# Bioengineered models to investigate lymph node stromal cell biomechanics in ageing and disease

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## INTRODUCTION

Lymph nodes are the sites within the body where immune responses are mounted. A hallmark of lymph nodes is swelling, they can swell up to ten-fold their original size to accommodate an influx of immune cells, then return to their original state with no apparent damage (Figure 1).

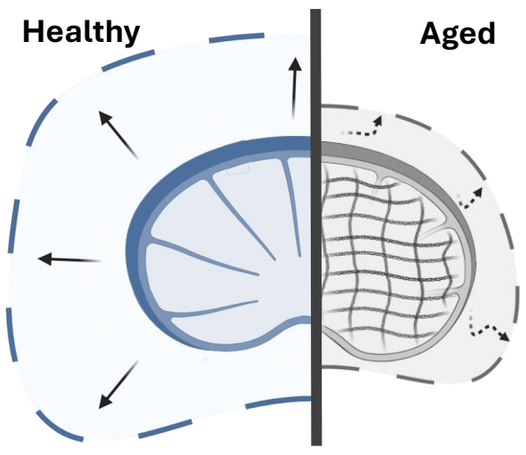



*Figure 1. LNs expand during immune responses; ageing leads to fibrosis and disruption of mechanical plasticity.*

This process requires structural plasticity, and a complete remodelling of the lymph node architecture, and is facilitated by Fibroblastic Reticular Cells (FRCs). Activation of the CLEC-2-podoplanin (PDPN) signalling axis induces FRC relaxation (figure 2). However, as we age our lymph nodes undergo profound biomechanical changes caused by the development of fibrosis and an increase in tissue stiffness, the impact of which is poorly understood. Here, we investigate the impact of age-related mechanical cues on mechano-immunological function using polyacrylamide hydrogels of different stiffnesses and primary FRCs to model ageing/fibrosis.

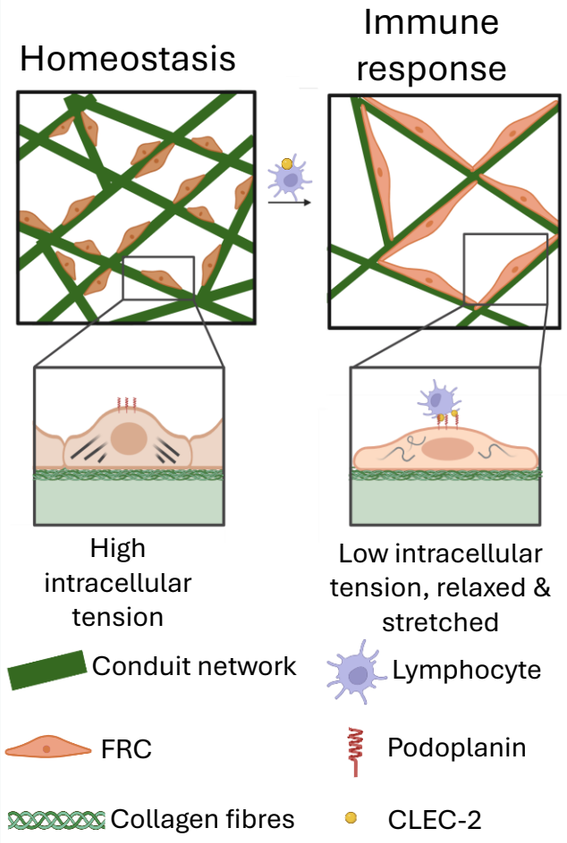



*Figure 2: Schematic of the FRC network. During an immune response (expanded), FRCs decrease intracellular tension, stretching upon secondary lymphoid organ expansion, allowing the conduit network to expand. Driven by the PDPN-CLEC-2 axis*

## METHODS

### Collagen functionalised 2D Polyacrylamide (PAAm) hydrogels

PAAm hydrogels with tuned elastic properties are synthesised by combining varying volumes of acrylamide (AAm) and bisacrylamide (Bis) cross-linker. Hydrogels are functionalised with collagen type-I. FRCs are seeded on top.

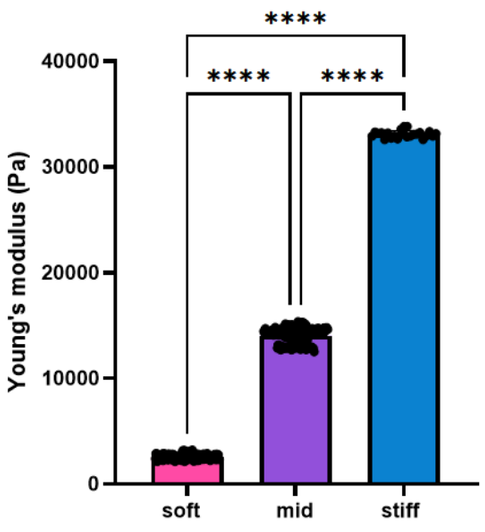
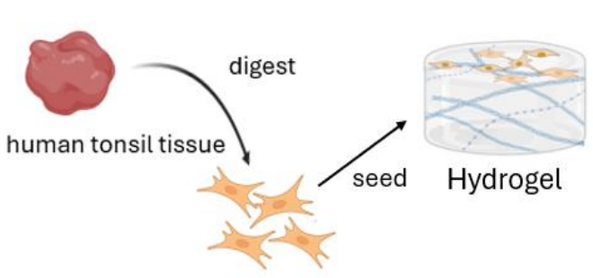



*Figure 3: Young's Modulus (Pa) measured by nanoindentation. Averages: Soft: 3kPa, mid: 13kPa, stiff 33kPa. Graph mean ± SD, n=3 hydrogel batches, 75 measurements/gel.*

### Isolation of secondary lymphoid organ cells

FRCs are isolated from human tonsil tissue, tonsils are a secondary lymphoid organ that is very similar in structure to lymph nodes.




