Electronic Supplementary Information

Bioimprint aided cell recognition and depletion of human leukemic HL60 cells from peripheral blood

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Size discrepancies between HL60 cells and PBMCs

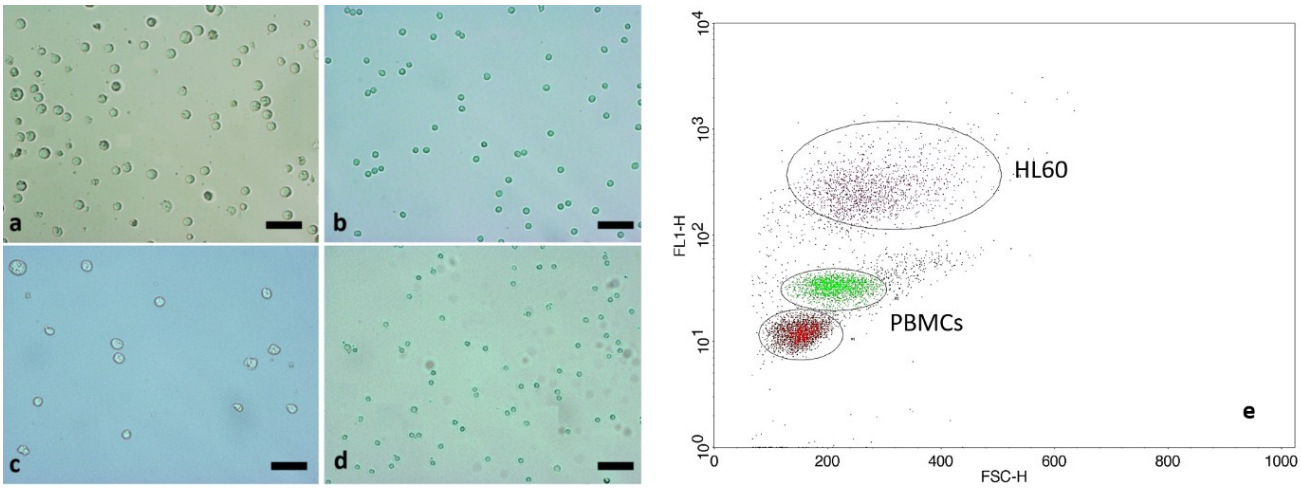

Figure S1 Bright field microscopy images of live (a) HL60, (b)PBMCs and fixed (c) HL60 and (d) PBMCs. (e) Flow cytometry dot plot showing mixed cell populations of PBMCs (lymphocytes red, monocytes green) and HL60 (purple) gated according to relative cell size (FSC- forward scatter) and fluorescent properties (FL-1). PBMCs were spiked with HL60 at a 20% target ratio (actual measured 20.28% HL60). Forward scatter (size) means were mean for PBMC is 185.7, compared with 272.7 for HL60 giving a mean size ratio of 1:1.467 for PBMC:HL60.

FL-1 is the detection channel used on the flow cytometer to detected auto/fluorescence. To identify HL60 cells and define the region HL60 or PBMC were first analysed by the flow cytometer as single populations. This allowed a gate or region to be set on those individual population for analysis purposes. HL60 and PBMC cells were then mixed in known ratios and analysed to ensure the identified regions were consistent for mixed cell populations.

The cells were fixed prior to use as the current methodology (i.e. making an imprint) can only be done with fixed cells. Since we are mostly concerned with the selectivity of the myeloblast bioimprints and their differentiation between HL60 and PBMC, for consistency we did all the experiments with cells that have been fixed in glutaraldehyde solution. This glutaraldehyde treatment completely preserves the cell shape and morphology of both HL60 and PBMCs but they are not viable after fixation. This was necessary, as we are working with large amounts of cells over long duration of the experiments and needed to avoid batch to batch variations due to loss of viability.

