Effects of mechanical load on cytoskeletal protein arrangement in scleral fibroblasts

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Purpose

- **Glaucoma**: Second most prevalent cause of blindness, with nearly 80 million affected and expected to reach 112 million by 2040[1]:
- **Peripapillary sclera** (PPS): Main contributor of force transfer from fluid intraocular pressure (IOP) and eye movements to the optic nerve head – implying a role in glaucoma[2].
- **Cytoskeleton**: Composed of 3 different protein networks, which provide biomechanical stability and signal transduction to the cell.
- **Previous research:** Extensive study of the extracellular component of the PPS connective tissue, while the cellular biomechanics require further elucidation.
- **This study:** We investigated the effects of cyclic tensile strain (CTS), mimicking IOP, on cultured scleral fibroblasts and associated alterations in the cytoskeletal fiber architecture.

Methods

- **Cells**: Scleral fibroblasts from PPS explants from young adult cattle.
- **Seeding**: 0.4×10^6/well onto type I collagen coated BioFlex™(FlexCell International) 6-well culture plates.
- **Load**: Equibiaxial CTS mimicking physiological IOP (0.26-1.8%, 1Hz), pathological IOP (0.6-4%, 1Hz) or unloaded state, applied to the cells for 1h using an FX 3000 tensile system (FlexCell International):
  - $\sigma = \frac{pr}{2t}$ — σ = in-wall stress (18.2kPa); p = IOP (3.6kPa)[3]; r = eye radius (16.2mm); t = eye tunic thickness (1.6mm).
  - $\varepsilon = \frac{\sigma}{E}$ — ε = strain; E = Young's modulus (1-7MPa)[4].
- **Fixation**: 2% Paraformaldehyde for 15min, 1h, 6h or 24h after CTS.
- **Immunocytochemistry**:
  - F-Actin – Alexa-488®phalloidin (1:40; Sigma-Aldrich).
  - β-Tubulin – Primary mouse E7 antibody (1:500; DSHB); Secondary goat anti-mouse IgG Alexa-594® antibody (1:400; Invitrogen).
  - Vimentin – Primary mouse V9 antibody (1:100; Sigma-Aldrich); Secondary goat anti-mouse IgG Alexa-594® antibody (1:400; Invitrogen).
- **Imaging**: Zeiss LSM 880 confocal microscope with Airyscan in Fast mode.
- **Image analysis**: ImageJ (FibrilTool).
- **Statistics**:
  - Distribution: Anderson-Darling test.
  - Location: One-way ANOVA.
  - Dispersion: Two-sample F-test.
  - Post-hoc: Tukey's test.

