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Purpose

Fibrosis at the human trabecular meshwork (HTM) has been associated with elevated intraocular pressure (IOP) in glaucoma. AR-13324 (netarsudil), a Rho kinase and Norepinephrine transporter inhibitor, is in Phase 3 trials for the treatment of glaucoma. This study explores the potential anti-fibrotic effects of AR-13324 in a 3D Bioengineered HTM model of steroid-induced glaucoma.

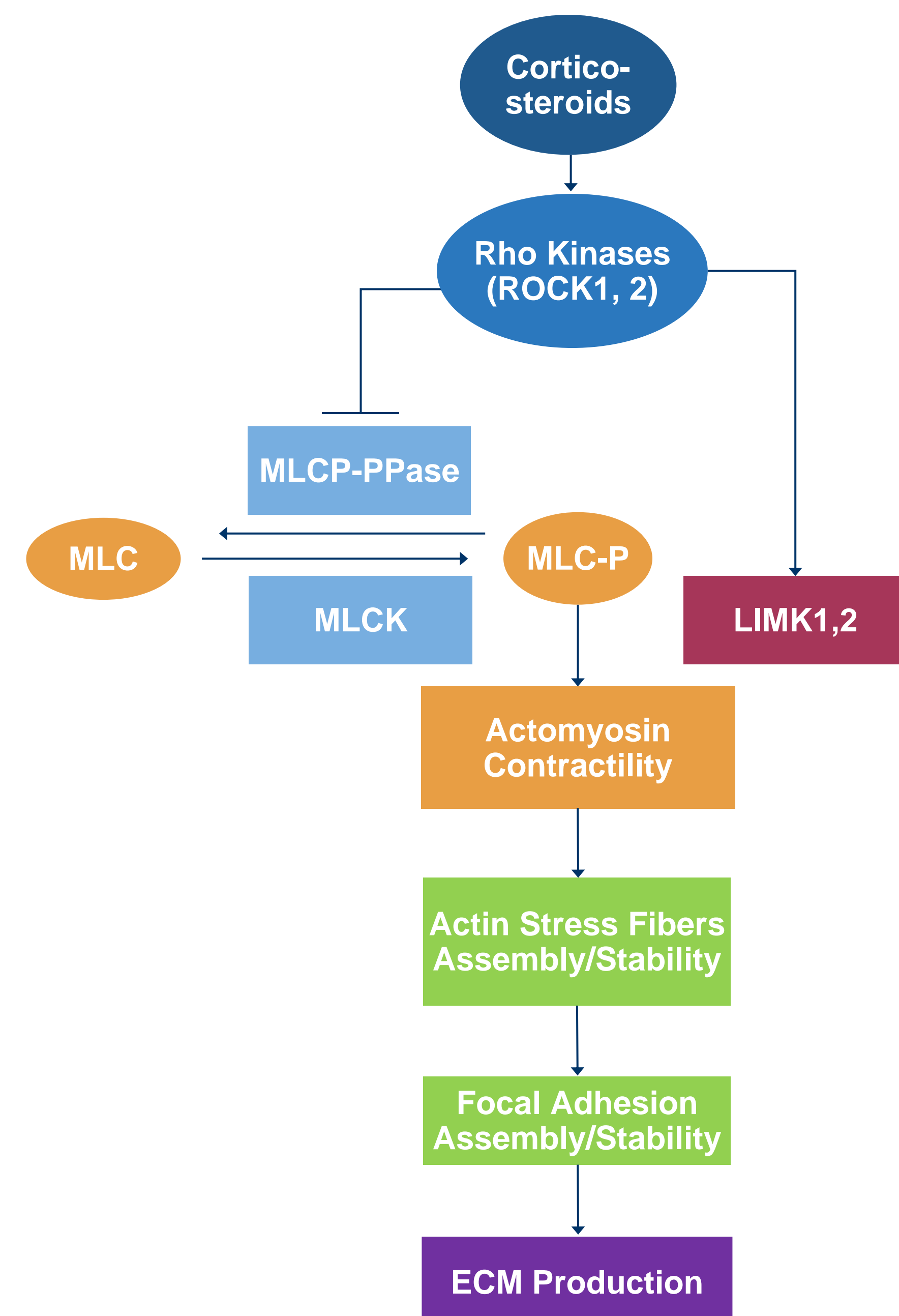
Methods

Prevention study: 3D HTM was treated with the steroid prednisolone acetate (PA) and simultaneously treated with 0, 100, 250 or 500nM of AR-13324.

