Electronic Supplementary Material (ESI) for Lab on a Chip.


**Electronic Supplementary Information**

*Materials*

SU-8 2150 was purchased from MicroChem (Westborough, MA). AZ 9260 was purchased from AZ Electronic Materials (Branchburg, NJ). RTV615 (PDMS) was purchased from R.S. Hughes (Sunnyvale, CA). Tygon tubing (0.020" ID) was purchased from Cole-Parmer (Vernon Hills, IL). Syringe needles (23G) were purchased from BD (Franklin Lakes, NJ). Phosphate buffered saline (PBS) was prepared from 10x stock solution purchased from Fisher Scientific (Pittsburgh, PA). Lysozyme and 10µL microcapillary tubes (Drummond Microcaps) were purchased from Sigma-Aldrich (St. Louis, MO). CBQCA Protein Quantitation Kit and Vybrant CFDA SE Cell Tracer Kit were purchased from Thermo Fisher Scientific (Waltham, MA). A hydrogen peroxide containing lens care product was received by GWU as a sample from an optometrist. Contact lens samples were obtained from an FDA Group IV lens material (Etafilcon).

*Mask and Mold Design*

The chip layout was designed in AutoCAD and photomasks were obtained by printing on transparency (CAD/Art Services, Brandon, OR). Three master molds (flow, control, and wells) were fabricated using photolithography on silicon wafers. For both flow and control layers, AZ 9260 was spun at 900RPM, soft-baked at 110°C for 5min, rehydrated for 30min, exposed at 1800mJ/cm$^2$, developed for 5min in AZ 400K 1:3 developer, and reflowed at 130°C for 1min (H = 19µm). For the well layer, SU-8 2150 was spun to a thickness of 230µm, soft-baked at 95°C for 1h, exposed at 1480mJ/cm$^2$ with long pass filter (PL-360-LP), post-exposure baked at 95°C for 20min, developed in SU-8 developer for 20min, and hard-baked at 155°C for 5min.

*Chip Fabrication*

To fabricate the PDMS well layer, RTV615 was mixed at a ratio of 10:1 (A:B), poured onto the well mold, degassed, and baked for 90min in a 75°C oven. After curing, the PDMS was peeled off the mold, cut into small squares, and bonded to a glass slide with air plasma (Electro-Technic Products, BD-20AC). To fabricate the control layer, RTV615 was mixed at a ratio of 5:1, poured onto the mold, degassed, and par-baked for 1h. To fabricate the flow layer, RTV615 was mixed at a ratio of 20:1, spun onto the mold at 1100RPM, and par-baked for 1h. The control layer was then peeled off the mold, cut into small squares, aligned on top of the flow layer mold, and baked for another hour before lifting off and baking overnight. Inlet/outlet ports were cored using a 0.75mm biopsy punch. Small (1mm diameter) contact lens samples were cored from a full-size lens using a biopsy punch. To assemble the chip, the top microfluidic layer and the bottom well layer were plasma treated, lens samples were placed into the wells, and the two PDMS pieces were aligned and sealed followed by a 10min bake.

*Protein Preparation*

Lysozyme was prepared from lyophilized powder at 4mg/mL in PBS and serial dilutions were made at 2, 1, 0.5, and 0.1mg/mL.

*Calibration Protocol*

Lysozyme (0.3µL) was deposited onto 1mm contact lens samples and allowed to soak in until no visible droplet remained (approximately 20min). The chip was then assembled as described above. CBQCA

reagent (1µL, 5mM) was injected into the device and allowed to incubate for 1h in darkness. After incubation, fluorescence from each well was imaged with an Olympus IX71 inverted microscope using a 4x objective.

*Tear Collection Protocol*

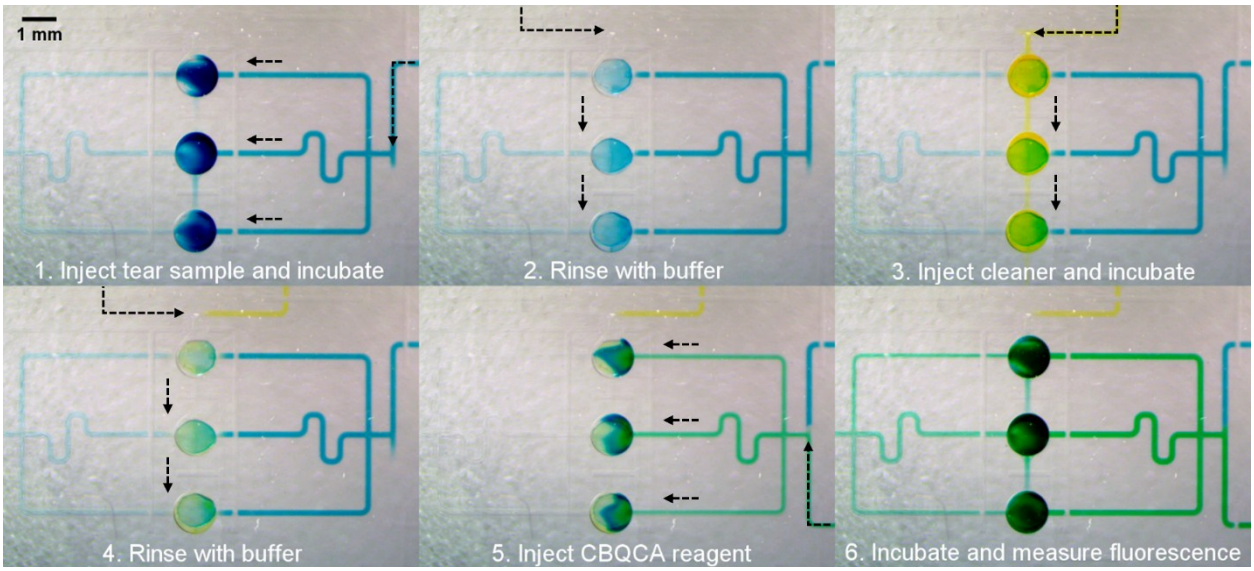
Tears were obtained by placing a microcapillary tube at the lateral canthus of each subject. After collection, the tear samples were dispensed into microcentrifuge tubes, refrigerated, and used within 72h.

