

Supplementary Information for

In vivo measurement of trabecular meshwork stiffness in a novel, corticosteroid-induced ocular hypertensive mouse model

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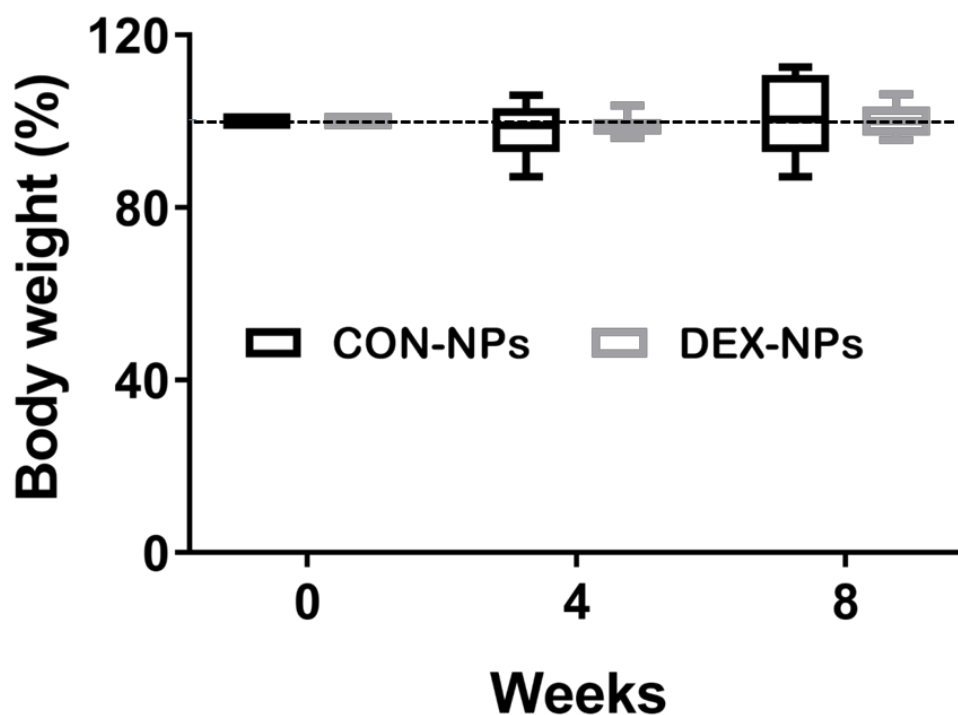


Fig. S1. **Body weight of mice treated with DEX-NPs or CON-NPs over time.** Two groups of 3 month-old C57BL/6 female mice were injected with identical amounts of CON-NPs or DEX-NPs (1 mg/20 μ l of particles containing no DEX or \sim 23 μ g of DEX, respectively) bilaterally once per week for eight weeks. Body weight was measured before treatment, and at 4 weeks and 8 weeks after the first injection. The quantity plotted on the Y-axis is body weight normalized to the value before treatments (N = 5-7). The data are displayed as box and whisker plots, with the box showing median, upper and lower quartiles and whiskers indicating highest and lowest observations. Dashed line indicates 100% of body weight before treatment.