## CONCLUSIONS & FUTURE WORK

We demonstrate that FRC morphology and mechanosensing are altered by matrix stiffness, comparing soft versus stiff hydrogels. Using CLEC-2 stimulation to mimic immune activation, we are beginning to assess how these changes influence immune function. We will next apply traction force microscopy to investigate if force transduction during immune stimulation is dysregulated by the mechanical microenvironment. This platform enables investigation of mechano-immunological mechanisms in ageing and has potential to support exploration of therapeutic targeting of the aged microenvironment.

## ACKNOWLEDGEMENTS

This work is supported by BBSRC Discovery Fellowship (BB/Z515000/1). Figures made on Biorender.com.

The University of Glasgow / University of Strathclyde
Centre for the Cellular Microenvironment

Biotechnology and Biological Sciences Research Council

## RESULTS

### Morphology of primary FRCs

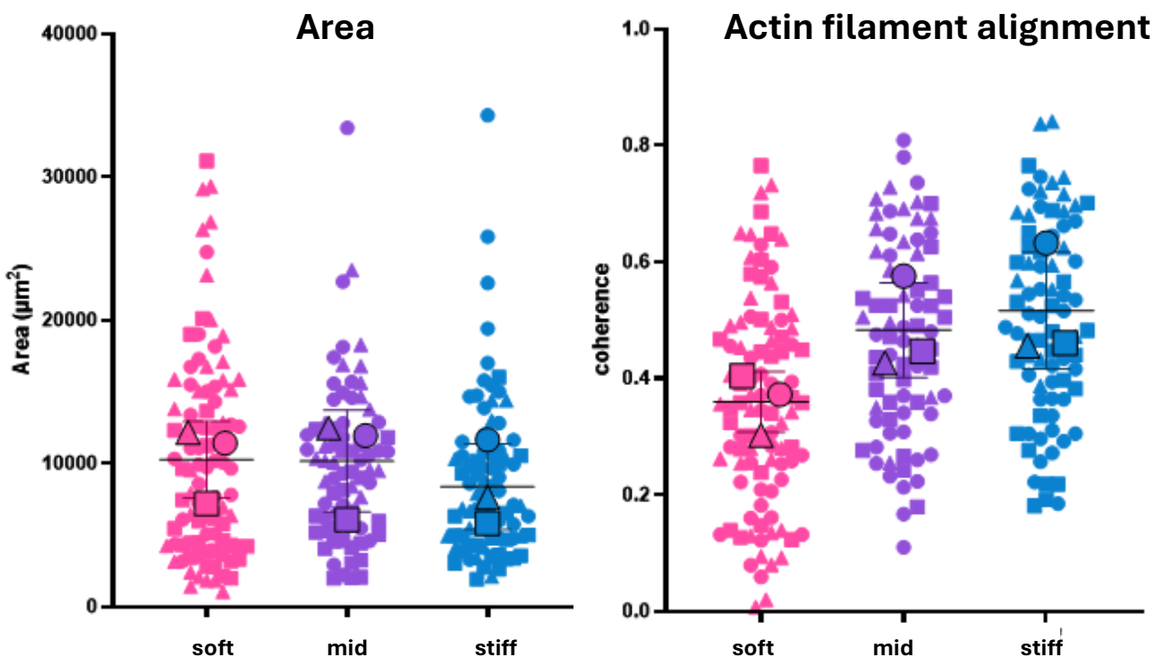
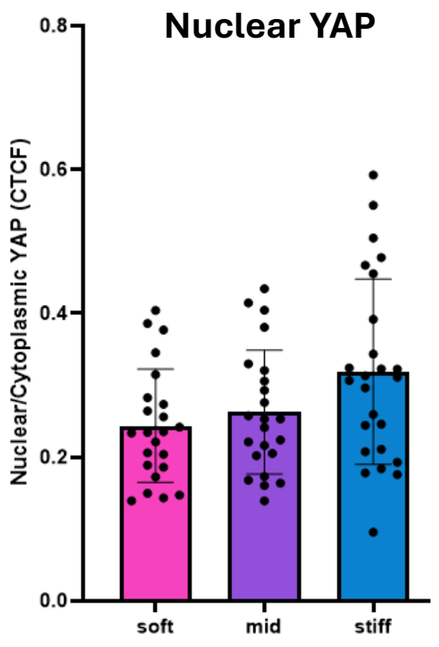



Figure 5: FRCs cultured on soft, mid and stiff PAAm hydrogels show stiffness-dependent changes in morphology. With increasing stiffness, cell area decreases, and alignment of actin fibres increases. Suggesting high intracellular tension.

