How to create a preferred collagen organization

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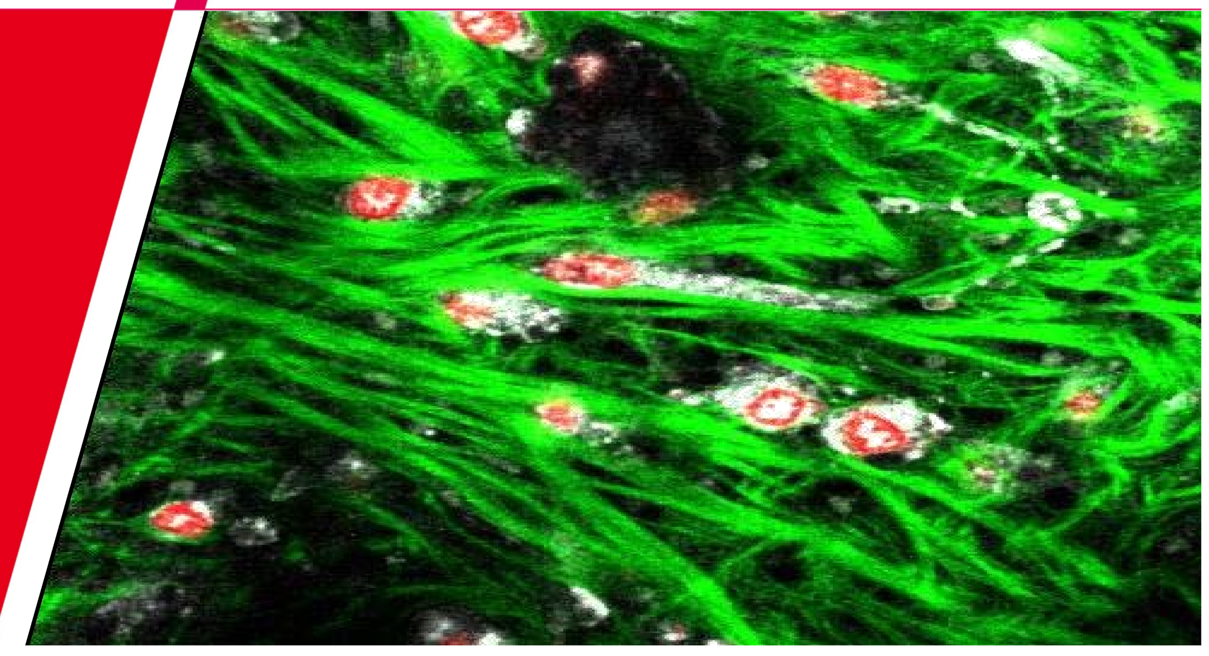


Introduction

- In vitro engineered fibrous tissues lack native-like matrix anisotropy, essential for in vivo functionality and durability.
- Experiments suggest actin-mediated cell traction and associated cellular orientation affect this anisotropy.
- Hence, the ability to manipulate stress-fiber orientation may be key to develop this preferred matrix anisotropy.

Research question

- Can we guide stress-fiber orientation via cyclic straining in 3D?, and expose the underlying mechanism?

Model system

- A small-scale tissue model system was developed to enable full 3D visualization of the specimen (Fig. 1).