Results

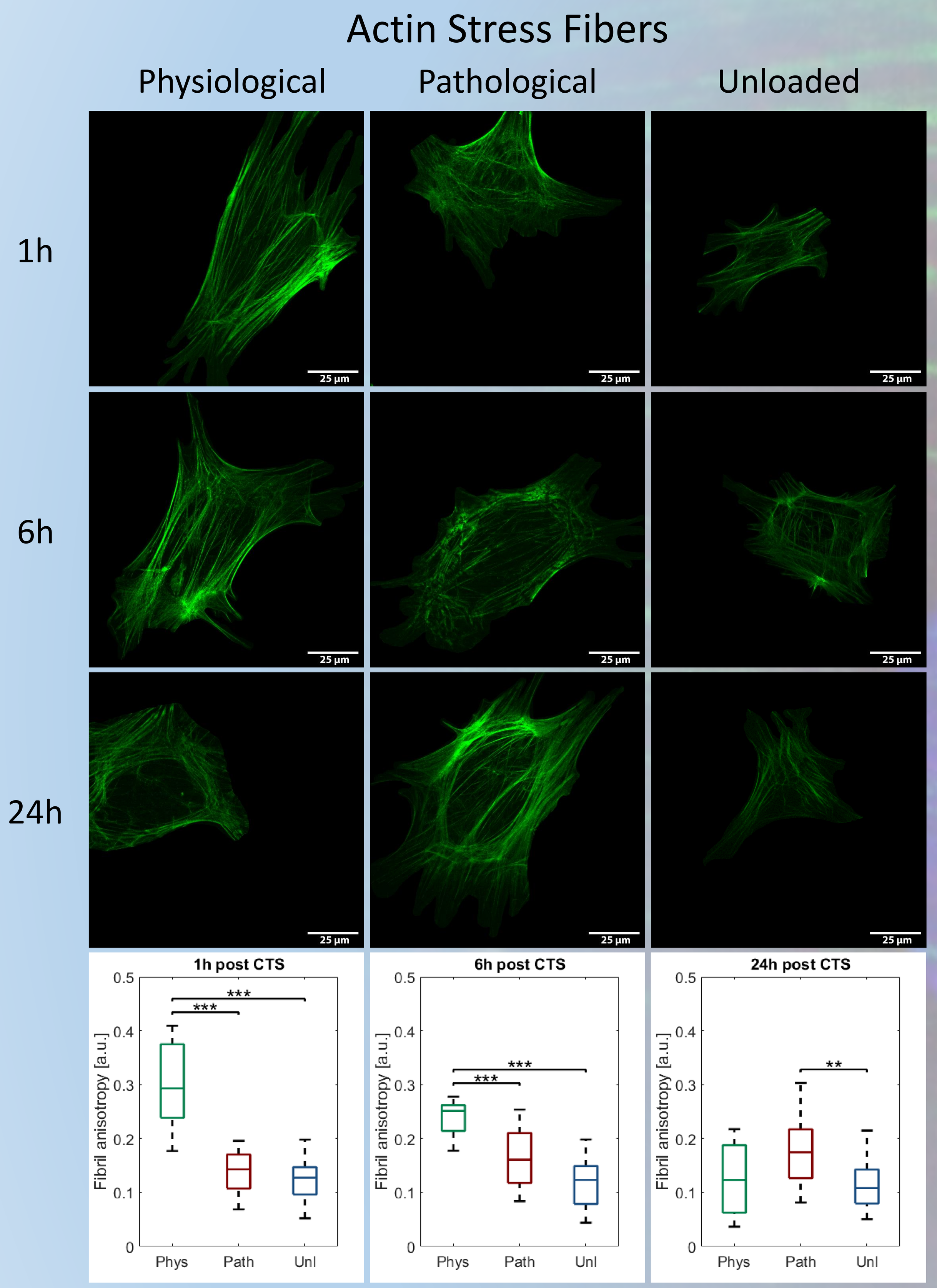

Figure 1: F-Actin cytoskeleton remodels 1h after physiological cyclic tensile strain (CTS) and at 24h after pathological CTS. Box plots of cytoskeletal fiber anisotropy assessed by FibrilTool. Results are grouped by applied CTS and relaxation time.