All experiments involving human subjects were performed in compliance with the relevant laws and The George Washington University (GW) Institutional Review Board (IRB) guidelines. Both human subjects provided informed consent and the study does not require IRB approval because the number of subjects is less than 4.

*Cleaning Protocol*

Control channels were filled with water to prevent air from entering the flow layer during pressurization. Flow channels were pre-filled with PBS buffer to rehydrate the lens samples and remove any air bubbles in the fluidic circuit. To avoid introducing bubbles into the device when interfacing the sample tubing with the inlet ports, the inlet ports were over-filled to form tiny droplets on the surface while the sample was pushed to the front of the tubing before connecting. Valves were actuated with a pneumatic system via MATLAB. The initial soiled pre-cleaning state of the lenses was quantified as follows: 1) tear fluid (1µL) was injected into the chip by syringe pump (NE-1000, flow rate = 2µL/min) with a 3mL syringe filling all three wells, 2) valves were actuated to isolate the wells and lens samples were incubated for 1h in the tear solution, 3) lens samples were rinsed with PBS at 30µL/min for 1min, and 4) CBQCA reagent (1µL, 5mM) was injected and incubated with the lens material. Fluorescence images of each well were taken after 1h. For the post-cleaning state of the lenses, Steps 1-3 above were repeated on a new chip, after which the lenses were rinsed with a commercial contact lens cleaning solution at 30µL/min for 1min. After rinsing, lenses were incubated in the cleaning solution for 1h. Lenses were then rinsed again with PBS buffer at 30µL/min for 1min. Fluorescence images of each well were taken after 1h incubation with CBQCA reagent (1µL, 5mM).

*Example Chip Operation*




*Bacterial Solution Preparation*

*Pseudomonas aeruginosa* (ATCC® 15442) were grown overnight in tryptic soy broth (TSB) and washed three times with phosphate buffered saline (PBS). Bacteria were then labeled with an intracellular fluorescent conjugate carboxy-fluorescein diacetate, succinimidyl ester (CFDA SE) (Vybrant CFDA SE Cell Tracer kit; Molecular Probes, Eugene, OR). The cells were then washed gently with PBS and filtered (5µm) to remove bacterial clusters. The solution was then re-suspended to a concentration of $10^7$ CFU/mL in TSB. The number of cells in the suspension after filtration was determined by plating.

*Microbial Bioburden Biofilm Assay*

For the microbial assay, the chip was prepared as above for the cleaning experiments. After soaking the lenses in 1µL of tear fluid for 1h, lenses were rinsed with PBS for 1min at 30µL/min. Next, *P. aeruginosa* was introduced into the chip and cultured for 4h at 37°C. Hydrogen peroxide (3%) was then injected by syringe pump (shear rate≈$4s^{-1}$) into the chip for 1h. On a separate control chip, PBS was injected in place of the hydrogen peroxide. Finally, lenses were rinsed with PBS and fluorescence imaging was performed.

*Shear Rate Calculation*

For a rectangular channel cross-section channel[1]:

$$\dot{\gamma} = \frac{3Q}{2\left(h/2\right)^2 w}$$

where $\dot{\gamma}$ is the shear rate ($s^{-1}$), $Q$ is the flow rate ($m^3/s$), $h$ is the channel height (m), and $w$ is the channel width (m).

To determine the shear across the lens surface, we approximate the effective channel height, $h_0$, as 50µm (20µm channel height + 30µm clearance) and the effective channel width, $w_0$, as the average diameter of the well. Given a well radius, $r$, of 0.6mm, and an incoming channel width, $w_c$, of 200µm, we can calculate $w_0$ as follows:

$$w_0 = \frac{2\int_{x=0}^{r_0} \sqrt{r^2 - x^2}}{r_0}$$

where $$r_0 = \sqrt{r^2 - \left(\frac{w_c}{2}\right)^2}$$

Using a flow rate of 0.1µL/min, this gives a shear rate of 4.2$s^{-1}$.

## References

1 H. J. Busscher and H. C. van der Mei, *Clin. Microbiol. Rev.*, 2006, **19**, 127–141.