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

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Supporting information: Monitoring transient cell-to-cell interactions in a multi-layered and multi-functional allergy-on-a-chip system

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Table of Contents: Materials and methods, Supplementary Figures S1, Experimental details.

1. Materials and methods

1.1. Cell culture

Human umbilical vein endothelial cells (HUVEC) and vascular smooth muscle cells (VSMC) (Lonza) were maintained in Endoprime Base Medium with 5 % FBS, 5 % HS, 0.2% VEGF and 0.2 % EGF (Endo prime kit, PAA Laboratories GbmH) under standard culture conditions. Histamine releasing rat basophilic leukemia cells RBL-2H3 were maintained in M200 LSGS medium (Thermo Fisher Scientific). Human Vascular smooth muscle cells (CRL-1999, ATCC) were maintained in DMEM (Sigma Aldrich) supplemented with 10% FCS.

1.2. Microfabrication and operation of the multi-layered lab-on-a-chip

Rapid prototyping of cyclo-olefin co-polymer microfluidic chips was described earlier[1]. In short, the production of micro structured microfluidic and pneumatic layers out of cyclo-olefin slides was realized using hot embossing. Vinyl adhesive masks for electroplating of nickel molds was cut directly from a CAD design using a Robo Pro CE5000-40 Cutting Plotter (Graphtec America, Inc.) and laminated onto steel wafers before nickel electroplating. The resulting nickel molds are used as masters for hot embossing to fabricate the pneumatic and fluidic layers of the micropump and microdegassing layers out of cyclo-olefin copolymer blanks. The microfluidic channels for cell cultivation were fabricated out of 100 µm thick PDMS sheets using the same plotter setup. Micropumps and degasser were fabricated by sandwiching a 250 µm PDMS membrane between the two hot embossed pneumatic and fluidic layers and bonded using UV/O3 oxidation (see Figure S-1A). Next, the microfluidic cell compartment was bonded to the bottom side of the liquid handling sandwich, which was in turn bonded to glass slides containing the thin film impedance gold electrodes (see Figure S-1B). Before on-chip cell experiments, the multi-layered microfluidic chips were placed on a transparent heating station and fixed with an aluminum manifold for connection of the fluidic and pneumatic lines (see Figure S-1C). All lab-on-a-chip systems were disinfected for 60 min with 70% ethanol. For degassing of the microchannels a constant negative pressure of -60 kPa was applied to the pneumatic interface of the degassing unit.

1.3. Impedance biosensing set-up

Microdevices containing 50 nm-thick gold thin film interdigitated electrodes (IDEs; 5 µm spacing x 5 µm finger width) passivated with 50 nm silicon nitride that were connected to a VMP3 multi-channel potentiostat (BioLogic) as described earlier2-3. Impedance spectra were recorded at a frequency range between 10 kHz and 900 kHz using the manufacturer's EC Lab software. Data were normalized and selected for the frequency with the highest sensitivity (ΔIZImax = IZI cells – IZImedium)

1.4. Activation and degranulation of basophil cells via antigen crosslinking

Rat basophilic leukemia cells RBL-2H3 were sensitized for 3 h in full culture medium using 0.3 µg/mL anti-rPhl IgE antibodies.

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For visualization of basophil degranulation, methylene blue assay was performed using 1 mg/mL methylene blue (Sigma Aldrich) in full culture medium. The basophil cells were stained for 30 min at 37°C and washed using thoroughly using PBS (Sigma Aldrich) for removal of excess dye. To trigger degranulation, basophils were exposed to 0.03 µg/mL recombinant rPhl antigen and the degranulation process was evaluated using time-lapse microscopy (Wilowert, Hund Wetzlar).

