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

Electronic Supplementary Information for the article **"Pressure-actuated monolithic acrylic microfluidic valves and pumps"** by Pablo E. Guevara-Pantoja, Rocío J. Jiménez-Valdés, José L. García-Cordero, and Gabriel A. Caballero-Robledo.

1 Thermoplastic properties

Thermoplastic materials		Physical properties				Optical transmissivity[b]		Injection micro molding behavior[a]			Micromilling behavior	
Acronym	Full name	Young´s Modulus (Gpa)	Glass temp. Tg (°C)[a]	Melting temp. Tm(°C)[b]	Density (Kg/m^3)[a]	Visible	UV	Reproduction	Filling	Separation	Surface roughness	Bonding time
PS	Polystyrene	3.4	80-100	240-260	1050	Excellent	poor	+	+	-	≥ 0.8 µm [c]	30 min [g]
PP	Polypropylene	1.3	0-10	160	900	Good	Fair	+	o	+		
PMMA	Polymethylmethacrylate	3	106	250-260	1190	Excellent	Good	+	o	-	≥ 0.5 µm [c]	30 min [e]
PE	Polyethylene	0.7	-	120-130	950	Fair	Fair					
COC	Cyclo olefine copolymer	2.4	138	190-230	1010	Excellent	Excellent				≥ 0.6 µm [c]	30 min [e]
PA	Polyamide	2.4	60	190-350	1130			++	+	+		
PC	Polycarbonate	2.0-2.4	150	260-270	1200	Excellent	poor	+	+	-	≥ 0.3 µm [d]	30 min [f]
								++ verygood, + good, o average, - bad				

a) H. Becker and C. Gärtner, *Electrophoresis* , 2000, **21**, 12–26.
b) C.-W. Tsao and D. L. DeVoe, *Microfluid. Nanofluidics* , 2009, **6**, 1–16.
c) D. J. Guckenberger, T. E. de Groot, A. M. D. Wan, D. J. Beebe and E. W. K. Young, *Lab Chip* , 2015, **15**, 2364–2378.
d) P.-C. Chen, C.-W. Pan, W.-C. Lee and K.-M. Li, *Int. J. Precis. Eng. Manuf.* , 2014, **15**, 149–154.
e) I. R. G. Ogilvie, V. J. Sieben, C. F. A. Floquet, R. Zmijan, M. C. Mowlem and H. Morgan, *J. Micromechanics Microengineering* , 2010, **20**, 65016.
f) D. Ogończyk, J. Węgrzyn, P. Jankowski, B. Dąbrowski and P. Garstecki, *Lab Chip* , 2010, **10**, 1324.
g) E. W. K. Young, E. Berthier, D. J. Guckenberger, E. Sackmann, C. Lamers, I. Meyvantsson, A. Huttenlocher and D. J. Beebe, *| Anal. Chem* , 2011, **83**, 1408–1417.

2 Membrane deformation

The milling machine imposes a limit to the minimum thickness of the membrane that can be achieved without deforming the membrane. A deformed membrane will not seal correctly the microfluidic channel and thus will not work. To determine the membrane deformation due to the milling process, we fabricated membranes of different thicknesses and evaluated their final deformation using the same membrane as a mirror. The image of a thin, straight copper wire was reflected on the membrane surface; the deformation of the membrane was estimated by assessing the deformation of the image by the wire. Figure S1 shows the results of the analysis where it can be observed that the membrane is not affected by the drilling process for thicknesses larger than 140 μm.