Stages of preparation of bioimprints from HL60 cell layers

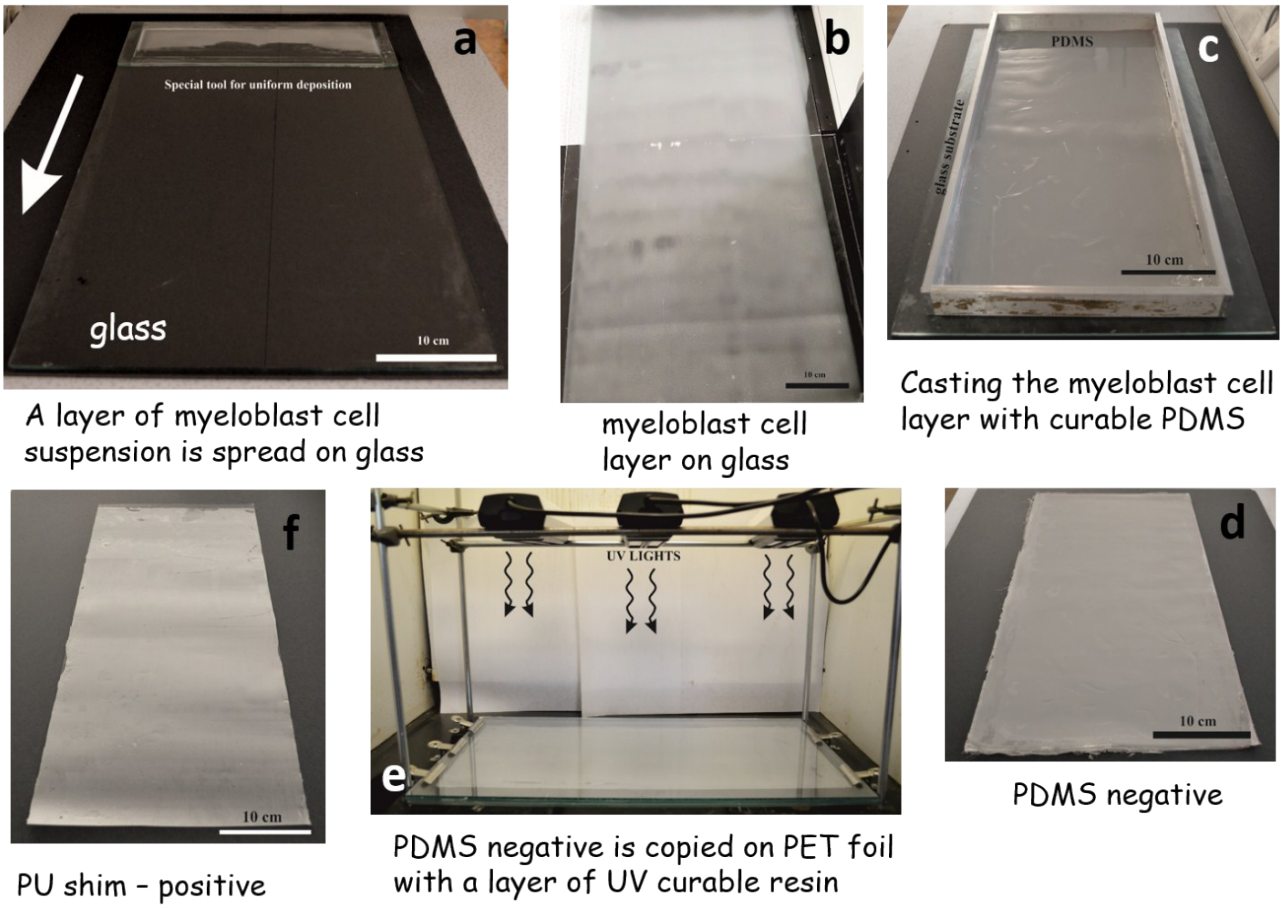

Figure S2 Photographs showing the different stages of bioimprint production, starting from (a) spreading of the HL60 cells (f) to the development of the PU positive shims.

Bioimprints were produced by immobilising a film of cells suspension onto activated glass substrates, bringing the layers to semi-dry state and impressing them in a curable silicone (PDMS). Fixed HL60 cells were spread from glucose solutions via a bespoke tool yield an even film of user defined size determined by the spreading tool gap. The layer of fixed HL60 cell suspension was allowed to dry with residual glucose acting as a protective layer which prevents cell clustering and acting as a filler of the intercellular voids. The negative bioimprint of the HL60 cell multilayer was obtained by casting the exposed part of the cells with curable silicone (PDMS), which was peeled off the cell layer after curing. The presence of glucose in the aqueous suspension also prevented the PDMS matrix material from penetrating the inter-cellular gaps, engulfing whole cells. The negative imprint captured accurately the size and the surface topographical information complementary to the templated HL60 cells. Positive imprint replica was produced by templating the PDMS replica with an UV photoresist resin which was cured under UV light. Further analysis of the cell shape was done directly on positive and the negative imprints to ascertain the quality and the uniformity of the cell shape and size replication. More importantly, via sequential negative-positive-negative copying, it is possible for unlimited replication of the imprinted areas (see Figure S3).