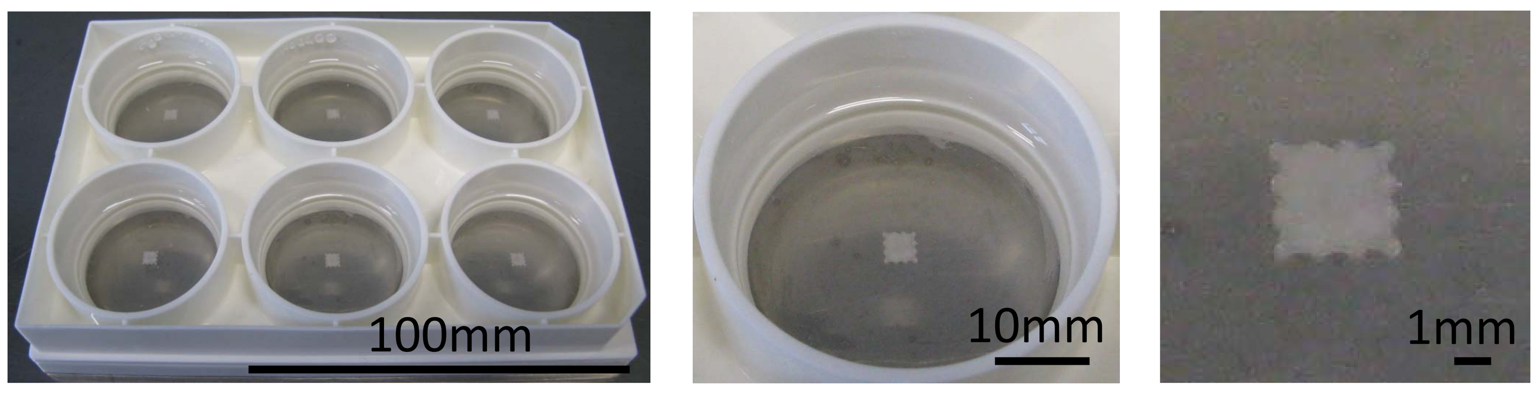
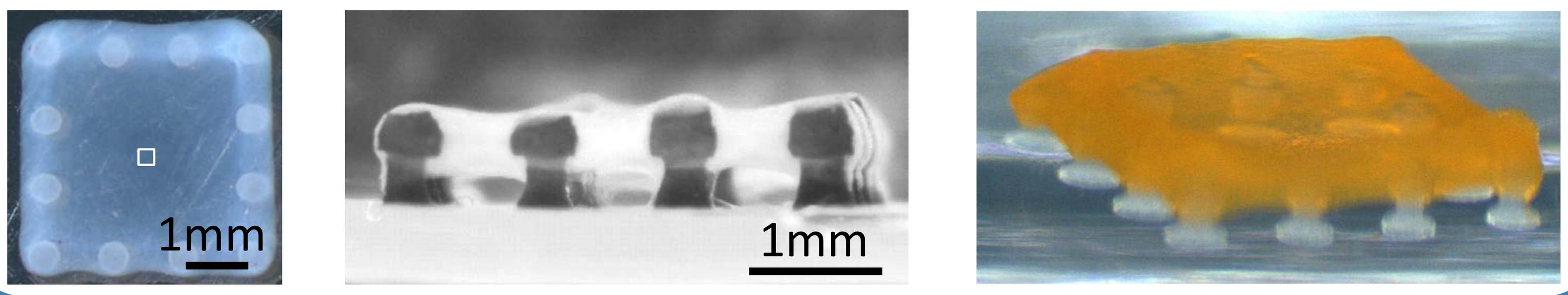


Fig.1: Flexible membranes (upper left) of Bioflex culture plates, were provided with an array of 12 silicone rubber posts (design enabled by TNO) serving as anchor points for a transiently contracting gel mixture of myofibroblasts (HVSC), collagen I and matrigel. Area scanned with confocal microscopy indicated in bottom left.



- Tissues were constrained and / or strained uniaxially (straining direction: ↔, i.e. along the 0 degrees axis; @10%; 0.5Hz) on the Flexcell system (Fig. 2).