*Graphs mean ± SD , n = 3 biological replicates*




### Mechanosensing in FRCs

Figure 6: FRCs respond to mechanical changes in their microenvironment. YAP is mechanosensitive transcriptional co-activator. Here, we see increased translocation to the nucleus at increased stiffness in FRCs.

*Graphs mean ± SD , n = 3 technical replicates*

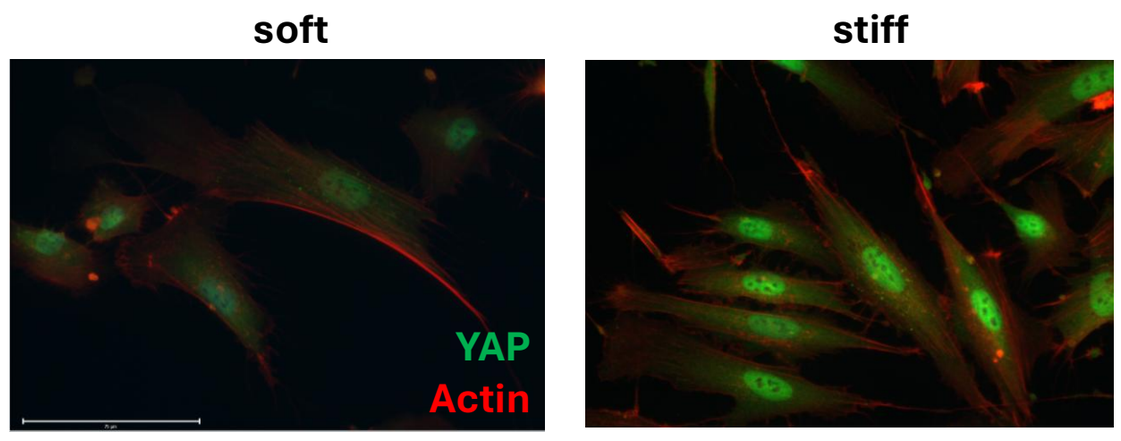
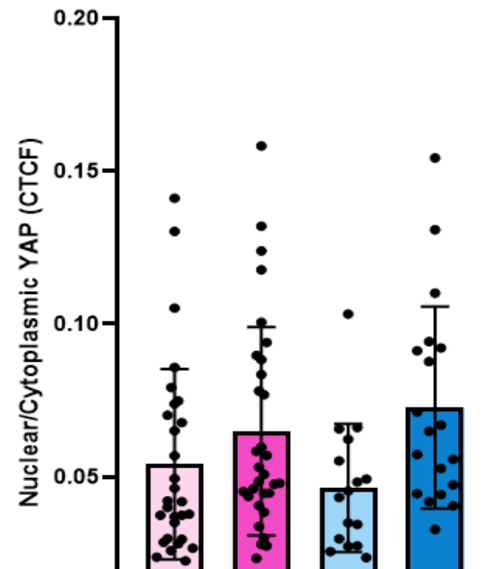



*Figure 7: Representative images of FRCs cultured on soft and stiff hydrogels for 24h. Shows increased nuclear YAP on stiff hydrogels. Scale bar is 75 µm.*

### Immune stimulation

Figure 8: FRCs cultured on hydrogels are stimulated with CLEC-2 protein to stimulate immune activation. Here, we see although nuclear YAP is increased on stiff substrates, it decreases to similar levels as those on soft substrates upon CLEC-2 stimulation. Suggesting that immune activation may reduce mechanoresponsive signalling.

*Graphs mean ± SD, n = 3 technical replicates*




### PDPN expression

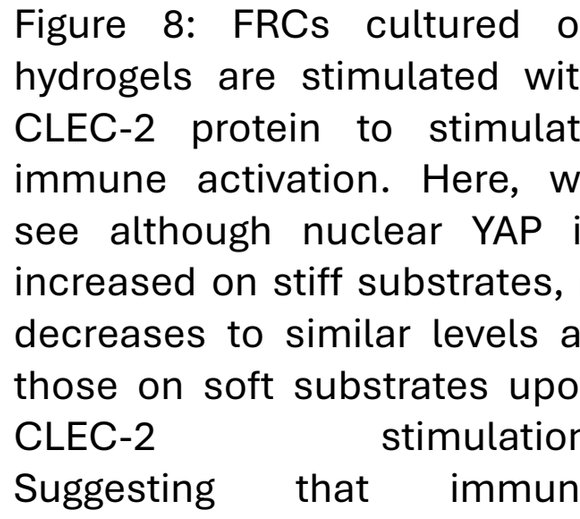



*Figure 9: Images of FRCs with PDPN stain. Scale bar is 100 µm.*




Figure 10: PDPN signalling drives FRC contractility in response to immune activation. On stiff hydrogels, PDPN expression is reduced, which may impair control of FRC contraction and limit the ability of lymph nodes to regulate organ swelling.

*Graphs mean ± SD, n = 3 technical replicates*