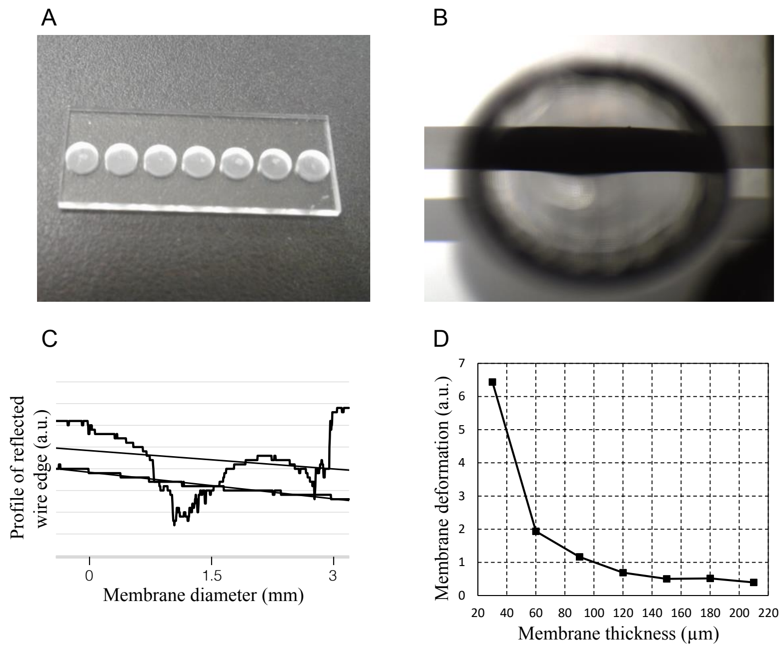

Figure S1. (A) Membranes of different thicknesses (30, 60, 90, 120, 150 and 210 μm) were fabricated in an acrylic sheet to evaluate their deformation. (B) The non-drilled side of the membrane was used as a mirror to reflect a straight wire. The image shows the wire reflected on the thinnest membrane. Image (C) shows the profile of the edge of the reflected wire obtained by image analysis for the cases of the thinnest and thickest membranes, in arbitrary units. The degree of deformation of the membrane was evaluated by calculating the standard deviation of the distances from the profile to a straight line. The plot in (D) shows the degree of deformation of the membranes as a function of thickness.

3 Membrane thickness repeatability

The drilling machine used for the fabrication of our devices has a mechanical resolution of 2 μm per step. To test the repeatability and accuracy across several samples, we initially set the zero position in the vertical axis (z-axis) on the surface of the workpiece table of the machine. The acrylic sheets to be drilled are positioned on this table where the zero position of the machine corresponds to the bottom of the acrylic sheet. Once the acrylic sheet is fixed, a circular pit is drilled with a 0.5-mm drill bit (Kyocera, 1610-0200L060) stopping the drilling 100 μm above the zero position (*i.e.* 100 μm above the bottom of the acrylic sheet). This ensures that the precision of the thickness of the membrane is limited almost solely by the positioning precision of the machine. If another membrane is to be drilled on the same acrylic sheet, or if a new acrylic sheet is placed on the table, the zero position remains the same and the thicknesses of the new membranes are as accurate as the machine's mechanical resolution. By specifying the zero of the vertical position at the bottom of the sheets the resulting thicknesses of the membranes are independent of possible variations on the thickness of the acrylic sheets, which could be another source of variation. The figure below shows twenty membranes fabricated on four acrylic sheets. We measured their thickness with a digital micrometer obtaining an average value of 97 μm with a standard deviation of 2 μm, which is consistent with the machine mechanical resolution.

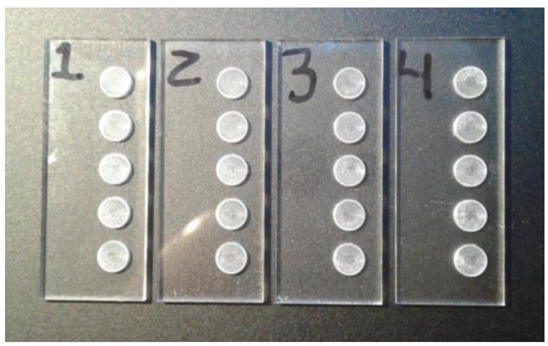

Figure S2. Twenty membranes were drilled on four acrylic sheets to assess the repeatability of the membrane thickness. The membranes were supposed to have a thickness of 100 μm. After fabrication, the thickness of all of them were measured using a digital micrometer resulting in a mean value of 97 μm with a standard deviation of 2 μm.

