Live Outflow Imaging in Porcine Eyes

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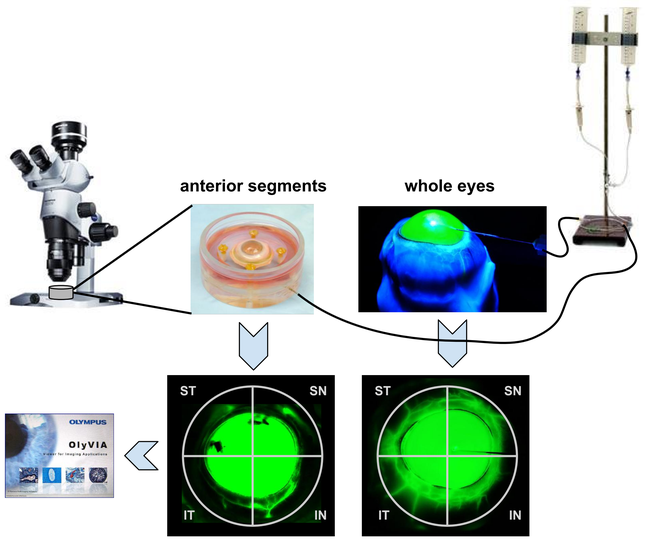
Purpose

Although outflow of aqueous humor can be estimated using fluorophotometry and tonography, no method exists to assess segmental outflow directly. This has hindered investigations into outflow resistance elements that are downstream of the trabecular meshwork and into causes of failure of canal-based minimally invasive glaucoma surgeries (MIGS). Here, we describe a method to observe and quantify conventional outflow directly in ex vivo whole eyes and an organotypic culture model using standard fluorescence visualization equipment.

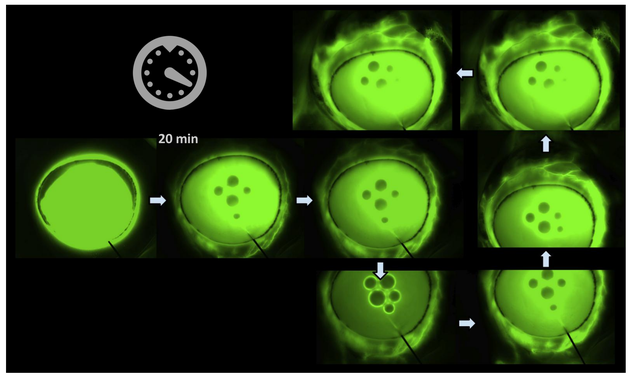
Methods

Pig eyes were obtained from a local abattoir and cultured within 2 hours. A 30 gauge needle was placed into the anterior chamber of six whole eyes. Six further eyes were placed into anterior segment culture systems after removal of uvea and lens. All eyes were perfused at a constant physiological IOP of 15 mmHg with Dulbecco's modified Eagle medium (DMEM) containing 0.017 mg/ml resorcinolphthalein. Chromophore flow patterns were visualized using a stereo dissecting microscope equipped for fluorescent imaging (Olympus SZX16 with GFP filter cube and DP80 Monochrome/Color Camera; Olympus Corp., Center Valley, PA). Images were obtained every 30 seconds for 20 minute for time lapse analysis (CellSens, Olympus Life Science). Following washout, anterior segments were imaged again on day 3 of culture. Whole eyes and anterior segment cultures were perfused with DMEM for three days and then reimaged.

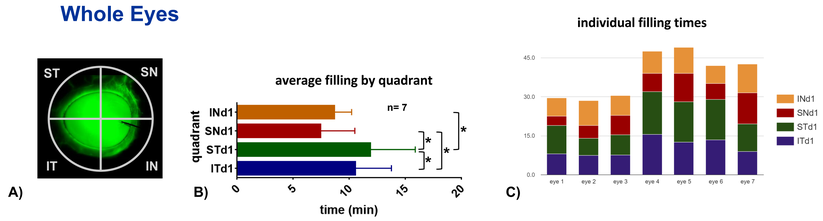


Results

Canalograms

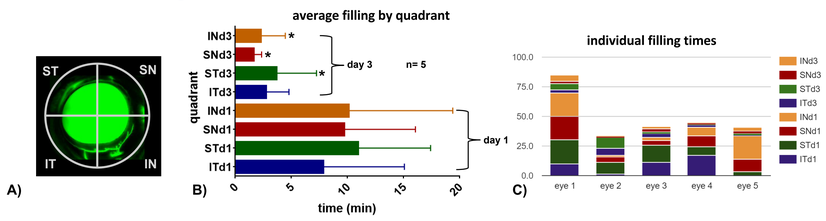


Whole Eyes



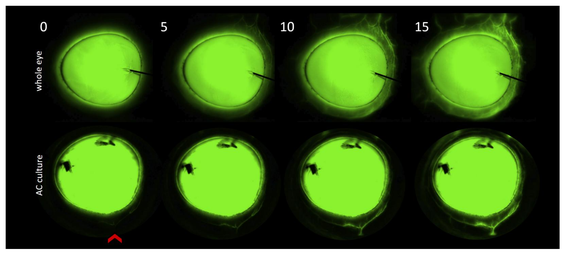
A) Analysis of average filling time by quadrant for whole eyes. **B)** Nasal quadrants (IN, SN) filled significantly faster than temporal quadrants (✻=p≤0.05) and IT filled faster than ST. **C)** Quadrant filling times for individual eyes. IN= inferonasal, SN= superonasal, ST= superotemporal, IT= inferotemporal quadrant.

Anterior Segment Cultures

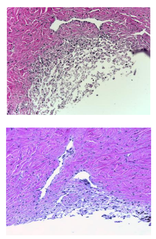


A) Analysis of average filling time by quadrant for anterior segment cultures. **B)** On d3, quadrants filled significantly faster than on d1 except in IT (✻= d3 significantly faster than d1 (p≤0.05)). In contrast to whole eyes, times for the four different quadrants were not significantly different within d1 or d3 groups. **C)** Quadrant filling times for individual eyes. IN= inferonasal, SN= superonasal, ST= superotemporal, IT= inferotemporal quadrant. D1= day 1, d3= day 3 of culture.

Occasional Reverse Filling of Day 3 AC cultures



Histology



Aqueous outflow paths could be distinguished from venous and arterial vessels. Intricate vascular tree filling patterns were observed down to estimated 50 micrometer diameter.

Whole eyes demonstrated significantly earlier filling of the nasal (SN, IN) than of the temporal quadrants (ST, IT). The fastest, superonasal quadrant filled 1.6 times faster than the slowest, superotemporal quadrant (p≤0.05). The inferotemporal quadrant also filled faster than the superotemporal quadrant.

In contrast, perfused anterior segment cultures had lost these characteristic filling time differences (p>0.05). One eye showed no outflow after 20 minutes of pressurization. After three days of culture, the same quadrant that was the fastest in whole eyes (SN), was 2.2 times as fast as the slowest (ST) but this did not reach statistical significance with the number of eyes tested. The eye without flow at 20 minutes did not recuperate during 3 days of culture.

Reverse filling was occasionally seen in d3 anterior segments (bottom row, red arrowhead). Whole eyes could not be reliably cultured for 3 days.

Discussion

Canalograms using a green fluorescent chromophore can be obtained with standard visualization equipment to estimate the local outflow function. Regionally different outflow patterns and filling times can be observed that match channel size equivalents in human eyes.

Outflow is impaired in fresh anterior segment cultures but then normalizes to the pattern seen in whole eyes.

The nasally increased flow may have implications for placement and study of MIGS.

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