**Hyaluronan generated during peritoneal dialysis does not drive fibrosis, but regulates inflammatory cell recruitment during peritoneal infection.**

**Introduction:** PD is a well-established form of RRT for ESRD patients. Longevity of treatment with PD is limited because of PD failure related to repeated episodes of PD peritonitis and constant exposure of the peritoneal membrane to bioincompatible PD solutions leading to peritoneal fibrosis. TGF-β1-driven transdifferentiation of mesothelial cells to myofibroblasts through mesothelial to mesenchymal transition (MMT) underlies peritoneal fibrosis. In solid organ fibrosis (kidneys & lungs), we previously showed that pro-fibrotic cell differentiation is driven by changes in synthesis and macromolecular organisation of the matrix polysaccharide hyaluronan (HA). Previous studies also demonstrate significant HA generation alongside peritoneal infection and inflammation. However, the role of HA in promoting peritoneal infection/inflammation and/or in driving MMT was not known. In this work, we aimed to determine the role of HA in driving peritoneal infection, inflammation and fibrosis. Specifically, we investigate if HA alterations identified as relevant in solid organ fibrosis can be applied in the peritoneum to promote and/or prevent PD failure.

**Methods:** Ex vivo PD effluent from patients with/without peritonitis were studied in parallel with human primary peritoneal mesothelial cells *in vitro*. In addition, a murine model of peritoneal inflammation (live attenuated *staphylococcus epidermidis* induced peritonitis) was investigated. An experimental model of TGF-β1-driven MMT was utilised for the *in vitro* studies. A chemical inhibitor of HA (PEP-1) was injected into mice to abrogate HA effects in *in vivo* studies, whilst *in vitro* HA manipulations were undertaken using siRNA and specific plasmid over-expression vectors. HA levels and associated inflammatory and fibrosis profiles were assessed using RT-qPCR, histological analysis and FACS.

**Results:**  PD effluent of patients with PD peritonitis had significantly increased HA concentrations day-1 after developing acute bacterial peritonitis compared to PD effluent from non-infected patients. In vitro studies demonstrated that whilst TGF-β1-driven MMT in primary human mesothelial cells was associated with significantly increased extracellular HA generation, in contrast to kidney and lung fibrosis this increased HA was not causally involved in driving pro-fibrotic cell differentiation (MMT). Furthermore, whilst our previous studies in solid organ (kidney, lung) fibrosis demonstrated that the HA Synthase-2 isoform (HAS2) was critical in driving fibrotic processes; in peritoneal tissues HAS2 was not significantly expressed or induced compared to the HA Synthase-1 isoform (HAS1). Increased HAS1 in peritoneal tissues and mesothelial cells was associated with enhanced neutrophil and macrophage recruitment, whilst blocking HAS1 driven HA synthesis (using intraperitoneal injection of PEP-1) led to delayed neutrophil clearance and monocyte recruitment indicating a delayed inflammatory response.

**Conclusion:** In comparison to solid-organ fibrosis where HA seems to have an important role in driving fibrosis through driving a HAS2 dependent myofibroblast phenotype, in the peritoneum HA does not drive peritoneal fibrosis through promoting TGF-β1-driven MMT. In contrast, HA in the peritoneum is released in response to infection, is driven by HAS1 and appears to be involved in regulating inflammatory cell recruitment in the peritoneal cavity.