

Electronic Supplementary Information (ESI):

Low cost lab-on-a-chip prototyping with a consumer grade 3D printer

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Materials and Methods

5 Unibody fabrication

3D printing was performed with a Miicraft® printer, which is a SL platform (2299 US\$) with 450ppi (~56µm) lateral resolution, and 50µm resolution in the vertical direction.

Unibody structures were designed with free CAD software for
10 Mac Os X 10.8.3 (Autodesk® Inventor® Fusion, Autodesk Inc.) on a MacBook Air computer (13-inch, late 2010, 1.86 GHz Intel Core 2 Duo, 4 GB 1067 MHz).

The CADs were exported as .stl mesh files and converted to
15 bitmap exposure patterns by the Miicraft® Suite software provided with the 3D printer. The Miicraft® Suite supports alignment and scaling of the .stl files, slicing and printer control. The slicing procedure transforms the 3D mesh in 2D cross sections (bitmap black and white files in .png format at 480 x 768
20 pixels resolution), which are resolved at 50µm steps in the vertical direction.

The .png files are accessible and can be edited with common image retouching software. Bitmap image edition was performed in Photoshop™ CS4, although any simpler software capable of single pixel edition, such as Microsoft Paint, is sufficient.

25 The Miicraft® Suite controlling the printing process enables to set the exposure time of each layer, the printing speed (2 or 3 cm/hour) and the vertical separation between exposed layers (50 and 100µm).

Upon optimization, unibodies were printed in the following
30 conditions of exposure time, printing speed and vertical step size: 7s, 2cm/hour, 50µm. After printing, the devices were sonicated (FinnSonic m15) in industrial grade ethanol for 20s, and air-dried.

The printer uses a proprietary resin (138 US\$ / 500 ml), which
35 composition is undisclosed, but the material safety data sheet¹ reports that is a modified acrylate oligomer and monomer in combination with an epoxy monomer, a photo initiator, and additives.

The average weight of each unibody in this work was 2.1gr,
40 which for the resin cost corresponded to 0.57 US\$/unibody.

Surface roughness was measured with a stylus profiler (Dektak 6, Veeco Instrument Inc.) along 1mm tracks resolved in 6000 points. The average roughness was 182nm for the finished
45 templates.

The devices in Fig. 1 were sealed with adhesive tape (3M Ruban Adhesive Scotch® Nastro Adhesive) on the backside, and seal1 and seal3 regions. Excess tape was trimmed with a cutter and the tape was pressed against the unibody surface with a

cotton tip in order to eliminate trapped air in small blisters.

50 The functionalized PDMS surface (see next section) was cut to the size of seal 2 and pressed in contact with the unibody surface. A tweezer tip was used to press the PDMS against the unibody surface, preventing pressure on the channels to avoid blockage with the functional coating. The PDMS was further secured with
55 adhesive tape on the outer surface.

The preparation of the PDMS² substrate followed a conventional procedure; Dow Corning Sylgard 184 base and curing agent were mixed in proportions 10:1 and stirred in a cup for 2 min. The mixture was degassed in a desiccator connected
60 with a rotary pump for 30 min, and afterwards poured on a clean glass surface and cured at 65 °C for 2h in an environmental oven (Gallem Kamp incubator).

The devices were completed with the manual insertion of silicone tubing (Esska.de GmbH, Hamburg) in the unibody
65 printed connector. The connector diameter, required for proper sealing with the tubing was: 1.2mm for a tubing inner diameter of 1mm.

Devices in Fig. 2 had the micro channels filled with cellulose
paste, made of cellulose powder (S3504 SIGMA Sigmacell Cellulose, Type 20, 20µm) mixed with water and squeegeed into
70 the channels with a metal spatula. Surface excess was removed with a humid cotton tip before the paste dried, and the channels were left to dry before pipetting the detection chemistry in the distal end of the structure. Once pipetted (Eppendorf Reference
75 0.5 - 10µl) the device was immediately dried with a N₂ jet to secure localization of the detection chemistry. The lateral flow devices did not require any further sealing or tuning. The samples were directly pipetted to the sample delivery zones.

Mixers (Fig. 3) were firstly front sealed with adhesive tape, and
80 in the model incorporating beads (G1145 SIGMA Glass beads, acid-washed, 150-212 µm (70-100 U.S. sieve)), these were delivered dry from the backside of the through hole. Excess beads were brushed away from the unibody and the backside immediately sealed with adhesive tape.

85 The devices were fixed with silicone tubing and placed in the imaging platform where color solutions (aqueous fluorescein and rhodamine B 1mM solution, Sigma-Aldrich, St. Louis, MO, USA) were circulated at 30µl/min (continuous infusion/ Dual NE-1000 syringe pump, from New Era Pump Systems Inc.,
90 www.syringepump.com).

