

# Collagen Mimetic Peptides and Restoration of Optic Nerve Head Stiffness in Experimental Glaucoma

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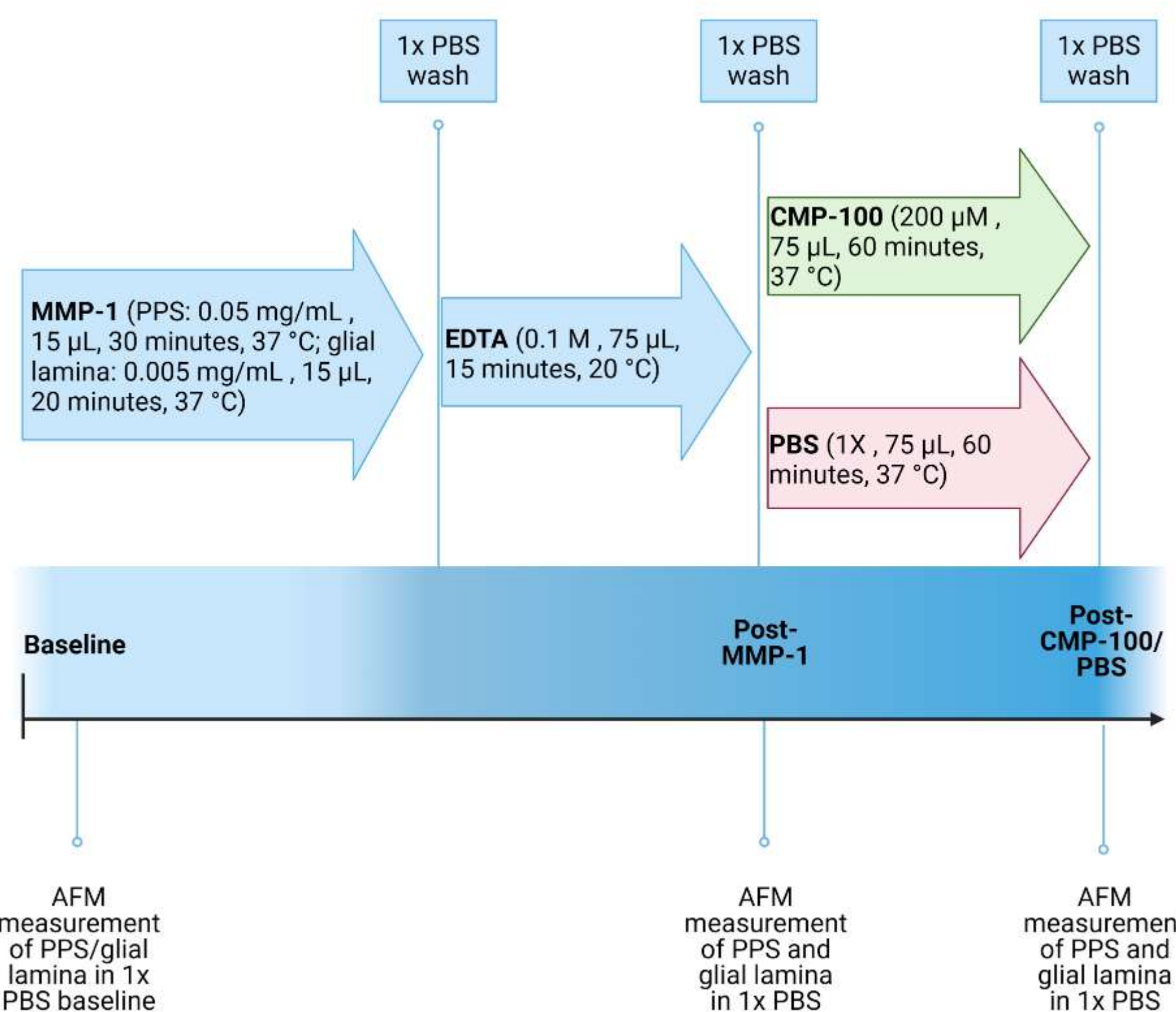
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## Introduction