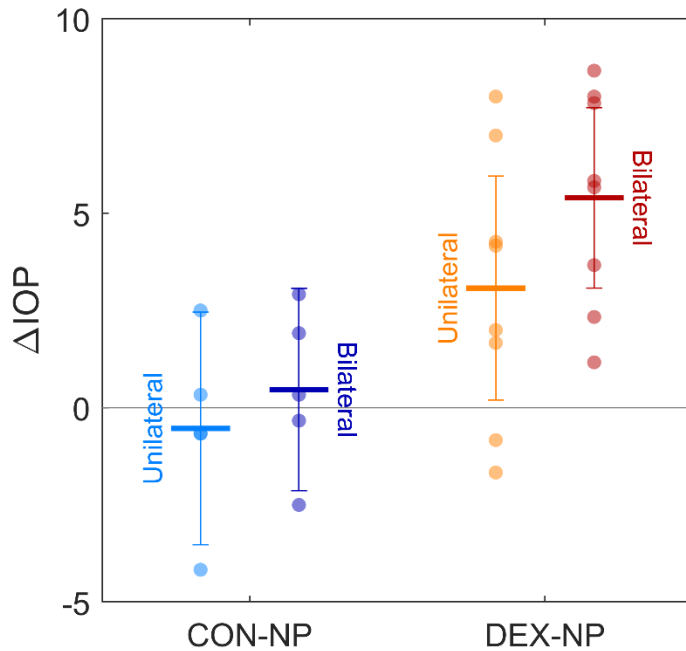


Fig. S2. **Effect of unilateral versus bilateral injections.** Two injections per month of DEX-NPs (N=8) or CON-NPs (N=5) were applied unilaterally, and compared to bilateral injections under the same conditions. Neither unilateral nor bilateral CON-NP injections resulted IOP elevation relative to baseline (Δ IOP). For DEX-NPs, unilateral injections resulted in a smaller average IOP elevation 3.1 [0.2, 6.0] mmHg than bilateral injections 5.4 [3.1,7.7] mmHg, but the difference was not statistically significant ($p = 0.16$)

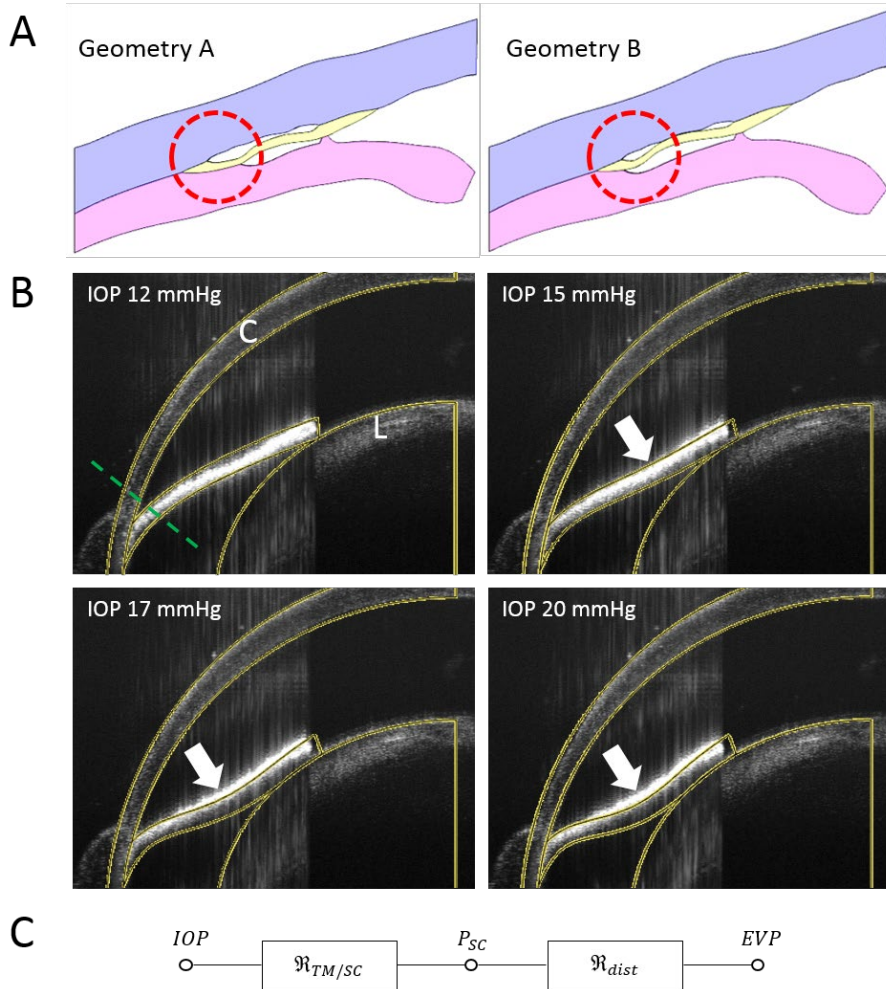


Fig. S3: **Details of finite element modeling.** (A) The specific anatomy of the iris-TM junction (red circles) influenced the extent and shape of SC collapse as predicted by FEM. For example, a more anteriorly-located iris-TM junction (Geometry A) led to minimal posterior SC collapse, in distinction to the situation with a more posteriorly-located iris-TM junction (Geometry B). Based on OCT imaging showing SC collapse both anteriorly and posteriorly, we chose Geometry B for all simulations presented herein. (B) OCT images of the anterior segment show that as the imposed IOP is increased, the iris (bright region) bows backwards (arrows) and the cornea-iris angle increases, which we took as evidence of formation of a lens-iris diaphragm and a pressure differential across the iris. C, cornea; L, lens. Yellow lines overlain on the OCT images show the iridial displacement predicted by a large-scale FEM, as described in the text. The green dashed line coincides with the right edge of the SC lumen-focused OCT image where effective shear/normal forces and moments were extracted and applied to the SC lumen-focused finite element model, as described in the text. (C) Equivalent electrical circuit representation of the conventional aqueous outflow pathway in mice, showing resistive elements (rectangles) and key pressures (nodes). See text for definition of symbols.