4 Cell staining

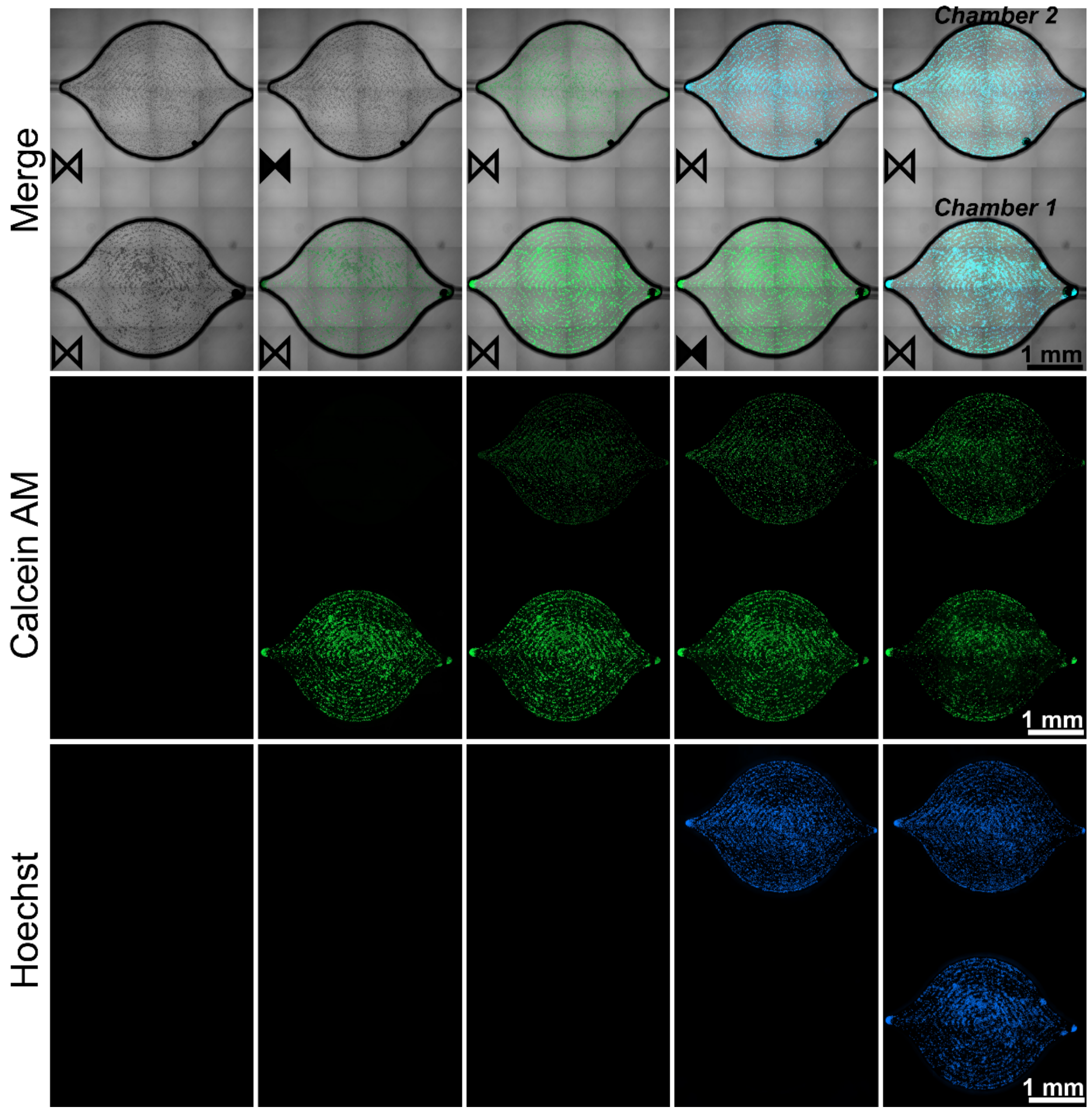

Figure S3. Independent cell staining inside the chambers of the device corroborates the functionality of the PMMA valves. Tiles images showing differential staining of cells contained in the chambers. Empty symbol, open valve; full symbol, closed valve.