- Biomechanical integrity of the sclera and optic nerve head (ONH) is critical for support of retinal ganglion cell (RGC) axons.
- In glaucoma, stress and strain at the ONH due to sensitivity to ocular pressure causes remodeling of the extracellular matrix including collagen reorganization.
- Collagen mimetic peptides (CMP) are short single-stranded peptides that bind with high avidity to damaged collagen, promoting structural and functional repair.
- Here we tested the capacity of a CMP to restore ocular tissue stiffness following matrix metalloproteinase (MMP) treatment ex-vivo.
- We also measured the stiffness of ONH from rats exposed to elevated ocular pressure to begin evaluating CMP use in vivo.

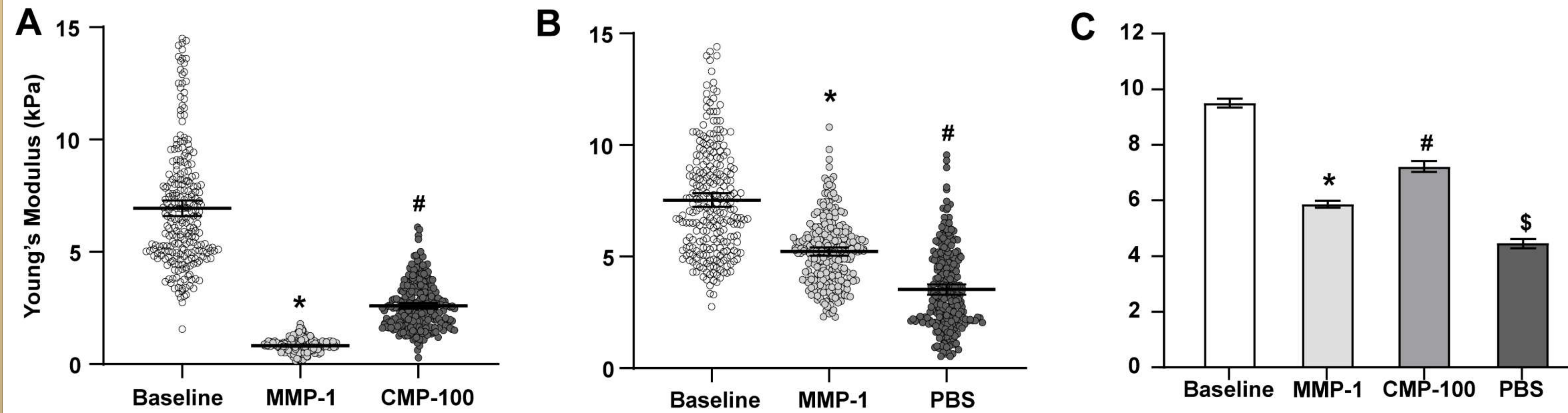
## Methods

- Using atomic force microscopy (AFM), we measured Young's modulus (tissue stiffness) in the glial lamina and peripapillary sclera (PPS) of fresh rat eye sections.
- First, we measured Young's modulus in naïve eye tissue at baseline, after MMP-1 digestion (30 min, 0.005-0.05 mg/mL), and after treatment with CMP-100 (60 min, 200  $\mu$ M (Pro-Pro-Gly)<sub>7</sub>) or vehicle (PBS).