R2R Nano-Imprinting Lithography for large scale bioimprint replication

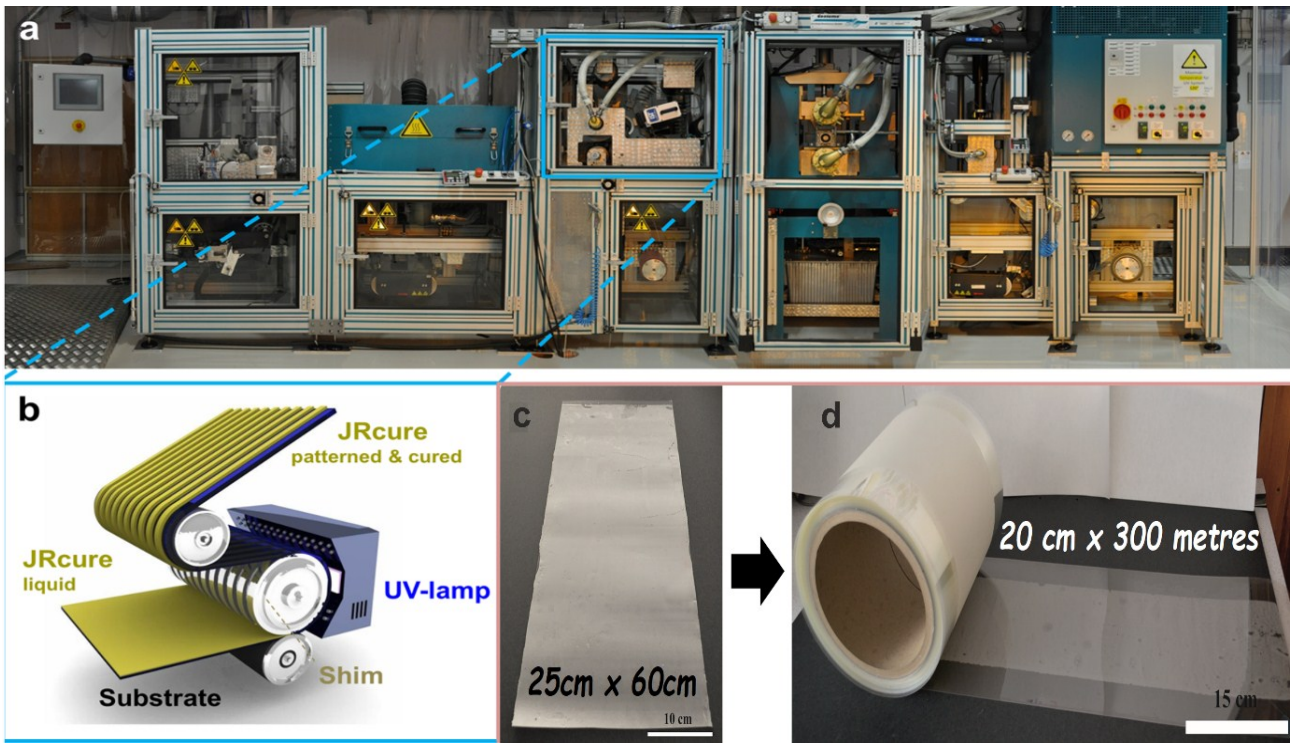

Figure S3 (a) The Roll-to-Roll-UV Nano-Imprinting Lithography (NIL) machine at our collaborator Joanneum Research FmbH (Weiz, Austria) printing facility with a speed up to 1 m min^{-1}. (b) Scheme of the R2R-UV-NIL unit. Photographs showing the production of (d) a roll of negative acrylate-based bioimprint on PET foil fabricated from (c) positive PU imprint shims on PET foil.[1] Figures S3a and S3b reprinted with permission from ref. 1. Copyright 2016 American Chemical Society.

Bioimprint fabrication: The positive shim imprint (Figure S3c) was made using an elastic resin formulated with a PU-acrylate oligomer, two acrylate diluents, a photo-initiator (3 wt%) and a polythiol (10 wt%) for suppressing the O_2-inhibition (from the PDMS). The resin for the final R2R negative imprint contains a hydrophilic acrylate, a photo-initiator (3 wt%) and a silicon surfactant (1 wt%) for easy demolding. The exact composition of the resins are proprietary of Joanneum Research FmbH (Weiz, Austria). The imprint base was a PET foil sourced from DuPont Melinex ST505 of thickness 125 µm.