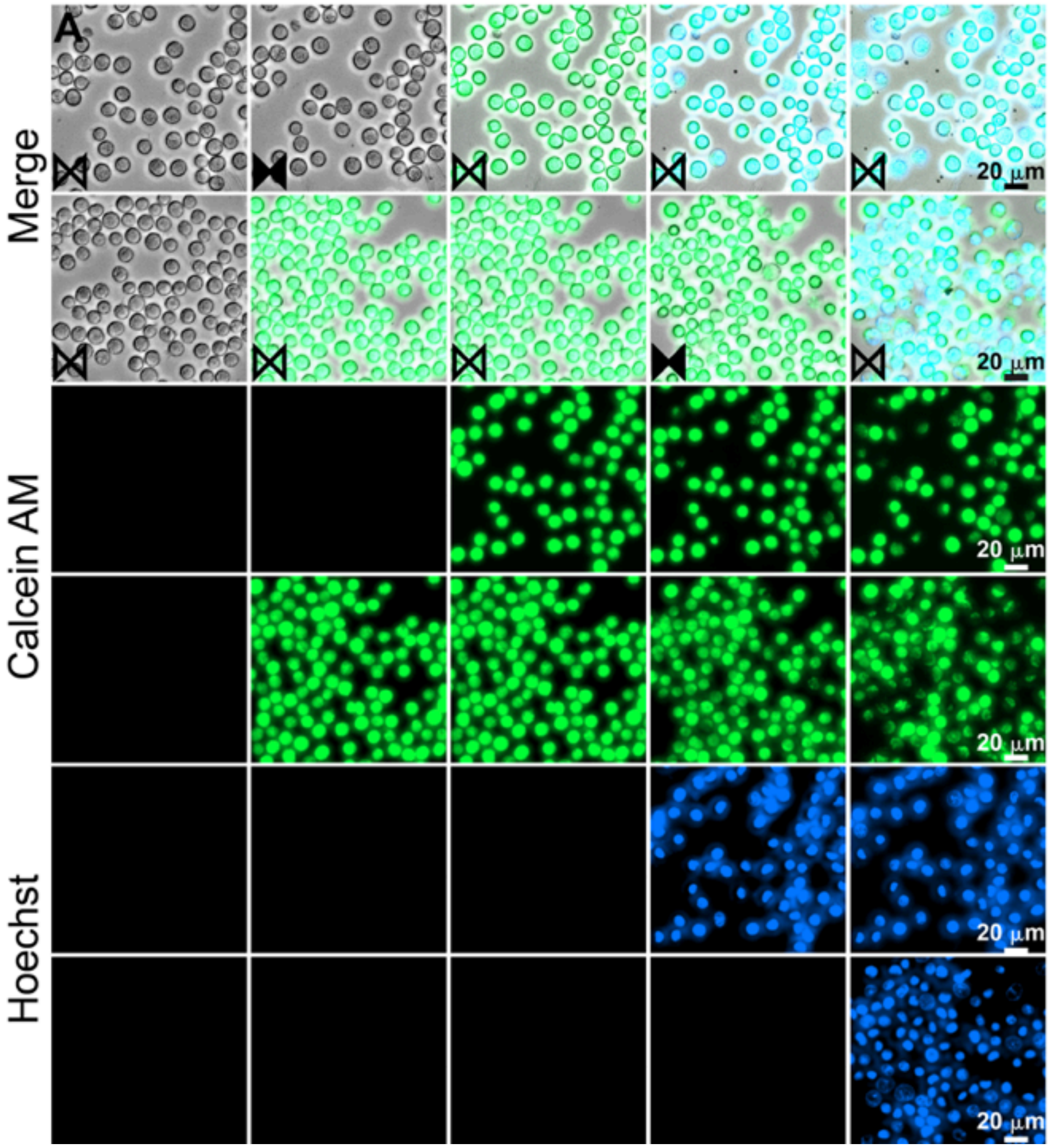

Figure S4: Close up on the cells of figure S3.