1.5. On-chip cell experiments of endothelial and smooth muscle cells

To evaluate the vascular dilation response of HUVEC to histamine, HUVECs were seeded into the microchambers using 40 000 cells/cm^2 and allowed to attach for 4h before initiation of perfusion using 3 µL/min (0.125 dyn/cm^2). Upon reaching confluence, the endothelial cells were stimulated using 30 µM histamine (Sigma Aldrich) and the response was monitored for 4 hours. Additionally, co-cultures of HUVEC with RBL-2H3 were established using HUVECs as described above and grown to confluence prior addition of IgE-activated basophils. Following a 4h of sedimentation period and equilibration, either 0.03 or 1 µg/mL rPhl antigen was applied and mediation of cell degranulation was recorded over a period of two hours. For two-layered co-culture of smooth muscle and endothelial cells, smooth muscle cells were injected into the microchip using 20 000 cells/cm2. HUVEC were stained using Calcein-AM according to the manufacturer's instructions to facilitate live cell tracking during on-chip cultivation and seeded on top of the confluent SMC monolayer. Antigen mediated degranulation was biochemically simulated by exposing the two-layered co-culture to 30 µM histamine and changes in cellular impedance was recorded for four hours.

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2. Figures

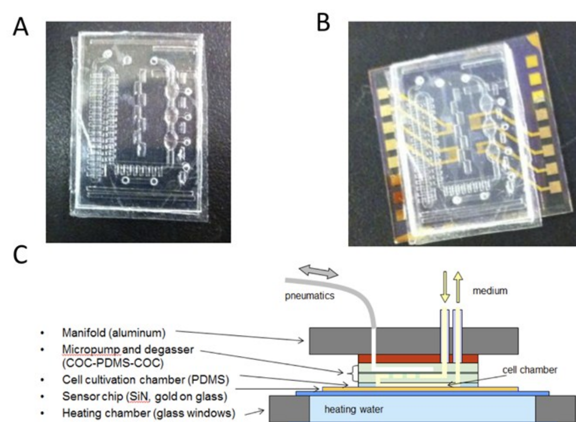

Figure S-1. (a) Pneumatic layers of the lab-on-a-chip system comprised of micropump and micordegasser. (b) Fully assembled lab-on-a-chip with integrated impedance biosensors and liquid handling. (c) Overview of the multi-layered microfluidic set up placed on a transparent heating station with fluid and pneumatic manifold.

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Figure S-2. (a) Peak flow profile of two parallel on-chip integrated micropumps for 75 ms actuation step time. (b,c) Peak flow and average flow rate of integrated micropumps for actuation pressure in the range of 10 – 45 kPa (d-1) Tuning of flow profiles by adjustment of micropump step time between 5 ms (e) and 100 ms (f).

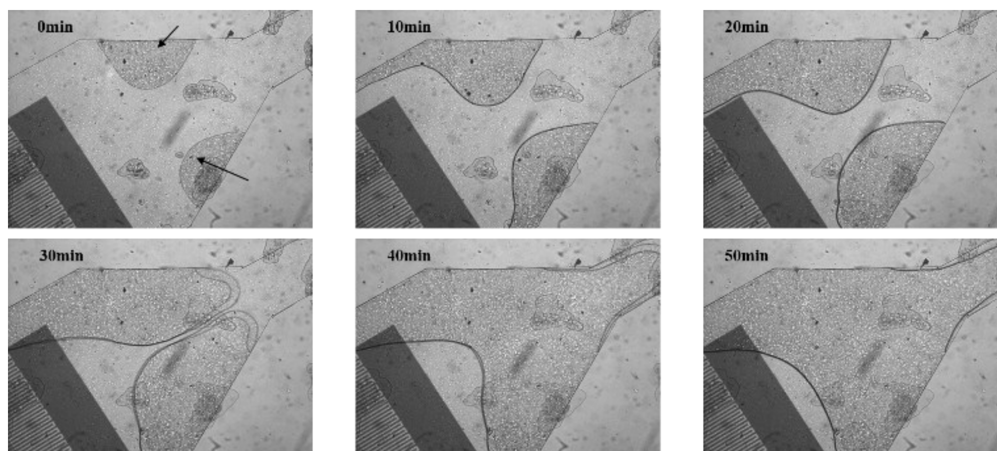

Figure S-3. Microscopic images of bubble nucleation and growth in the absence of microfluidic degassing.