Bioimprint surface modification: The negative HL60 imprint was treated with Oxygen-plasma and then coated with Branched PEI (bPEI). It is known that bPEI has certain low toxicity when used as gene-transfer vector in solution. This has been widely researched and commented on by a number of authors[2,3] for multiple cell types exposed to a solution of bPEI. The same polymer has been extensively used for coating nanocarriers for delivery of chemotherapeutic agents due to its relatively low toxicity.[4] However, in the case of our bPEI surface treated bioimprints, there is no free bPEI in the solution as bPEI is bound to the bioimprint. In addition the surface is further treated with Pluronic surfactant (Poloxamer 407) which is routinely used to passivate surfaces in biomedical equipment (catheters, etc.) and minimise non-specific adsorption. Hence during the bioimprint selectivity experiments, the PBMCs do not come in direct contact with bPEI which may otherwise potentially impact their viability in solution.

Architechture and setup of the microfluidic bioimprint chip

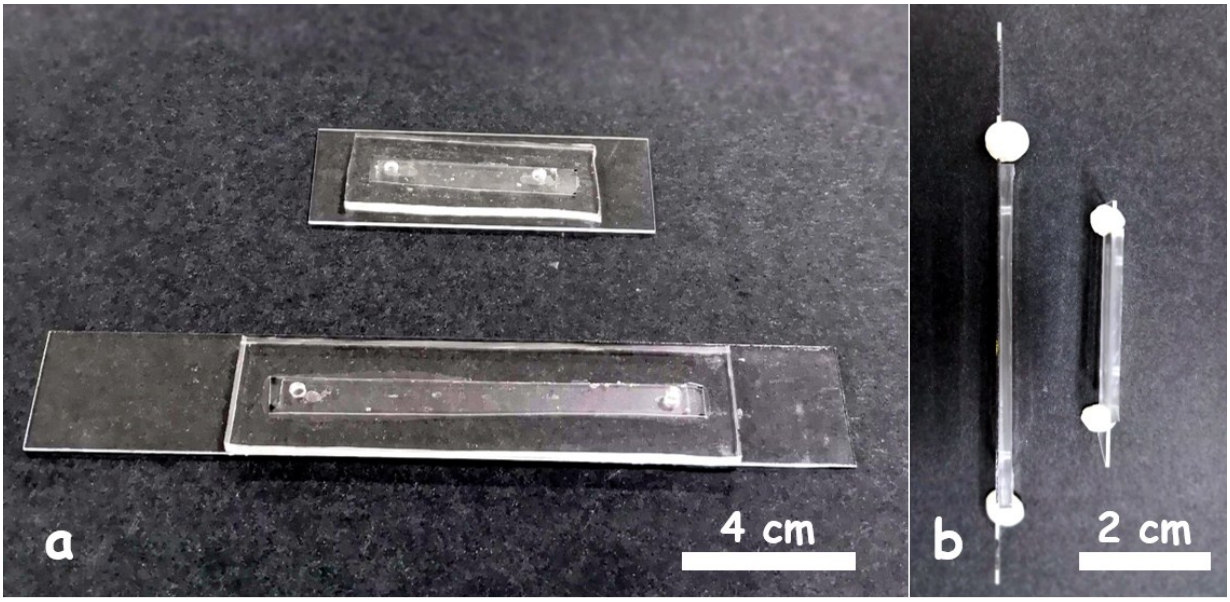

Figure S4. Photographs showing the (a) horizontal and (b) vertical views of the two different 4 cm and 8 cm PDMS based chips used for the bioimprint selectivity experiments.