H₂O₂ assay

The assay solution contained: 10 units/ml horseradish peroxidase (HRP) and 0.3 mM trehalose in physiological saline (0.9% NaCl

in water). In order to facilitate the assembly of the functional surface with the unibody, the assay was conditioned as a surface film on a PDMS slab, which could be cut in small areas and supplied to the devices.

5 A PDMS film was framed with PDMS trimmings of the same film and plasma treated to render the surface hydrophilic, whereas the frame, after removing the PDMS stripes, remained hydrophobic.

10 The liquid assay solution was delivered on top of the hydrophilic PDMS surface and placed in a chamber at 35% relative humidity (RH) for 12h. The assay exposed to these conditions became a sticky thin gel coating PDMS surface, which was cut to seal 2 size and transferred to the unibody.

15 The fluorescent substrate Ampliflu Red (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in DMSO to a concentration of 1 mg/ml. For the test solutions, Ampliflu Red stock solution was added to 0.9% NaCl normal saline (5 μ l Ampliflu Red in 995 μ l 0.9% NaCl), and this preparation was directly used as control, and to produce 0.1 μ M and or 1 μ M hydrogen peroxide solutions, which resolve the lower limit of H₂O₂ in urine for whole body oxidative stress monitoring.³ The test solutions were prepared fresh directly before the experiment.

Glucose assay

25 Unibody channels were filled with cellulose paste. Type 20 cellulose (20 μ m particle size) from Sigma was mixed with deionized water (40% in weight) forming a thick paste, which was squeegeed into the channels and let to dry.

30 The glucose assay reagent contained 120 units/ml of glucose oxidase enzyme activity, 30 units/ml of horseradish peroxidase activity, 0.6M potassium iodide, and 0.3M trehalose in 0.1M phosphate buffer, pH 6.0, and it was pipetted (0.2 μ l) to the detection zones.

35 Glucose solutions of 10mM and 5mM (90 and 180 mg/dl respectively) were prepared in 0.1M phosphate buffer, pH 6.0 and the same buffer without glucose was used as control.

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

40 Imaging

Imaging of unibody printouts was carried out with an Olympus SZ60 stereo zoom microscope, provided with a Canon EOS 500D DSLR camera (15 MP, APS-C Cmos sensor).

45 Bright field and epi-fluorescence imaging was performed on a Zeiss Axiovert 40 cfl inverted routine microscope. Fluorescence excitation was provided by a Zeiss HBO50 illuminator, housing a mercury vapor short arc lamp HBO 50. The same Canon EOS 500D camera was used in both microscopes.

50 Imaging of mixing flows (Fig. 3b-d) was carried out with a Samsung Galaxy Note 2, 8 Mpix rear side camera in full HD video acquisition mode (30fps). The camera in this case was positioned facing the devices, which were sitting on a glass stage back illuminated using an iPod Touch (4th generation, 960 x 640 pixel screen resolution, with iOS 6) running a Led Torch v1.37 app (www.smalte.ch), set to pure white (rgb 255, 255, 255).

H₂O₂ fluorescence response (Fig. 1), for C, S and H concentrations, was time-lapsed captured at 10s interval with a

Canon EOS 500D mounted on the Zeiss Axiovert 40 cfl. The image sequence was processed in Matlab R2008b with a dedicated script developed for this purpose. Signal noise was extracted from linear curve fitting to the response in the interval [10,12] min and used to compute the detector resolution.

65 Glucose color response (Fig. 2), for C, S and H concentrations, was time-lapsed captured at 20s interval, with the Samsung Galaxy Note 2 rear camera controlled by the Tina Time-lapse 4.1.0 free app. The image sequence was processed in Matlab R2008b.

70 Time response was extracted with a manual grid of regions of interest (ROI) (control and 2 glucose concentrations, and equivalently for the H₂O₂ assay). Average intensities, within ROIs from the green camera channel were used to evaluate the glucose response, and the red camera channel for hydrogen peroxide. The procedure was automatically repeated in all pictures extracted from the original acquisition. The time response data was copied and pasted to Microsoft Excel 2011 for mac, for final edition.

Figures in this paper were contrasted and composed in Apple Keynote 09, v5.3.

80 Notes and references

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† Electronic Supplementary Information (ESI) available

¹ http://miicraft.com/web/assets/2012/07/MiiCraft_Blue-Resin_MSDS1.pdf

² L. Ugozzoli, *Clin Chem*, 2004, **50**, 1963.

³ J. Yuen, I. Benzie, *Free Radic Res*, 2003, **37**, 1209.