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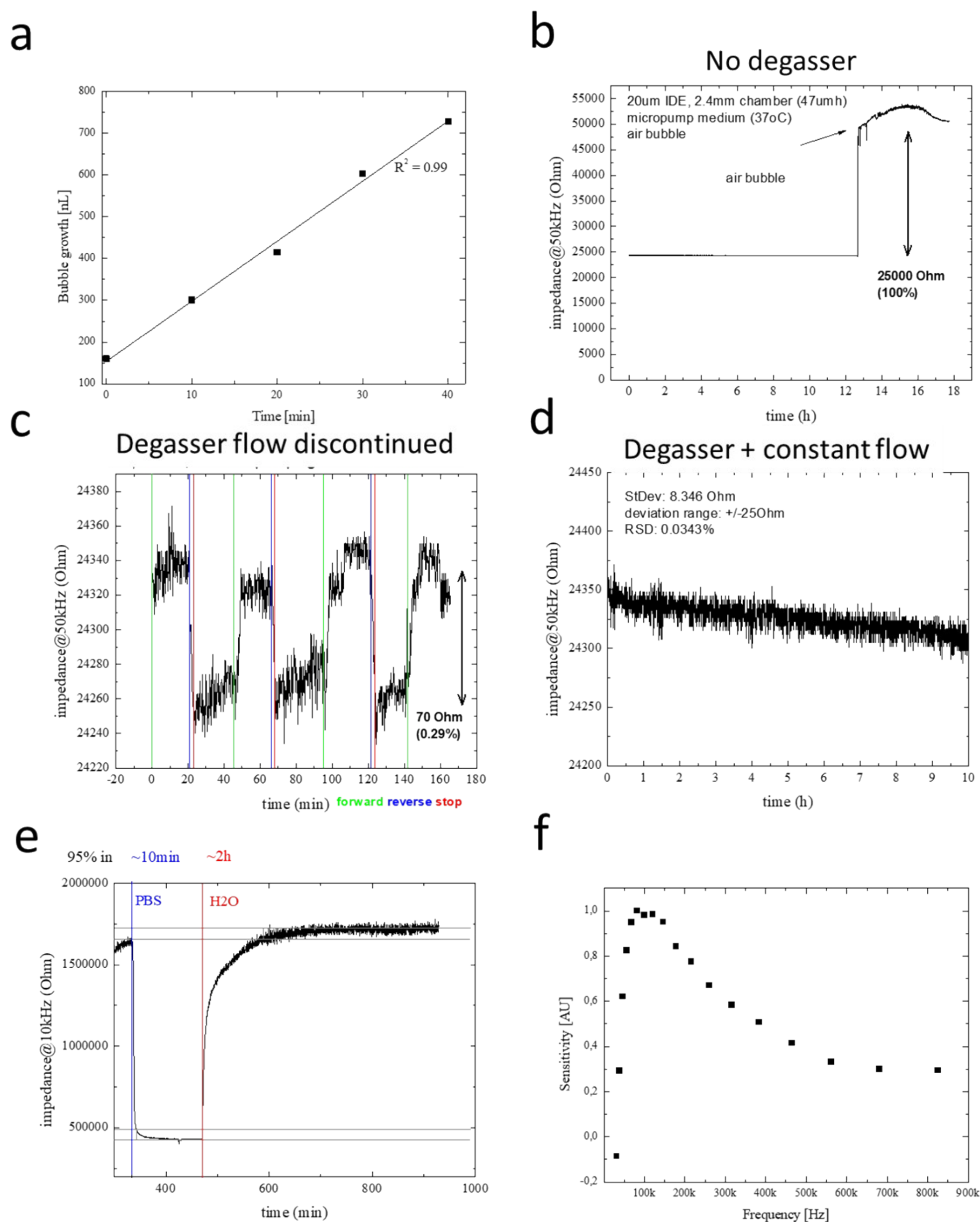

Figure S-4. (a) Linear bubble growth in vapor permeable PDMS microfluidic micro-channels. (b) Impedance time trace of nucleating and growing air bubbles at 37°C. (c,d) Influence of stop-forward as well as continuous medium perfusion on impedance biosensing.(e) Response of the 20 µm IDEs impedance biosensors for rapid medium exchange from water to 1x Dulbecco'S PBS using integrated micropumps. (f) Sensitivity of the interdigitated thin film impedance biosensor towards human umbilical vein endothelial cells in the frequency range of 40 kHz to 900 kHz