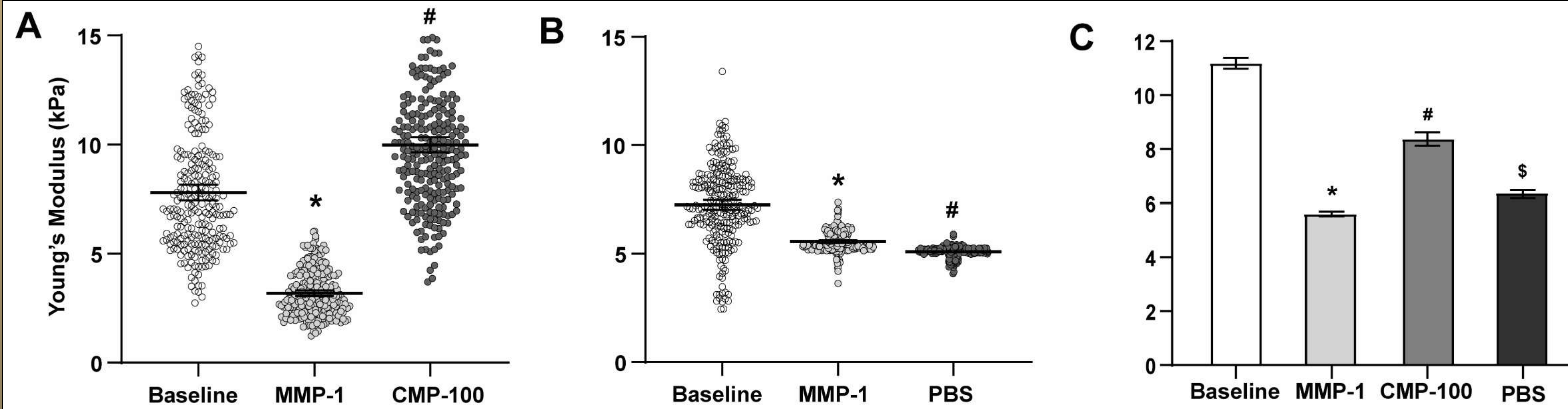
- Next, we induced ocular hypertension (OHT) in rats using microbead occlusion and measured Young's modulus after 4 weeks compared to controls (Figure 2).

## 1) CMP-100 Partially Restores PPS Stiffness after MMP-1 Treatment



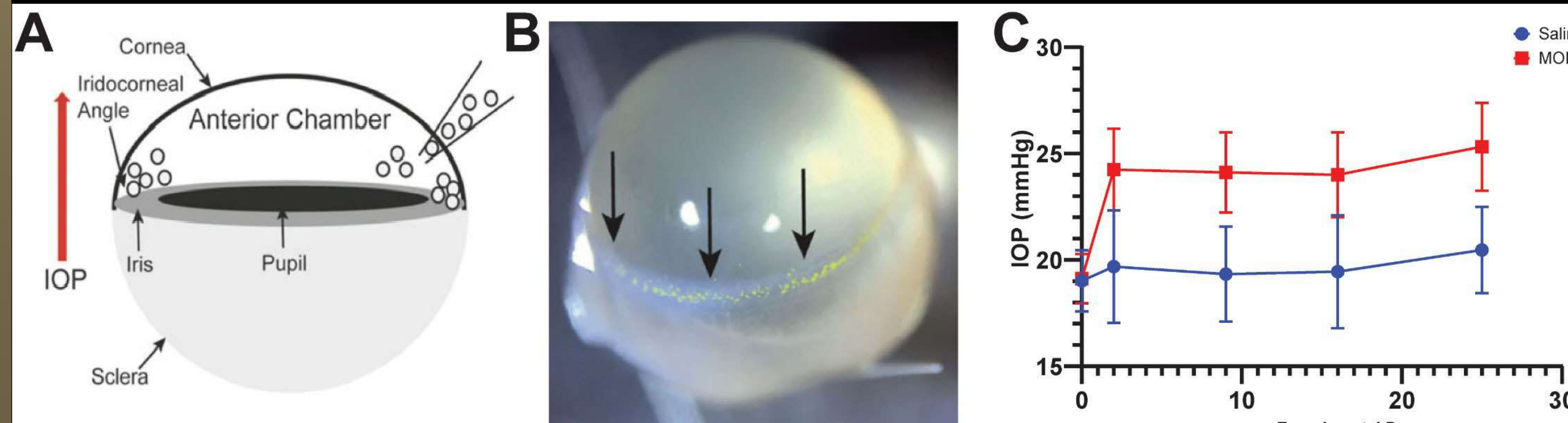
**Figure 1.** (A) Representative Young's moduli measurements in PPS from n = 1 of 3 animals. MMP-1 treatment reduced the stiffness of the PPS (\*, p ≤ 0.001), while CMP-100 partially restored tissue stiffness (#, p ≤ 0.001). (B) Representative Young's moduli measurements in PPS from n = 1 of 3 animals. The addition of vehicle (1x PBS) after MMP-1 treatment gradually reduced tissue stiffness (\*, #, p ≤ 0.001). (C) Pooled Young's moduli mean values from n = 3 animals show that MMP-1 treatment significantly reduced PPS tissue stiffness compared to baseline (\*, p ≤ 0.001). Treatment with CMP-100 after MMP-1 digestion partially restored tissue stiffness (#, p ≤ 0.001), while the addition of vehicle after MMP-1 treatment further reduced tissue stiffness (\$, p ≤ 0.001).