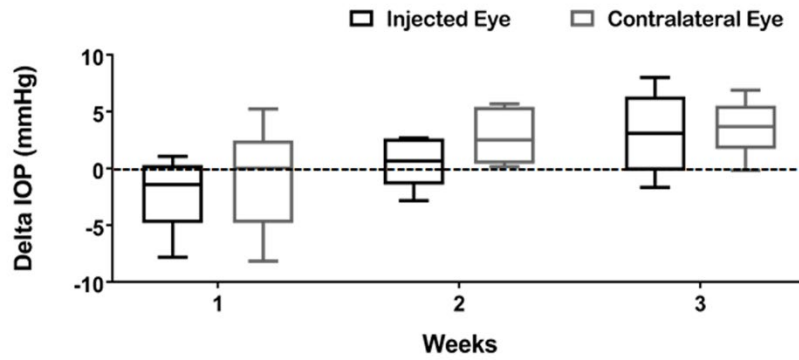


Fig. S4. IOP elevation in contralateral eyes following unilateral DEX-NP injections. Three month-old C57BL/6 male mice were injected with DEX-NPs (1 mg/20 μ l of particles containing \sim 23 μ g of DEX) into right eyes twice/month. IOPs were measured in both eyes. Data shows average IOP elevation from baseline readings at indicated time points on both eyes. N = 8 mice. The data are displayed as box and whisker plots, with the box showing median, upper and lower quartiles and whiskers indicating highest and lowest observations.

