Scleral collagen remodeling and repair assessed in intact eyes through Second Harmonic Generation

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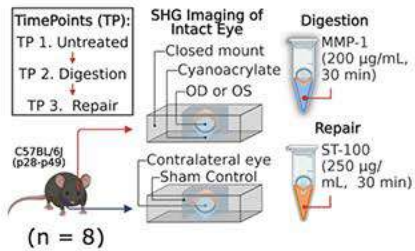
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Background

- Remodeling of the sclera during myopia progression is initiated through the activation of matrix metalloprotease (MMPs)[1,2].
- Collagen-mimetic peptides have shown reparative capabilities after collagen digestion[3].
- **Our goal is to capture microstructural changes in the sclera.**

Methods

Experimental overview

(n = 8)

- The effect of the treatments were compared by structural analysis of the collagen network in intact eyes[4].
- The effect of dissection was also compared (n=5).

SHG Microscopy

A custom-built two-photon SHG microscope (Fyla, Spain, 950-1150nm, 20 fs, 80MHz) was used to measured the backscattering signal (λ_0=525 nm BW=100 nm) of scleral fibrillar collagen 500 µm away from the optic nerve.

Structural Analysis

- The Alignment Index (AI) measures the distribution of the collagen bundles based on 2D-Fourier transform[5].
- The AI will be higher when the frequency is more concentrated at a certain orientation.
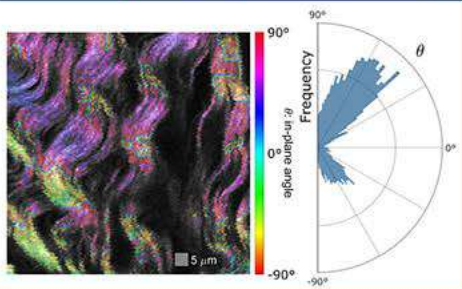
Statistical Analysis

One-way ANOVA to test statistical significance in alignment parameter across treatments and specimen state.

Results

Effect of treatments

- MMP-1 (0.30 ±0.05) increased the alignment of collagen bundles (p<0.05) with respect to the virgin state (0.25 ±0.01).
- ST-100 induced a significantly different (p<0.05) reduction of the alignment (0.24 ± 0.01).

Untreated   MMP-1   ST-100
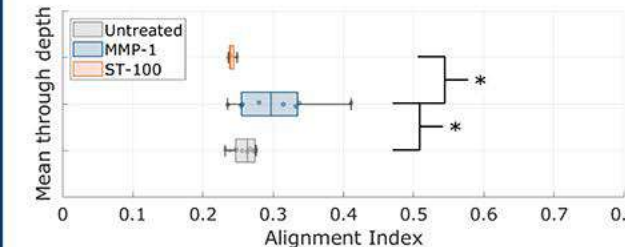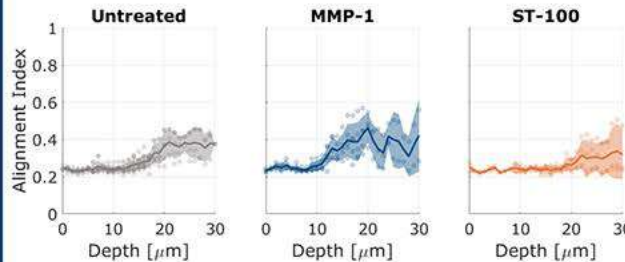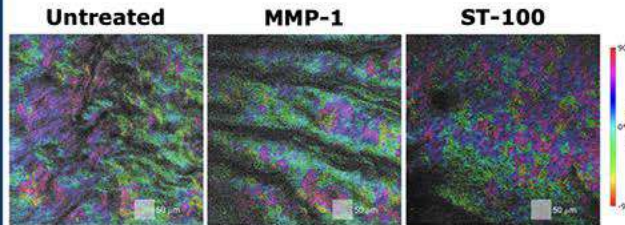
With respect to control: *p <0.05; ** p < 0.01

Effect of dissection

Dissection increased scleral thickness and increased the alignment of collagen bundles (0.39 ± 0.07) with respect to intact eyes (0.28 +0.01).
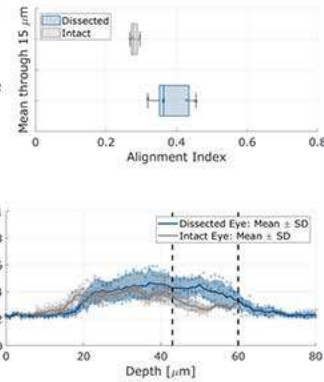
Conclusions

- SHG microscopy allowed characterization of scleral collagen organization in murine intact eyes, avoiding tissue dissection which altered collagen organization
- This method is well suited for future characterization of scleral collagen in myopic animal models, which was mimicked by MMP digestion
- ST-100 restores collagen alignment close to virgin state values in ex vivo experiments, holding promise as a therapeutic agent for myopia.

References

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Commercial Relationship

RB and ES (Stuart Therapeutics, E)