## 2) CMP-100 Partially Restores Glial Lamina Stiffness after MMP-1 Treatment



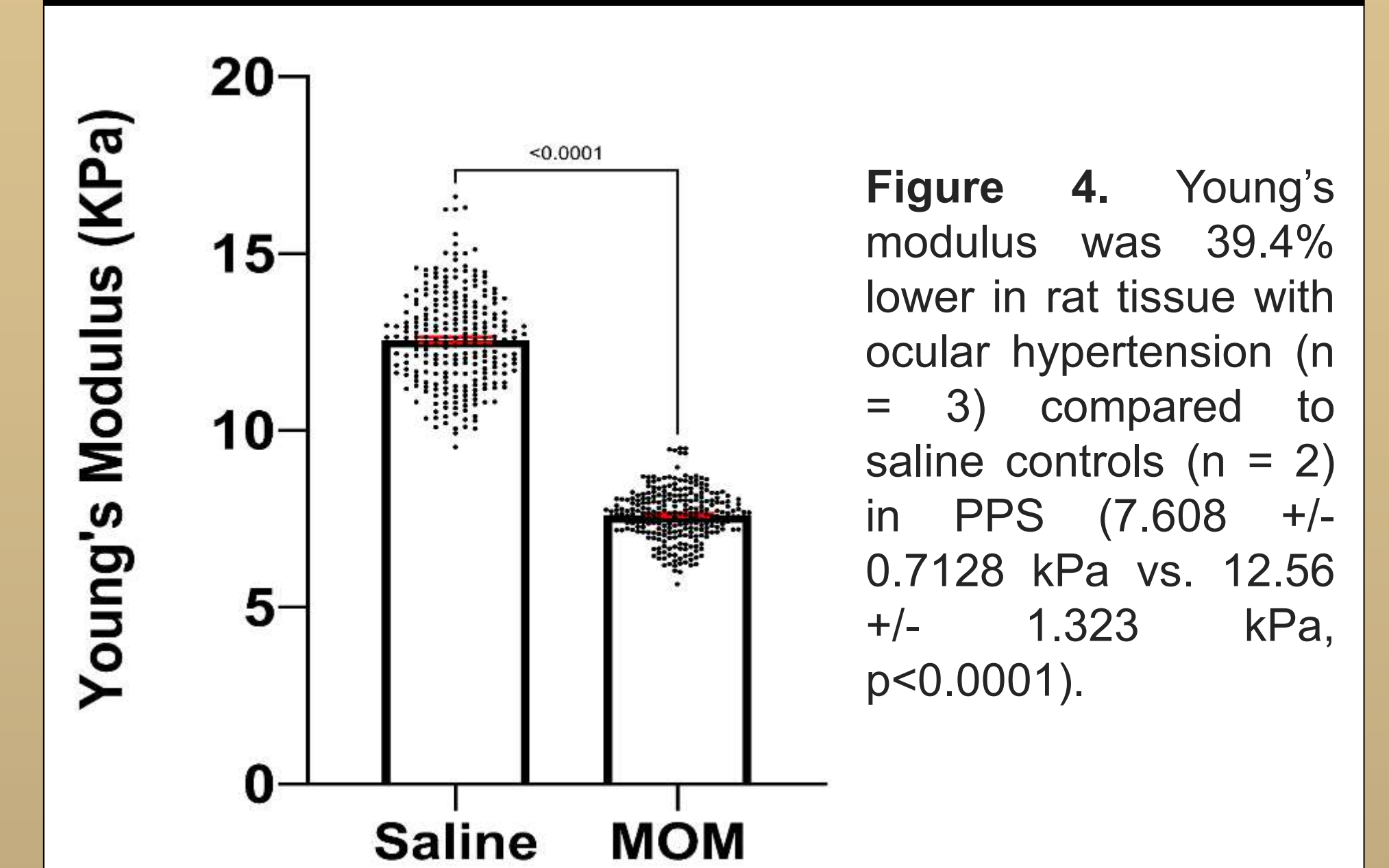
**Figure 2.** (A) Representative Young's moduli measurements in glial lamina from n = 1 of 3 animals. MMP-1 treatment reduced stiffness of the PPS (\*, p ≤ 0.001), while CMP-100 restored tissue stiffness (#, p ≤ 0.001). (B) Representative Young's moduli measurements in glial lamina from n = 1 of 3 animals. The addition of vehicle (1x PBS) after MMP-1 treatment gradually reduced tissue stiffness (\*, #, p ≤ 0.001). (C) Pooled Young's moduli mean values from n = 3 animals show that MMP-1 treatment significantly reduced glial lamina tissue stiffness compared to baseline (\*, p ≤ 0.001). Treatment with CMP-100 after MMP-1 digestion partially restored tissue stiffness (#, p ≤ 0.001), while the addition of vehicle after MMP-1 treatment increased tissue stiffness to a smaller extent (\$, p ≤ 0.001).