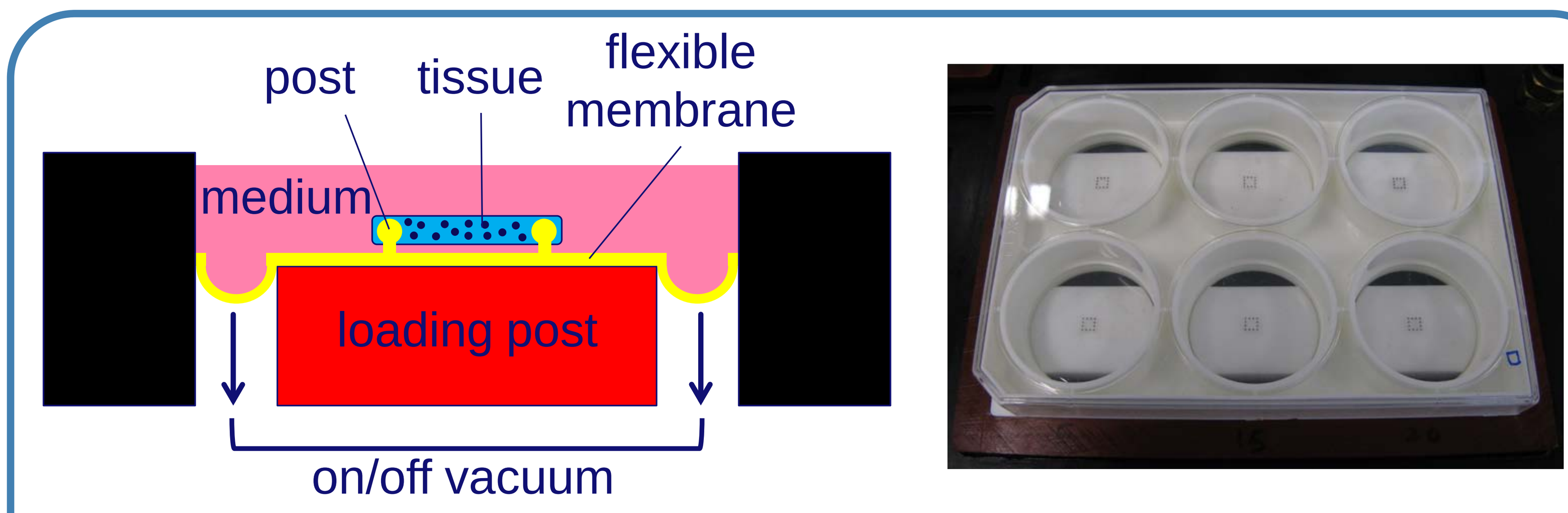


Fig.2: Loading posts were applied below the membrane. By applying on/off vacuum, tissues were subjected to uniaxial cyclic strain.

Analysis

- Confocal microscopy was used to visualize cell nuclei & stress-fibers; a measure for cell traction).
- Preferential stress-fiber orientation (α_i±std) was quantified using fiber tracking software and bimodal fitting.

Results & Discussion

- Transient contraction followed by uniaxial cyclic strain induced a biaxial stress-fiber orientation, preferentially towards the constraint direction (Fig. 3, core).
- However, surface layers displayed strain-avoidance (Fig. 3, bottom & top), absent at ROCK-inhibition (decreasing mechanosensitivity) or at static constraint (Fig. 4 & 5).