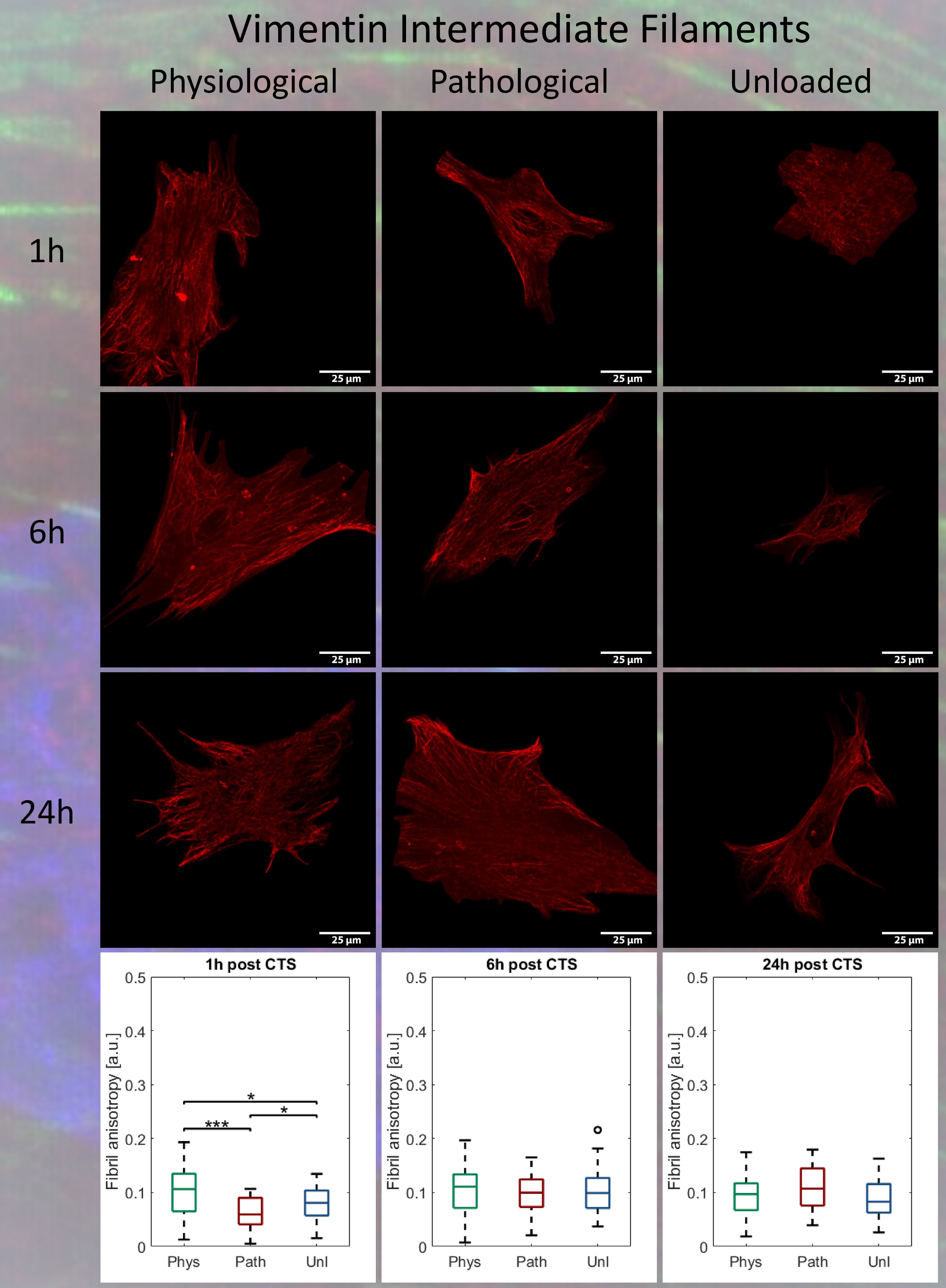

Figure 2: Vimentin cytoskeleton remodels 1h after physiological cyclic tensile strain (CTS). Box plots of cytoskeletal fiber anisotropy assessed by FibrilTool. Results are grouped by applied CTS and relaxation time.

