Engineering a zonal collagen/chondroitin sulfate scaffold to recapitulate the depth-dependent microarchitecture of articular cartilage

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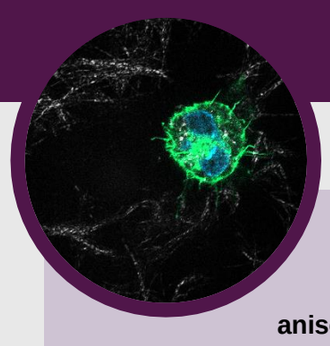
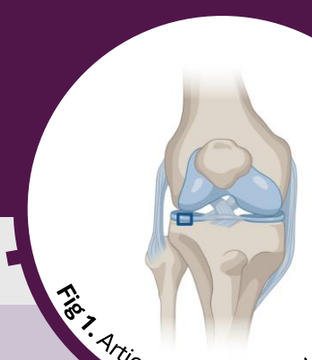


Introduction and project aims

Articular cartilage resides in our load-bearing joints including the knee **(Fig 1).** Cartilage tissue exhibits **anisotropy**, achieved through the spatial orientation of cartilage proteins and collagen fibres as well as cellular organisation (**Fig 2**). Collagen fibres are organised parallel to the interface with synovial fluid, yielding a low friction coefficient to allow smooth movement of the joint. Disruption to the homeostatic regulation of the tissue leads to degradation of the ECM within the joint which cause disease such as **osteoarthritis (OA)**. Tissue engineering is a promising approach to model and mimic cartilage disease.



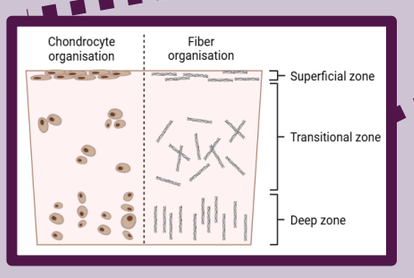
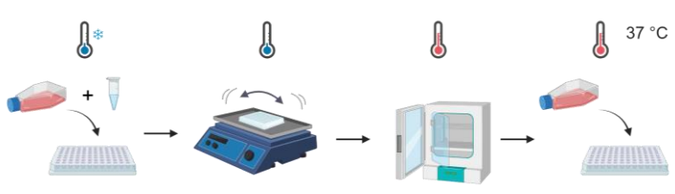
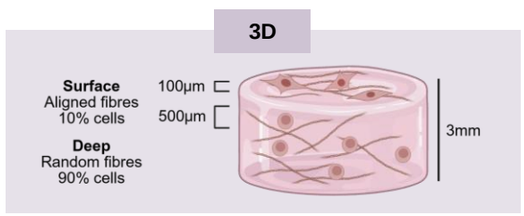


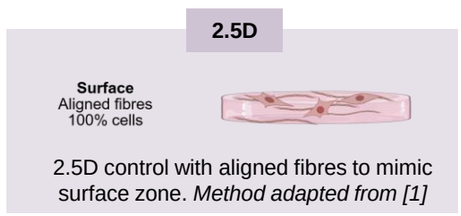
Fig 2 (left). Zone-dependent organisation of chondrocytes and collagen fibres in articular cartilage.

This project aims to fabricate tissue models that mimic anisotropy and alignment patterns for in vitro studies of cell behaviour and cartilage mechanics in healthy and disease microenvironments

Materials and methods





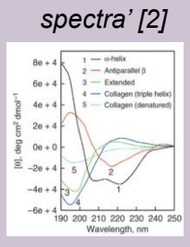


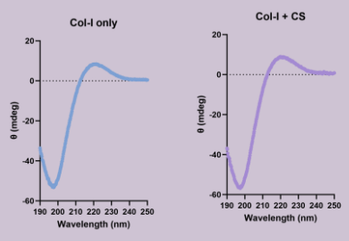
2.5D control with aligned fibres to mimic surface zone. *Method adapted from [1]*