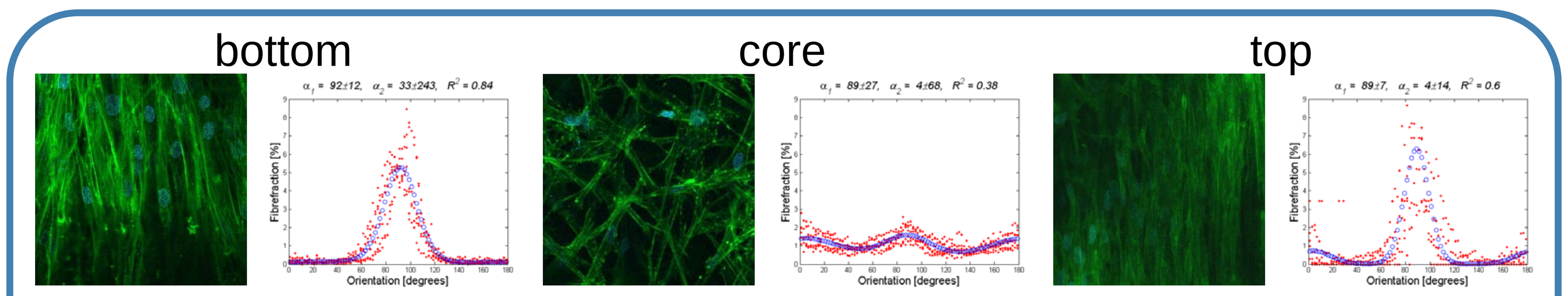


Fig.3: Static constraint 3d + cyclic stretching 3d

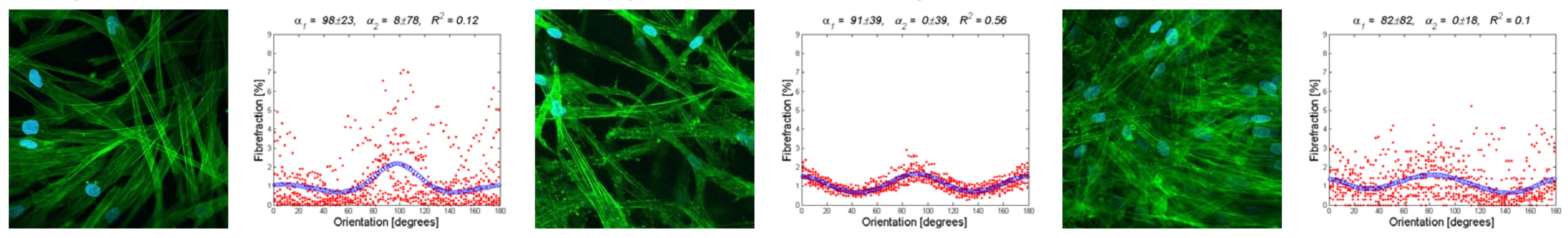


Fig.4: Static constraint 6d

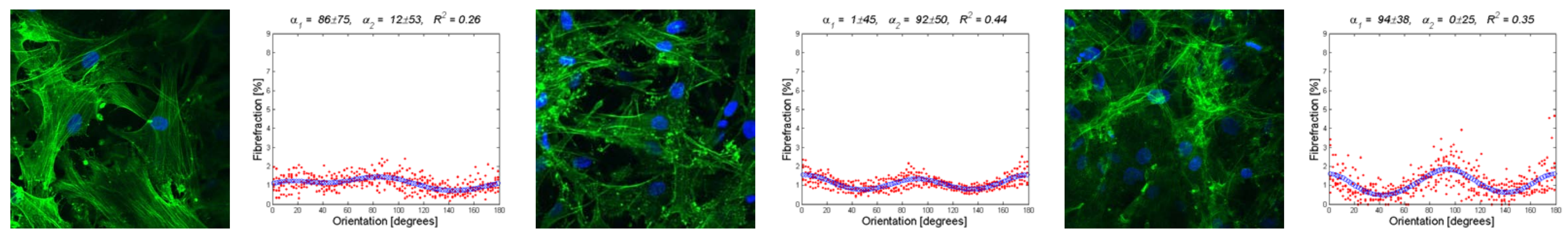


Fig.5: Static constraint 3d + cyclic stretching 3d (+ROCK inhibitor)

- Absence of strain-induced stress-fiber orientation in the tissue core, made us hypothesize that collagen contact guidance prescribes stress-fiber orientation.
- Support was obtained by decreasing collagen integrity, i.e. by lowering collagen density, addition of MMP-1 or MMP-1 + ROCK (Fig. 6). The former two increased strain-avoidance (Fig 6, left & middle), while ROCK-inhibition counteracted this effect (Fig. 6, right).