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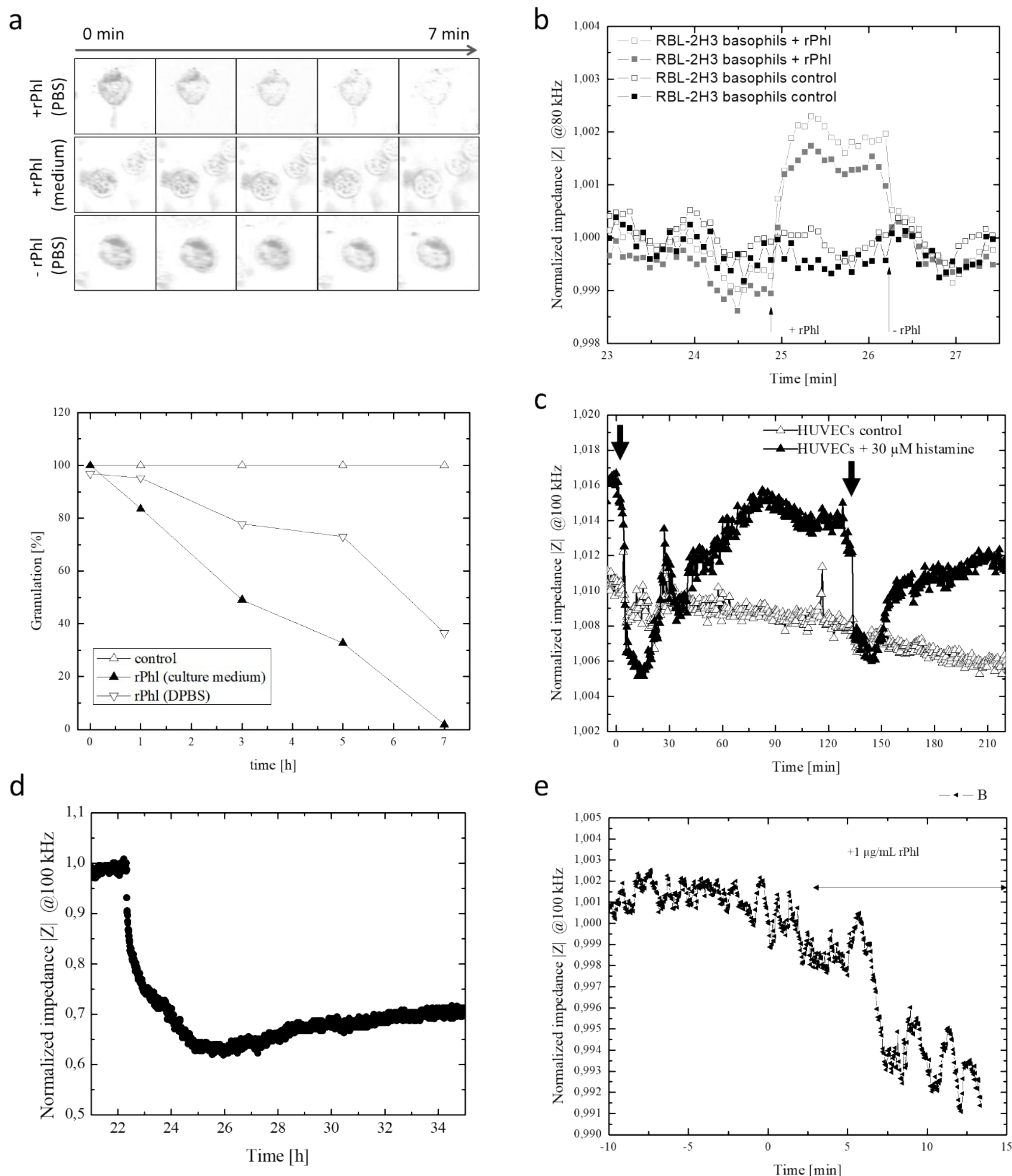

Figure S-5. (a) Influence of medium selection on rPhI antigen-mediated degranulation of RBL-2H3 basophils at 37°C. Cells were stained with methylene blue to track degranulation, where 60 individual cells were analyzed for each condition. Degranulation events result in loss of methylene blue, thus contrast. (b) Impedance time-trace of rPhI antigen-mediated degranulation of RBL-2H3 basophils at a frequency of 80 kHz. (c) Impedance time-trace of histamine-mediated hyperpermeability of HUVEC endothelial cell barriers at a frequency of 100 kHz and a histamine concentration of 30 µM. Black arrows indicate histamine administration. (d) Impedance loss of HUVEC monolayers over two hours upon serum starvation (0% FCS). (e) Impedance time-trace of HUVEC endothelial response to 1 µg/ml rPhI antigen-mediated degranulation of RBL-2H3 basophils at a frequency of 100 kHz.