- **Collagen-I** and **chondroitin sulfate** (Col-I/CS) scaffolds were fabricated in in custom PDMS moulds using **temperature casting**
- **ATDC5 cells** were seeded in a ratio of 90:10 (embedded: surface) and cultured for **14 days** in differentiation medium

Col-I/CS secondary structure

'Gold standard spectra' [2]

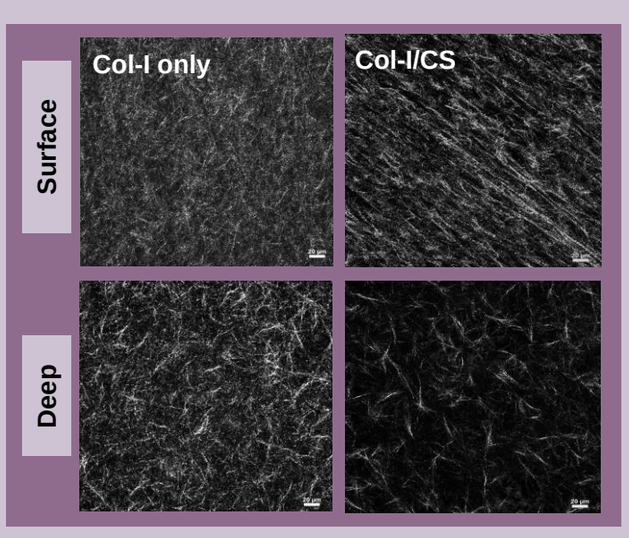




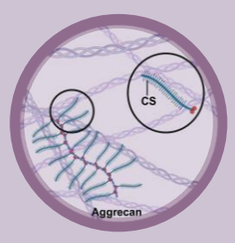
Circular dichroism (CD) spectra shows Collagen triple helices conformation in both conditions

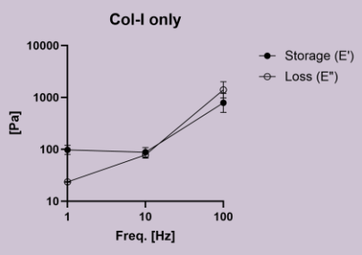
Depth-dependent scaffold characterisation, cell distribution and morphology