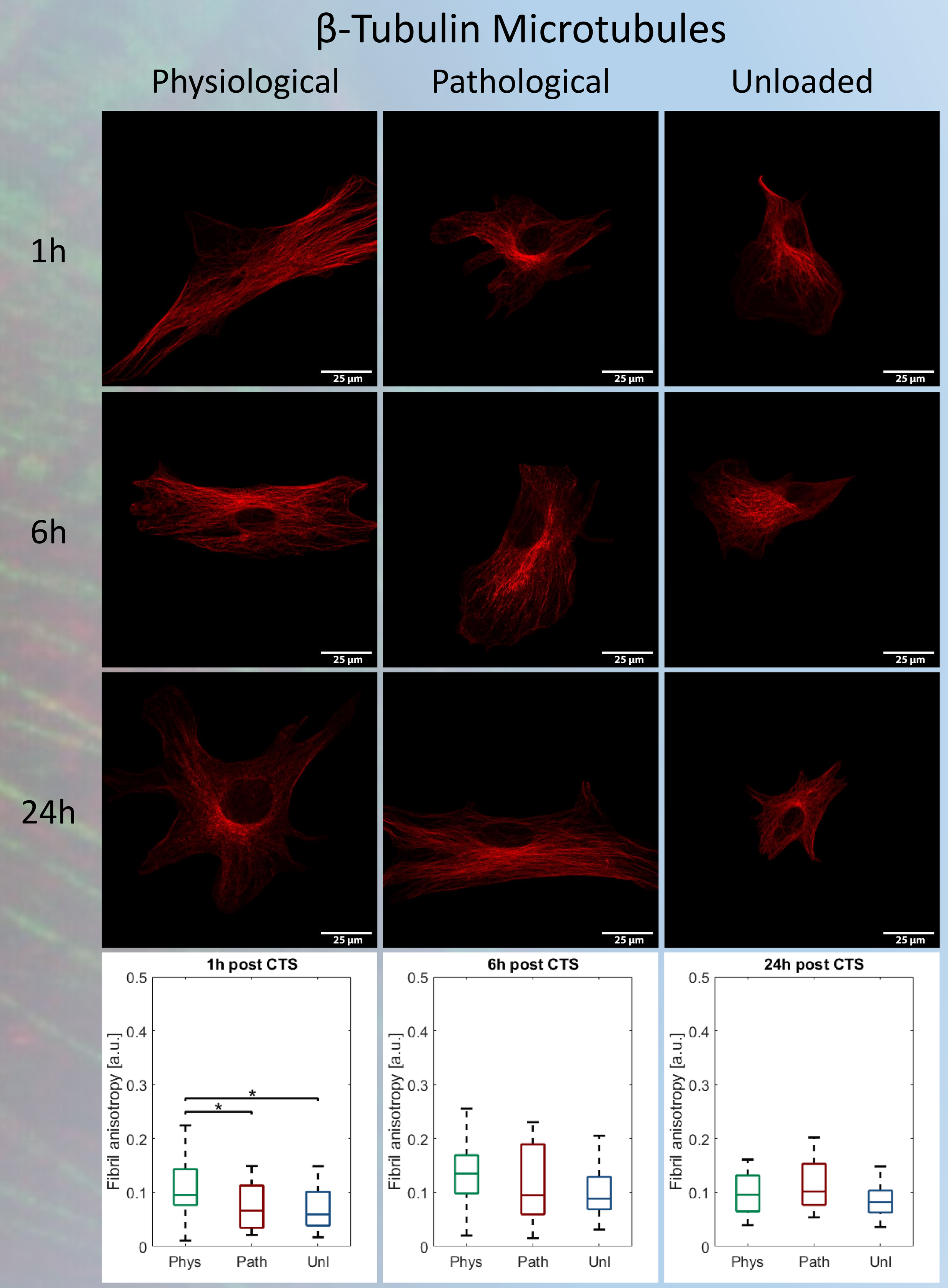

Figure 3: β-Tubulin cytoskeleton remodels 1h after physiological cyclic tensile strain (CTS). Box plots of cytoskeletal fiber anisotropy assessed by FibrilTool. Results are grouped by applied CTS and relaxation time.

Study Outcomes

Time	Physiological Strain			Pathological Strain		
	Alignment	Eccentricity	Area	Alignment	Eccentricity	Area
1h	⬆	⬇	⬆	–	–	↑
6h	↑	↓	↑	–	–	↑
24h	–	–	↑	↑	↓	⬆

Table1: Effects of physiological (normal) and pathological (glaucomatous) cyclic tensile strain (CTS) on bovine scleral fibroblasts. Comparison of changes of fiber alignment, cell eccentricity (width/length) and area against unloaded group.

Note: Arrow size correlates the amount of alterations.
↑ – Increase; ↓ – Decrease; – – No Significant Change.

- **Unloaded**: Low alignment, high eccentricity, small area. No exhibited changes as time progresses.

Time	Eccentricity [a.u.]			Area [µm²]		
	Phys	Path	Unl	Phys	Path	Unl
1h	0.441 ±0.131	0.553 ±0.121	0.586 ±0.157	4430 ±1476	3966 ±1336	2021 ±934
6h	0.495 ±0.166	0.561 ±0.148	0.648 ±0.129	3994 ±1872	4529 ±1641	2129 ±967
24h	0.604 ±0.154	0.492 ±0.168	0.633 ±0.149	3554 ±1589	4840 ±2331	1944 ±883

Table2: Cell eccentricity (width/height) and area of bovine scleral fibroblasts. Measurements taken with ImageJ. Results are grouped by applied CTS and relaxation time.

Conclusions

- **Physiological CTS/IOP**: Causes high alignment of the cytoskeleton, specifically the actin stress fibers. 24h after CTS the effect has not completely disappeared.
- **Pathological CTS/IOP**: Inhibitory influence on protein orientation. 24h the fibers reorganize to the initial physiological phenotype.
- **Future work**: Incorporation of cytoskeletal protein organization in finite element models to study glaucoma.

Acknowledgements

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Disclaimer

- The authors declare that there is no conflict of interest.

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