## 3) Microbead Injection Effectively Elevated Intraocular Pressure



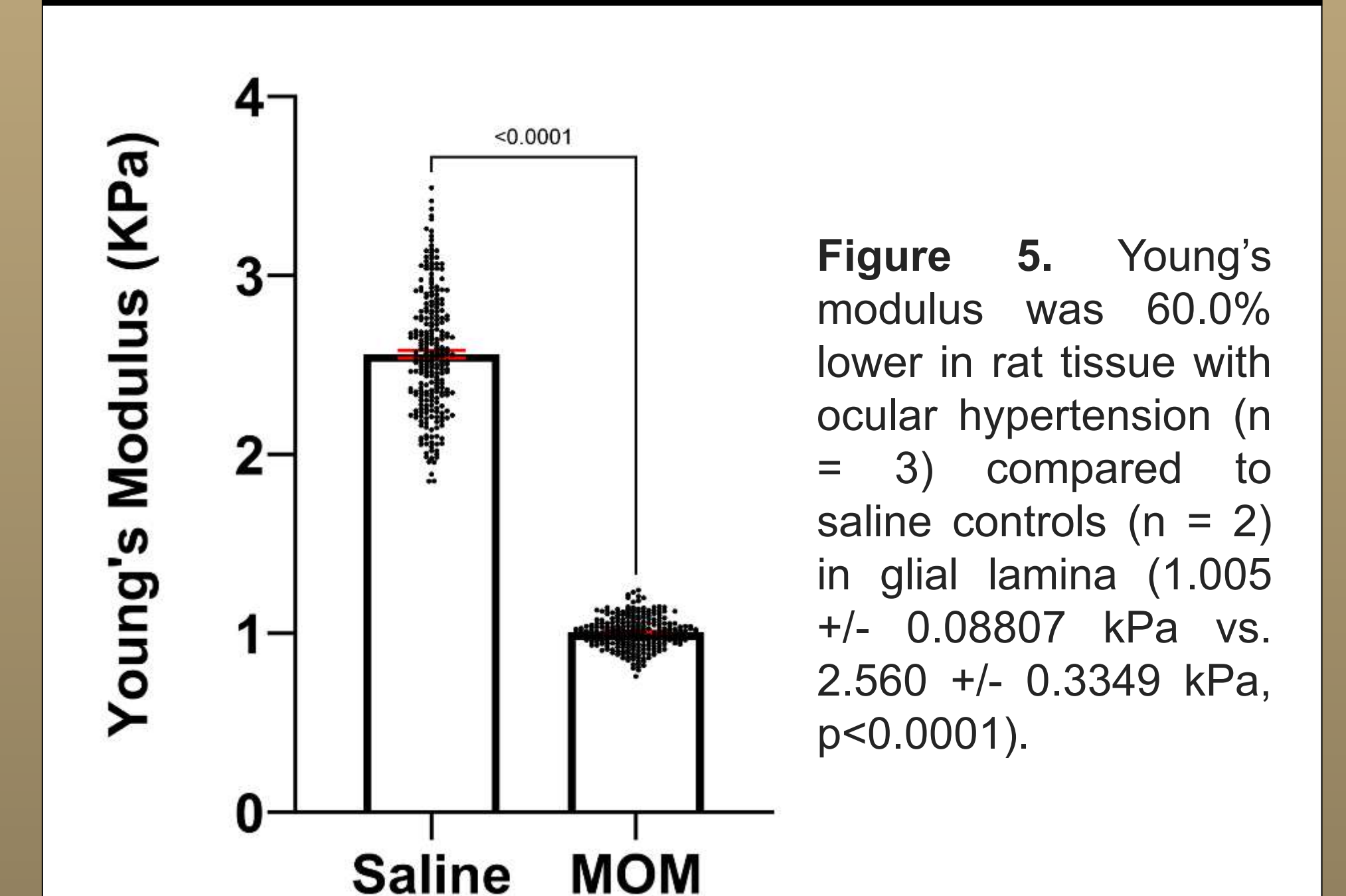
**Figure 3.** (A) Schematic of microbead injection. (B) Microbeads in the iridocorneal angle cluster near the point of aqueous outflow (arrow). (C) Plot of intraocular pressure (IOP) measurements for saline (n = 4) and microbead injected (MOM) eyes (n = 6) in rats for the 4-week cohorts. Data plotted as mean ± SEM.

## 4) PPS Stiffness is Lower with OHT



**Figure 4.** Young's modulus was 39.4% lower in rat tissue with ocular hypertension (n = 3) compared to saline controls (n = 2) in PPS (7.608 ± 0.7128 kPa vs. 12.56 ± 1.323 kPa, p < 0.0001).

## 5) Glial Lamina Stiffness is Lower with OHT



**Figure 5.** Young's modulus was 60.0% lower in rat tissue with ocular hypertension (n = 3) compared to saline controls (n = 2) in glial lamina (1.005 ± 0.08807 kPa vs. 2.560 ± 0.3349 kPa, p < 0.0001).

## Conclusions

- Together, our results indicate that CMP-100 can restore stiffness of MMP-1-digested ONH tissue. Furthermore, ONH tissue stiffness is reduced with ocular hypertension in rats.
- Since MMP-1 expression increases in ONH in glaucoma, ongoing experiments are evaluating the potential of CMPs to restore ONH collagen homeostasis. These experiments highlight a potential novel avenue of restoring tissue integrity in glaucoma, which may impact RGC survival.

## References

<sup>1</sup>Sappington *et al*, *Investig. Ophthalmol. Vis. Sci.* 2010, 51, 207-216.

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- The study received funding from Stuart Therapeutics, Inc