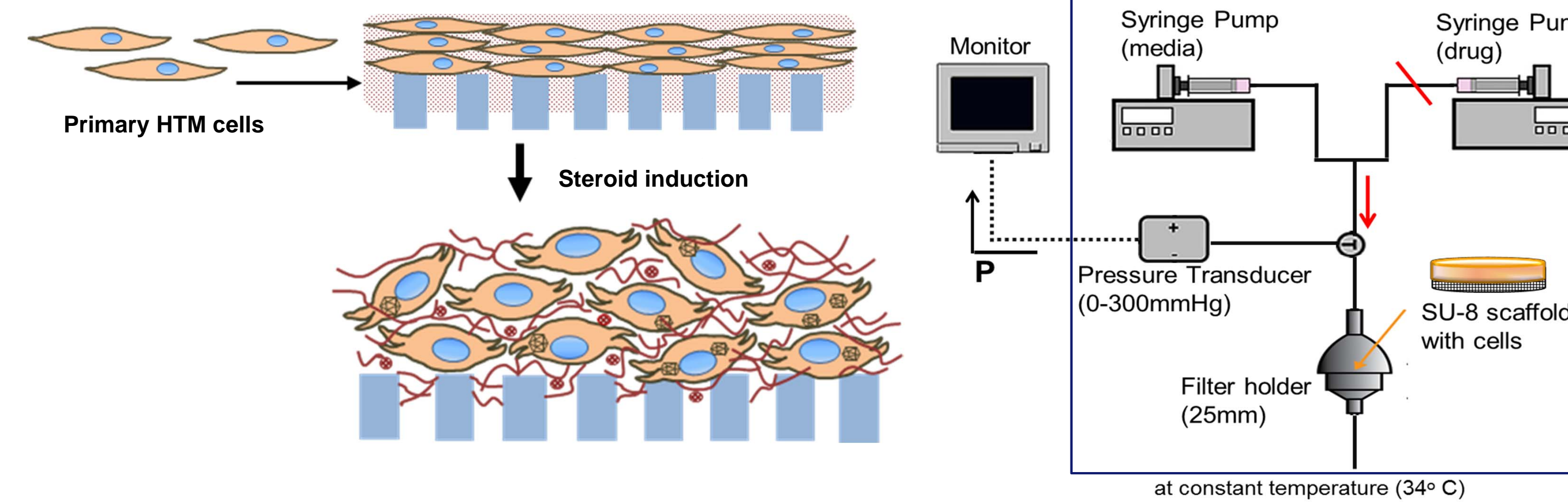
Treatment study: 3D HTM was treated with PA for 144 hours, with 100nM AR-13324 added after the first 72 hours of PA treatment.

Both studies: HTM samples were analyzed by immunocytochemistry and confocal microscopy for expression of paxillin (focal adhesions), F-actin stress fibers, and the extracellular matrix (ECM) proteins fibronectin and collagen IV. Transcriptional and protein expression levels of fibronectin and collagen IV were also evaluated. Scanning electron microscopy (SEM) was used to investigate the morphology and topography of the 3D HTM. Perfusion studies were performed to measure the outflow facility of 3D HTM.

Corticosteroids and Rho Kinase Promote HTM Cell Contraction, Overproduction of Fibrosis-related ECM Proteins



3D Bioengineered HTM and Perfusion System (Glauconix)

