Supplementary Material

Purpose: To quantify pressure difference across 50µm glass pipettes at various intraocular pressure (IOP) levels under steady state condition.

Methods:
Pressure difference across a 50 µm glass pipette can be quantified by multiplying the aqueous outflow rate ($F_a$) by the resistance to Hanks’ balanced salt solution (HBSS) flow in the pipette ($R_p$).

Resistance is given by the pressure (mmHg) required to produce a unit of fluid flow (µL/min). The flow rate through the tubing system alone ($R_t$) and of the total tubing plus pipette system ($R_{t+p}$) were calculated by measuring volume flow over a fixed time period (µL/min) with reservoir maintained at various heights corresponding to a range of pressure levels (mmHg). Pressure was then plotted against flow rate, and the gradient of such pressure-flow relationship gives the resistance (mmHg per µL/min). Resistance to flow through a 50 µm glass pipette ($R_p$) was calculated by subtracting the resistance of the tubing system from the resistance of the total tubing plus pipette system

$$R_p = R_{t+p} - R_t$$
All pipettes were made and beveled according to methods previously described. (John SW, IOVS, 1997).

Aqueous outflow rate in mice has been previously described as the sum of IOP-dependent conventional outflow ($F_c$) and uveoscleral outflow ($F_u$) (Aihara, IOVS 2003) where:

$$F_c = 0.0051 \pm 0.0006 \mu \text{L/min per mmHg}$$
$$F_u = 0.148 \mu \text{L/min}$$

Results:
Resistance to flow in the tubing system alone ($R_t$) was measured as 0.012 mmHg/ (µL/min) and resistance to flow in the tubing system plus pipette ($R_{t+p}$) was 0.24 ± 0.01 mmHg/ (µL/min) (n = 3 needles). Therefore resistance due to 50µm glass pipette alone ($R_p$) was 0.23 ± 0.01 mmHg/ (µL/min).

Total aqueous outflow flow ($F_a$) at an IOP of 80 mmHg was calculated to be 0.56 ± 0.05 µL/min ($F_c + F_u$). From this, the pressure difference across 50 µm glass pipettes was calculated to be 0.13 ± 0.01 mmHg. Thus when IOP is measured to be 80 mmHg manometrically, we expect the true IOP to be 0.13 ± 0.01 mmHg less.

For an IOP of 30 mmHg and 50 mmHg, the pressure difference was calculated to be 0.07 ± 0.01 µL/min and 0.09 ± 0.01 µL/min, respectively.

Conclusion:
Under steady state conditions, continuous aqueous outflow can induce a pressure difference across 50 µm glass pipette due to resistance across the pipette. This pressure difference is governed by IOP and type of fluid used for infusion. This pressure difference will need to be taken into consideration in situations where 50 µm glass pipettes are used for simultaneous IOP adjustment and IOP measurement.