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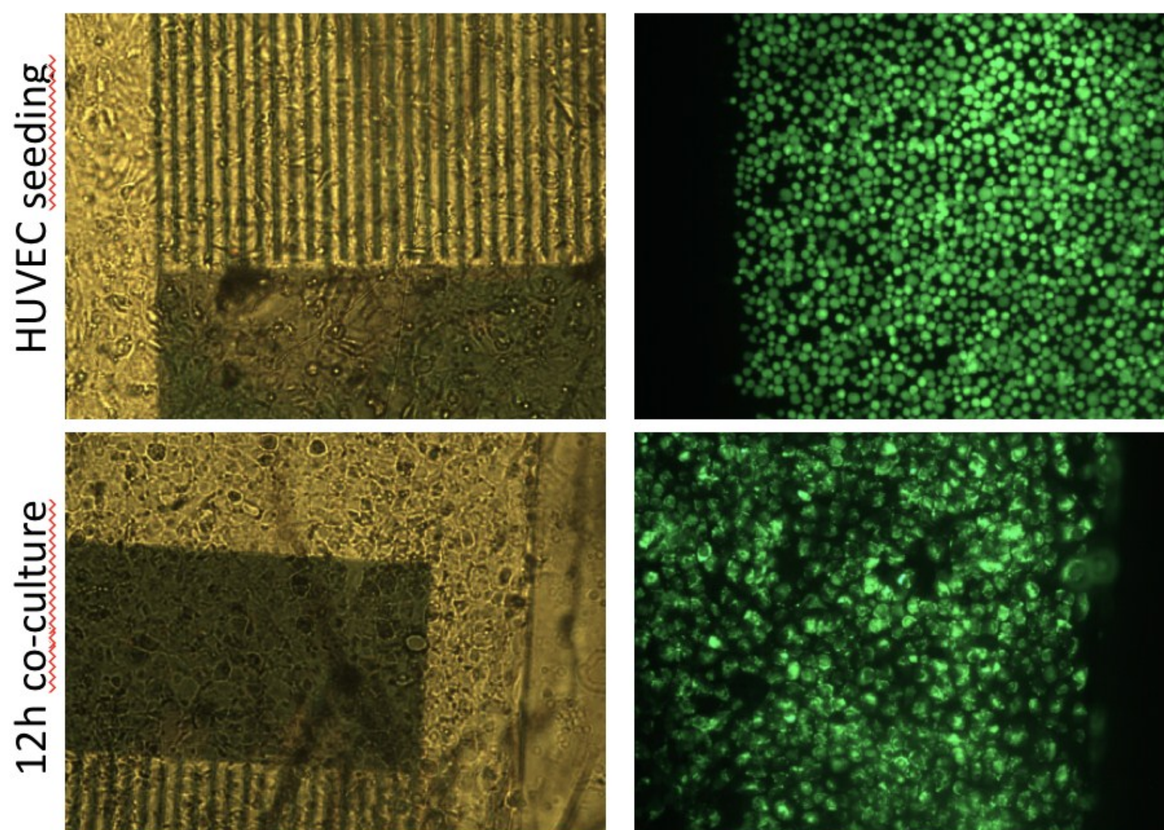

Figure S-6. Bright-field and fluorescence images of two-layered co-culture of HUVEC endothelial with VSMC smooth muscle cells during seeding and 12 h after adhesion of the second endothelial cell monolayer.

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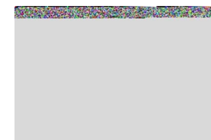

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Author contributions

All authors conceived and discussed the manuscript. P.E., R.A.M., R.N., V.C., H.W. and D.S. designed and performed the experiments. V.C., D.S. and M.R. analyzed data. M.R., B.B. and P.E. wrote the manuscript and all authors edited the manuscript. P.E. and R.A.M. supervised the project.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

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