibit disease-modifying properties and modulate cartilage degradation [2]. However, recent studies have demonstrated a strong association between synoviitis, capsular thickening and the severity of knee pain [3]. To date the effects of intraarticular HA therapy on synovial pathology in OA have not been well documented. We have established a model of OA in sheep induced by meniscectomy, where progressive focal cartilage erosion, subchondral bone sclerosis and osteophytosis develops over 6 months recapitulating the changes observed in post-traumatic OA in humans [4]. The initial aim of this study was to evaluate and quantify the synovial pathology in this model and investigate potential pathological mechanisms. We then determined the ability of Hyalgan® (Fidia Farmaceutici) and a novel amide derivative of HA (HYADD4®-G, Fidia Farmaceutici) to modulate the pathologic changes in ovine synovium induced by meniscectomy.

**Materials and Methods:** Eighteen aged (6-7 years) Merino ewes had bilateral lateral meniscectomy to induce OA. Six other animals were used as non-operated controls (NOC). Four months after meniscectomy the animals were divided into 3 groups of 6 receiving either intra-articular saline or Hyalgan® weekly for 5 weeks or intra-articular HYADD4®-G every two weeks for 3 injections. All sheep were sacrificed six months after meniscectomy. A sample of synovium from the suprapatellar fold of each joint was processed to paraffin and sections were stained with haematoxylin and eosin, coded and scored blind for synovial OA pathology (intimal hyperplasia, inflammatory cell infiltrate, vascularity and sub-intimal fibrosis). Intimal cell numbers and depth of intimal fibrosis (μm from the surface) were quantified using a graticule. Serial sections were immunostained for the collagen chaperone heat shock protein-47 (HSP-47), transforming growth factor-β (TGF-β), connective tissue growth factor (CTGF), the HA-receptor CD44, tumor necrosis factor-α (TNFα), and inducible nitric oxide synthase (iNOS). Synovial fibroblasts were isolated by enzymatic digestion from each meniscectomised joint and the HA synthesised by these cells quantified by Streptomyces hyaluronidase-sensitive 1H-glucosamine incorporation.

**Results:** Subintimal fibrosis, vascularity and aggregate score were all markedly higher in OA joint synova compared with NOC (p < 0.005 for all analyses) (Table 1). Intimal cell numbers and depth of fibrosis as measured by graticule were increased in OA synovium (p < 0.01) compared with NOC. Immunostaining for HSP-47, TGF-β and TNF-α were no different between NOC and meniscectomised joints. In contrast CD44, CTGF and iNOS immunostaining was stronger in the synovial lining cells and/or subsynovial tissue of OA synovium compared with NOC (p < 0.005 for all analyses) (Image 1).

**Discussion:** This study has demonstrated that there is significant measurable synovial pathology in this meniscectomy-induced model of OA that mimics the changes reported in human OA synovium [1]. These changes included intimal cell hyperplasia, increased vascularity and both intimal and subintimal fibrosis. In contrast, there was no consistent increase in plasma/inflammatory cell infiltration, which together with the lack of increased staining for TNF-α indicates minimal synovial inflammation at this 6-month time point in this model. However, the increase in immunostaining for CD-44 was similar to that reported in OA in man, where it correlates with the degree of inflammation [5]. Increased iNOS staining has also been associated with increased inflammatory cytokine production [6]. The increase in CTGF, a downstream regulator of TGF-β, was consistent with the increased fibrosis observed. Intraarticular HA reduced the overall pathology of synovia from meniscectomised joints, with Hyalgan® principally reducing vascularity and depth of intimal fibrosis and HYADD4®-G having its predominant positive effects on vascularity, intimal hyperplasia, and HMW HA synthesis. It is clear from the present study that the actions of intraarticular HA in OA are multifactorial, nevertheless the effects on synovium we have observed are a disease modification that would be expected to improve joint mobility and function in OA. Further studies are necessary to elucidate the processes by which different intraarticular HA treatments generate a positive effect in OA synovium.

**References:**

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<table>
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Table 1. Histological scores of synovium sampled from NOC and OA sheep subjected to various intraarticular treatments. MWU = Mann Whitney U test

**NOC** = Normal OA synovium

**O&A** = OA synovium

**HA** = Hyaluronan

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