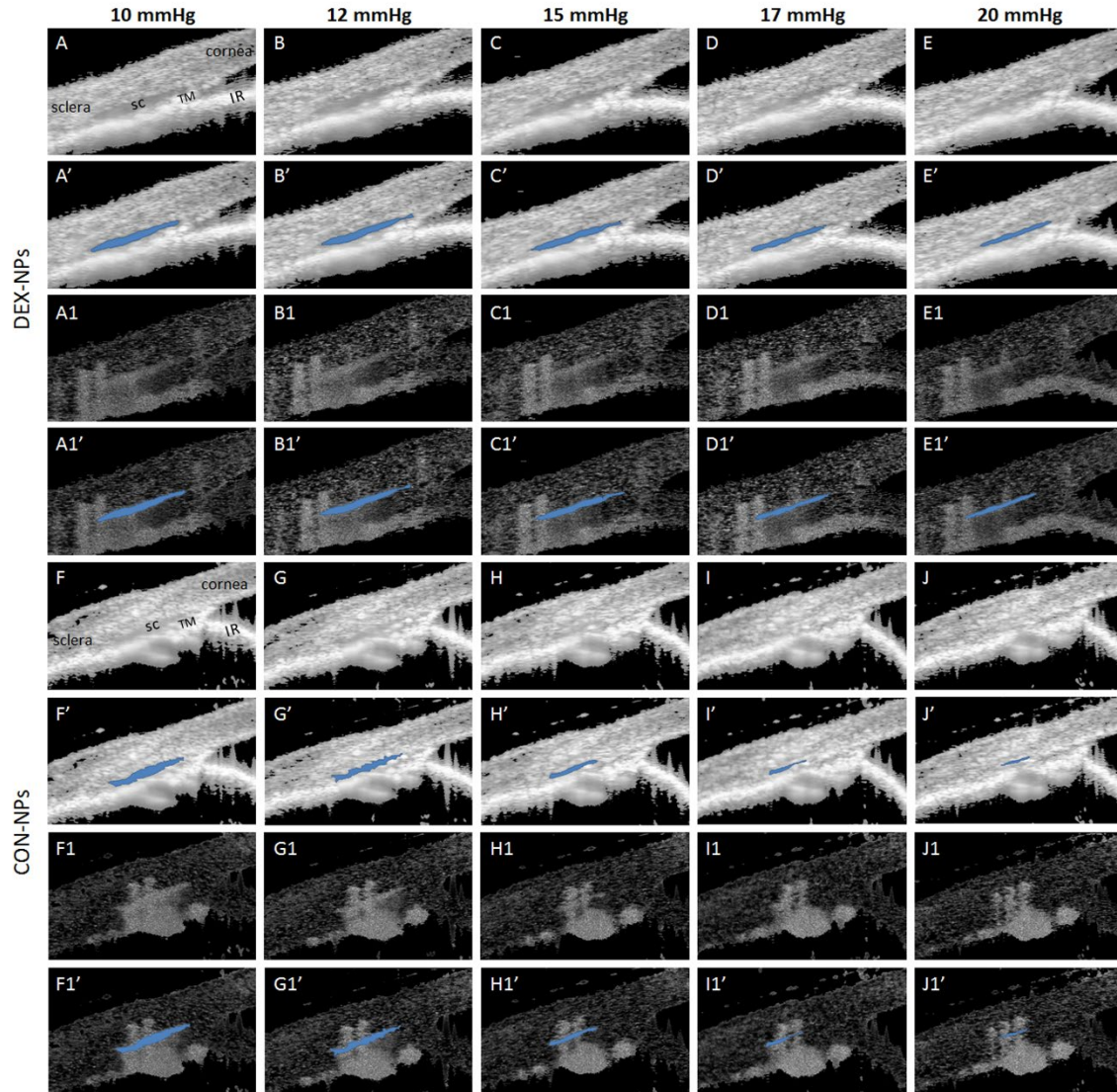


Fig. S5. Changes in conventional outflow tissue behavior in living mice treated with DEX-NP and monitored by OCT. Living DEX-NP- or CON-NP-injected mouse eyes were cannulated to control IOP, and were subjected to increasing pressure steps (10, 12, 15, 17 and 20 mm Hg) while imaging conventional outflow tissues in cross section by OCT. Representative averaged OCT images for a single DEX-NP-injected eye at the indicated IOP levels are shown in the top row. The third row shows speckle variance images from the same mouse eye as in the top row. The second and fourth rows show semi-automatic segmentation of Schlemm's canal lumen (blue) using SchlemmSeg software, overlaid on averaged and speckle variance images, respectively. A single mouse eye treated with CON-NPs is shown in rows 5-8 at different IOP levels, with the same pattern of display as described for DEX-NP eyes. SC, Schlemm's canal; TM, trabecular meshwork; IR, iris.

Table S1. Segmented cross-sectional area (pixels) of Schlemm’s canal lumen at clamped intraocular pressures (IOP, mmHg)

IOP	10	12	15	17	20
DEX-NP					
1	1952	1842	1552	979	851
2	1822	1760	1599	1490	1153
3	2222	2096	1712	1469	1210
4	2121	1979	1641	1280	953
5	2064	1878	1757	1568	1190
6	1820	1405	1339	1102	589
7	1985	1934	1476	1370	960
8	1985	1888	1661	1481	1145
9	1459	1382	1275	894	770
10	1510	1398	1273	1068	859
mean	1894	1756.2	1528.5	1270.1	968
SD	235.6	250.6	170.2	229.5	195.5
CON-NP					
1	1882	1707	1148	596	286
2	1019	818	683	393	165
3	1884	1507	n/a	736	389
4	1030	838	718	360	155
5	1028	926	689	512	245
6	1999	1506	1392	1045	705
mean	1473.7	1217	926	607	324.2
SD	492.6	398.8	325.9	254.7	205.4

DEX: dexamethasone, CON: control, NP: nanoparticles, n/a: not available, per manufacturer’s specifications, one pixel is approximately 1.15 μm^2

Table S2. Numbers of mice and eyes used for experiments

Measurements	CON-NPs treatment	DEX-NPs treatment	For figures	Condition
IOP	28 mice, 51 eyes	37 mice, 66 eyes	1A, 1B, S2,S4	In vivo
Facility	12 mice, 23 eyes	13 mice, 25 eyes	1D	Ex vivo (globe)
DEX level	-	12 mice, 12 eyes	1C	Ex vivo (globe)
Histology	7 mice, 8 eyes	14 mice, 17 eyes	2	Tissue
OCT	6 mice, 6 eyes	10 mice, 10 eyes	3, 4, S3	In vivo
Body weight	5 mice	7 mice	S1	In vivo

IOP: intraocular pressure, OCT: optical coherence tomography, DEX: dexamethasone, CON: control, NP: nanoparticles

Table S3. Estimated pressures within SC lumen as a function of set (“clamped”) IOP levels

Clamped IOP (mmHg)	Schlemm’s canal luminal pressure (P_{SC} mmHg)	
	CON-NP	DEX-NP
10	7.2	6.7
12	8.0	7.3
15	9.2	8.1
17	10.0	8.7
20	11.2	9.6

IOP: intraocular pressure, DEX: dexamethasone, CON: control, NP: nanoparticles