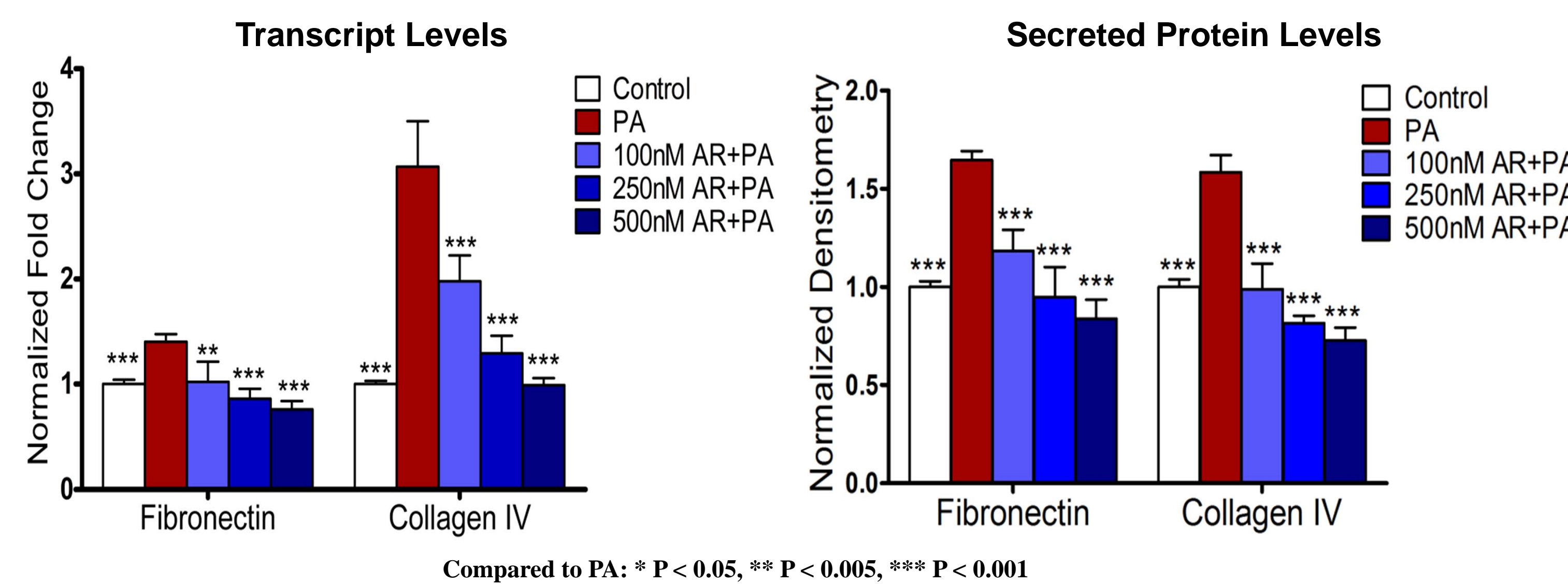


Results

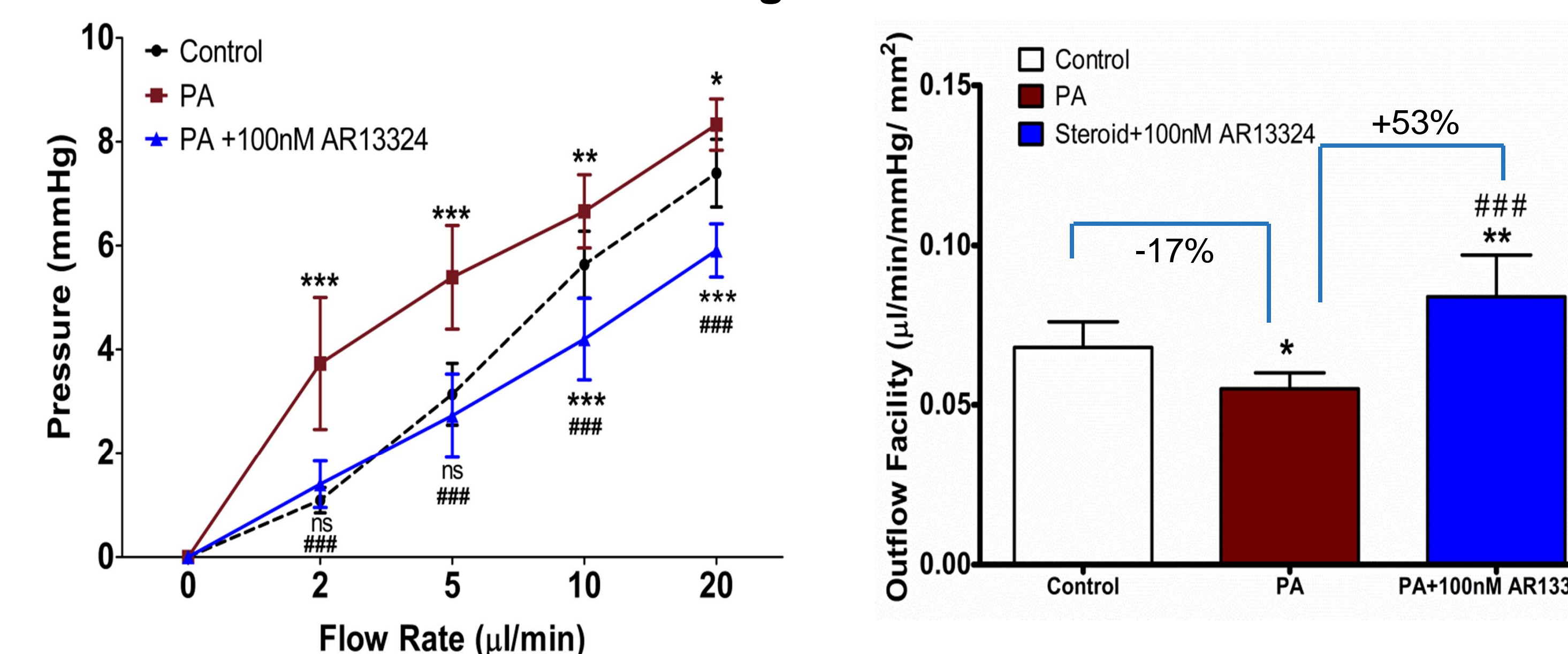
Prevention Study

The PA-treated 3D HTM responded to AR-13324 treatment in a dose-dependent manner. Compared to PA-only treated samples, the presence of AR-13324 significantly decreased the levels of fibronectin and collagen IV transcripts ($p < 0.01$) and protein expression and secretion ($p < 0.001$). Treatment with AR-13324 also prevented