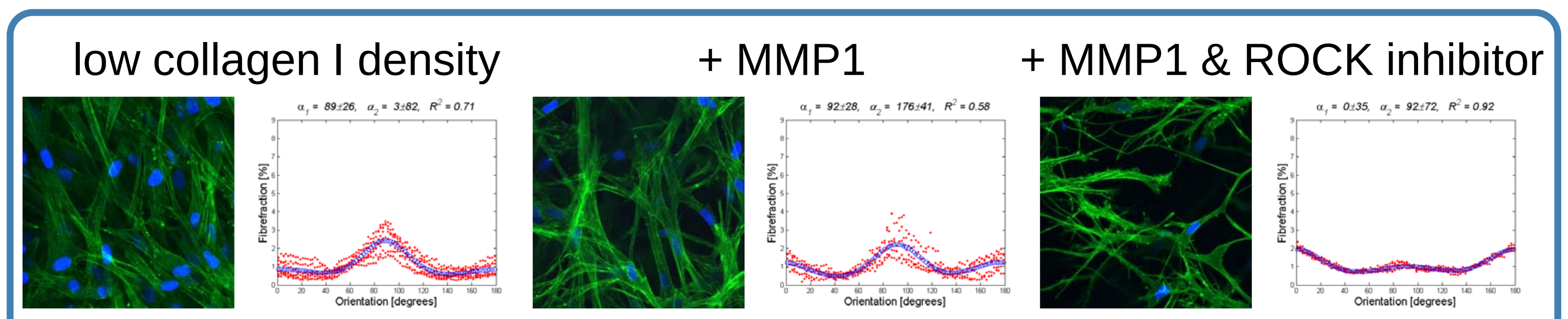


Fig.6: Static strain 3d + Cyclic stretching 3d (only tissue core is shown)

- Because the response was moderate, we eliminated collagen contact guidance via immediate cyclic stretching, i.e. before collagen polymerization.
- Immediate stretching resulted in a strong strain-avoidance of stress-fibers & collagen! in all tissue layers, which were both abolished by adding a ROCK-inhibitor (Fig. 7).

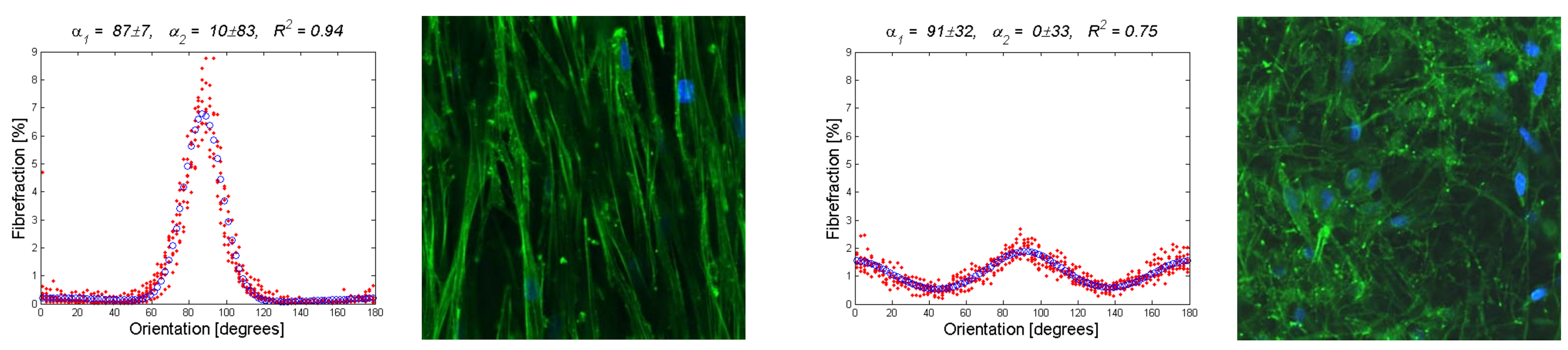


Fig.7: Cyclic stretching 6d. Left: no agents. Right: + ROCK inhibitor

Conclusion

- In 3D, cells orient perpendicular to imposed cyclic strain (strain avoidance), however, collagen contact guidance can dominate over strain avoidance.
- Results indicate the significance of scaffold directionality for obtaining a preferred matrix anisotropy in tissue-engineering applications.

/ Department of Biomedical Engineering

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