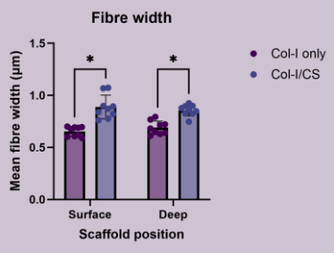
Confocal reflectance microscopy

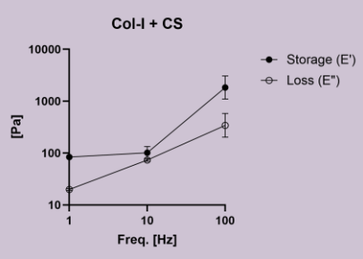


Fibre characterisation and microrheology

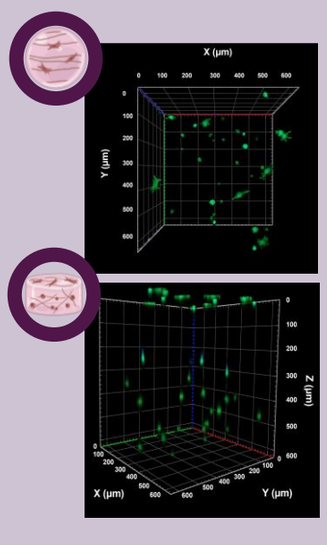


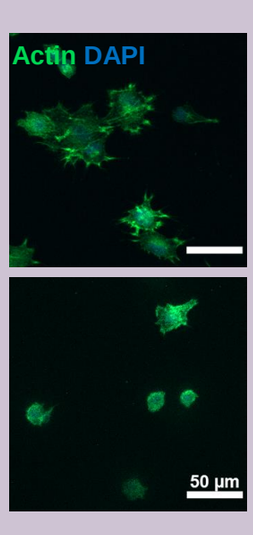


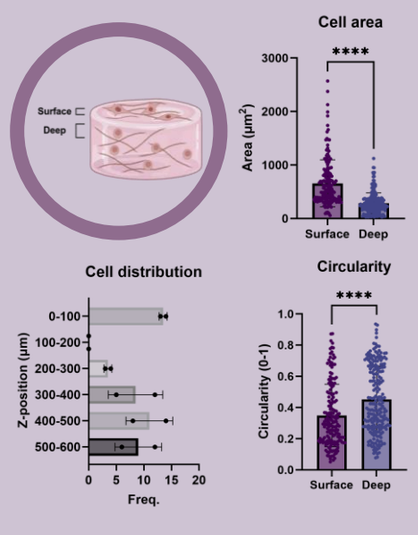




Immunofluorescence in 3D scaffolds



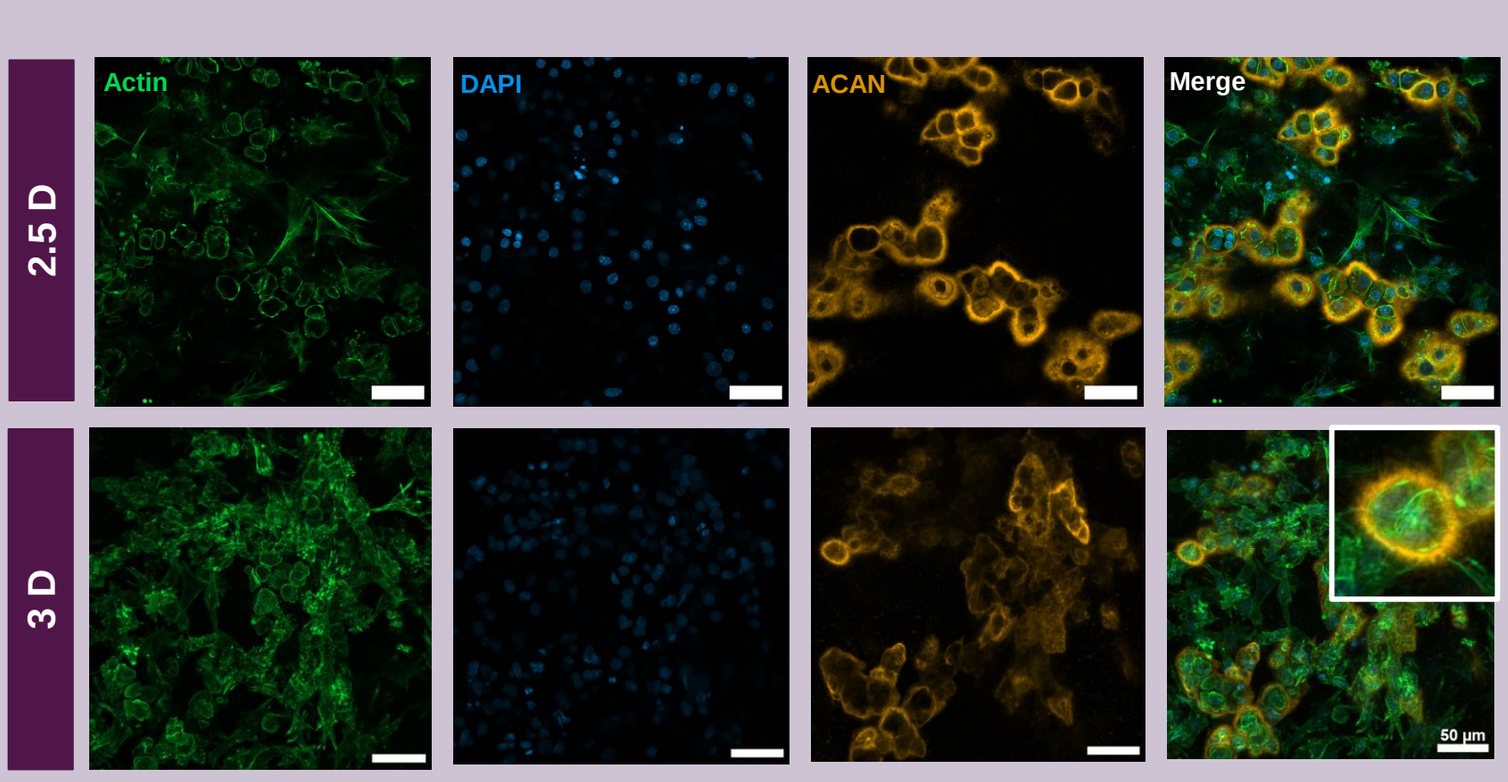


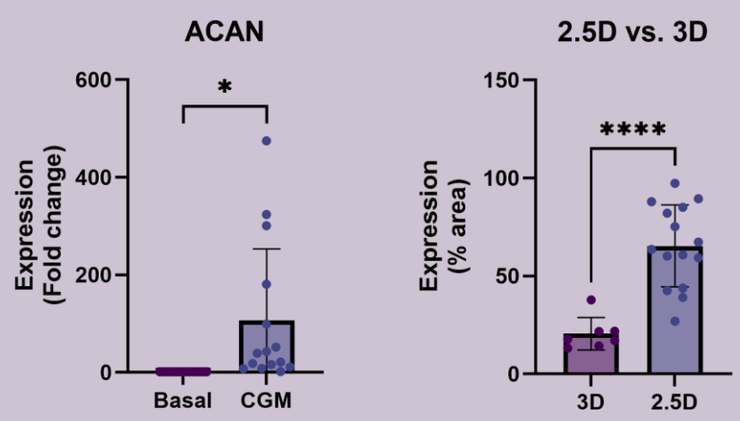


Confocal reflectance microscopy (CRM) images show fibre alignment at the surface of the scaffold which is more conserved in the Col/CS condition. CS has a significant effect on **fibre width** ($p<0.00002$) and mesh size but does not have a significant impact on elastic (E') and viscous (E") moduli, as shown by **AFM** data

ATDC5 cells are evenly distributed in two distinct scaffold zones. Embedded ATDC5 cells showed significantly smaller size and higher circularity than surface cells ($p<0.0001$)

Aggrecan expression in 2.5D and 3D cartilage models





We compared Aggrecan (ACAN) expression in 3D and an aligned 2.5D scaffold to mimic 'deep' and 'surface' zones, respectively

Both scaffolds supported deposition of ACAN compared to growth medium controls (Basal) ($p<0.02$)

2.5D scaffolds supported higher expression of Aggrecan, normalised to cell number ($p<0.0001$). Further analysis will investigate formation and thickness of **pericellular matrices (PCM)** and cell clustering

Discussion and conclusion

- Self-assembled 3D collagen scaffolds were fabricated with **biomimetic gradients in fibre alignment**
- Soft 2.5D and 3D scaffolds support ATDC5 differentiation and **expression of cartilage proteoglycans**
- 2.5D and 3D scaffolds may support development of chondrocyte-like **PCM,** which are not often well-established in 2D cell culture
- ATDC5 cells show significant morphological differences in response to depth, which can be correlated to **fibre orientation,** mesh size, stiffness gradients and hydrostatic pressure gradients

Future work

- The project will build on this work to correlate expression behaviour and morphology with mesh size and mechanical cues
- Remodelling studies will characterise focal adhesions, contractility and expression of cartilage metalloproteinases
- To validate this in vitro scaffold, we will investigate disease behaviours by simulating inflammatory environments to mimic OA disease environments

References

[1] Sapudom, J. et al. Collagen Fibril Orientation Instructs Fibroblast Differentiation Via Cell Contractility. Adv. Sci.2023, 10, 2301353.
[2] Greenfield, N. Using circular dichroism spectra to estimate protein secondary structure. Nat Protoc 1, 2876–2890 (2006). https://doi.org/10.1038/nprot.2006.202
- Figures and schematics created in BioRender

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