increased accumulation of focal adhesions and actin stress fibers induced by PA.

AR-13324 Blocks Prednisolone Acetate-induced Expression of ECM Proteins in Bioengineered HTM



AR-13324 Blocks PA-induced Reduction of Outflow Facility in Bioengineered HTM



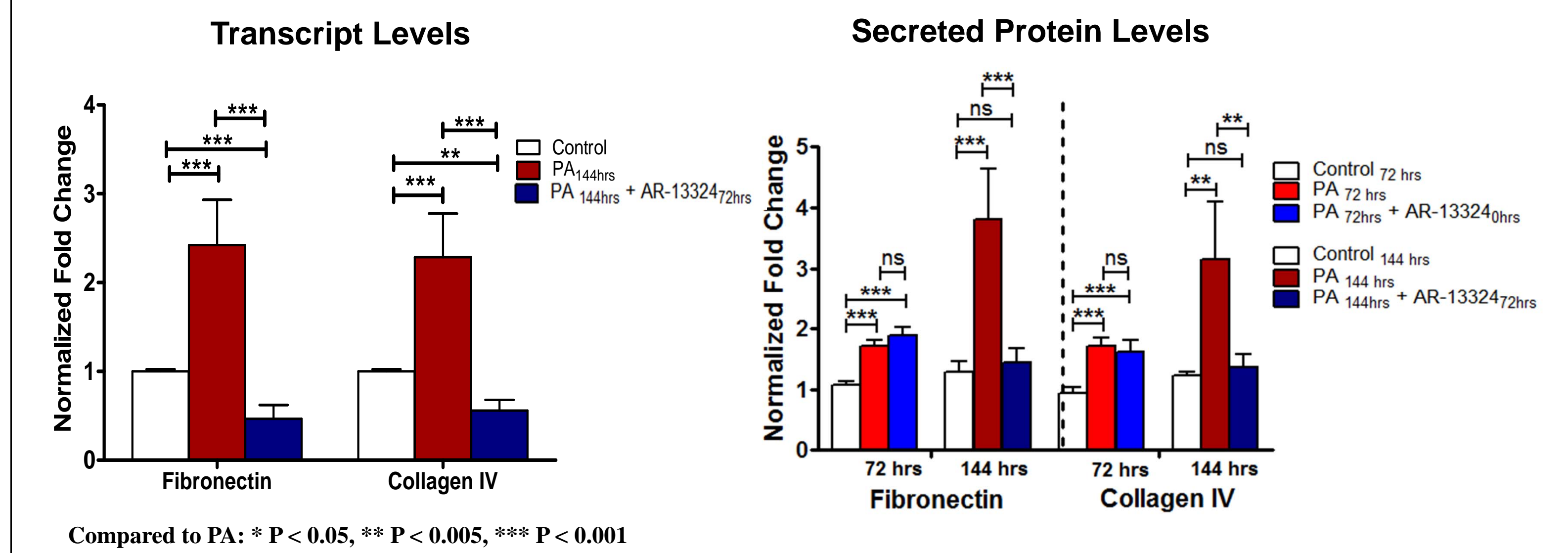
Compared to controls: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$. Compared to PA: # $P < 0.05$, ## $P < 0.005$ and ### $P < 0.001$

	Control	PA	PA+100nM AR13324
Outflow facility ($\mu\text{l}/\text{min}/\text{mmHg}/\text{mm}^2$)	0.066 \pm 0.009	0.055 \pm 0.006	0.084 \pm 0.013

Treatment Study

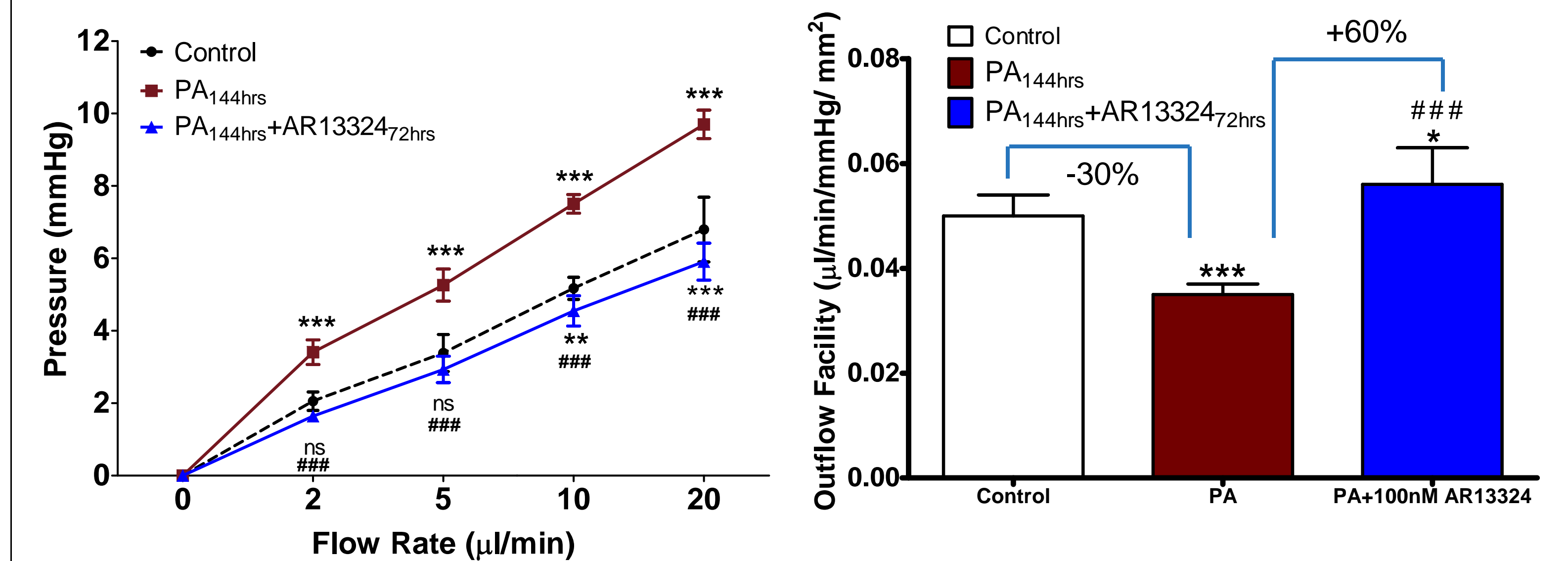
The addition of AR-13324 to HTM that had been pre-treated with PA for 72 hours reversed the PA-induced changes in ECM protein expression. Perfusion studies showed that 100nM of AR-13324 also reversed the decrease in outflow facility caused by pretreatment of HTM with PA.

AR-13324 Reverses PA-induced Expression of ECM Proteins in Bioengineered HTM



Compared to PA: # $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$

AR-13324 Reverses PA-induced Reduction of Outflow Facility in Bioengineered HTM



Compared to controls: * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$. Compared to PA: # $P < 0.05$, ## $P < 0.005$ and ### $P < 0.001$

	Control	PA	PA+100nM AR13324
Outflow facility ($\mu\text{l}/\text{min}/\text{mmHg}/\text{mm}^2$)	0.050 \pm 0.004	0.035 \pm 0.002	0.056 \pm 0.007

Conclusions

Corticosteroids cause ocular hypertension and secondary glaucoma as a consequence of impaired aqueous humor outflow through the trabecular meshwork. AR-13324 prevented steroid-induced overproduction of ECM, focal adhesions and actin stress fibers in 3D bioengineered HTM. The biochemical and morphological changes induced by AR-13324 correlated with an increase in outflow facility in PA-treated HTM. AR-13324 countered the effects of steroid treatment on the HTM whether added prior to or after the onset of the steroid-induced changes.