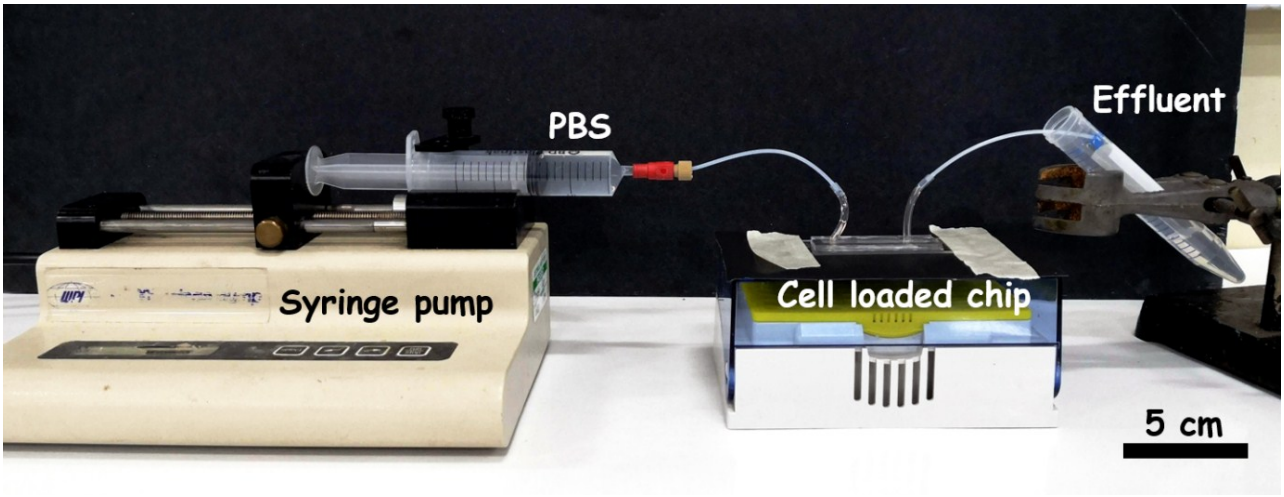

Figure S5. Photographs showing the setup used for the static experiment using the syringe pump the cell loaded chip and the collected effluent.

Note regarding the cell incubation times on the chip

We have tried much shorter incubation times of the cell mixture on the chip but choose 1 h of incubation as it improves the reproducibility of the experimental results. This long incubation time allows all cells to effectively fully settle down on the imprint which slightly improves the capturing rates. Therefore for standardisation of the cell recognition experiment we fixed the incubation time to 1 h in order to study the effect of the flow rate and the bioimprint coating. We do not have systematically collected data for shorter incubation times.

ImageJ macro for processing of fluoresence microcopy images of FITC-tagged HL60 cells and TRITC-tagged PBMCs on the HL60 bioimprits

Image J macro used to automatically count the FITC-tagged HL60 cells from the fluorescence microscope images of the bioimprint.

```
//run("Threshold...");
run("8-bit");
setOption("BlackBackground", false);
run("Make Binary");
run("Watershed");
run("Analyze Particles...");
run("Set Scale...", "distance=0 known=0 pixel=1 unit=pixel global");
run("Analyze Particles...", "size=180-Infinity show=Masks display clear summarize");
run("Close");
run("Close");
```

Percentage of decrease of HL60 cells and PBMC on the bioimprits

Equations used to calculate the percentage of HL60 and PBMC decrease:

$$\%\ of\ cell\ decrease = \frac{\left(No\ of \frac{cells}{m^2}\right)_{before} - \left(No\ of \frac{cells}{m^2}\right)_{after}}{\left(No\ of \frac{cells}{m^2}\right)_{before}}$$

$\left(No\ of \frac{cells}{m^2}\right)_{before}$ is the number of cells per m^2 loaded on the bioimprint chip before flushing.

$\left(No\ of \frac{cells}{m^2}\right)_{before}$ is the number of cells per m^2 left on the bioimprint chip after flushing.

Selectivity of HL60 bioimprints with respect to HL60 cells

Equations used to calculate the percentage of HL60 and PBMC decrease:

$$Selectivity\ (\%) = \frac{(\%\ HL60\ cells)_{before} - (\%\ HL60\ cells)_{after}}{(\%\ HL60\ cells)_{before}}$$

$(\%\ HL60\ cells)_{before}$ is the percentage of HL60 cells in the HL60:PBMC cell mixture seeded on the imprint before flushing.

$(\%HL60\ cells)_{after}$ is the percentage of HL60 cells in the HL60:PBMC population left on the imprint after flushing.

References

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3. L. Castan, C. J. da Silva, E. F. Molina, R. A. dos Santos, *J. Biomed. Mater. Res. B: Appl. Biomater*., 2018, **106B**, 742-750.

4. F. ud Din, W. Aman, I Ullah, O. S. Qureshi, O. Mustapha, S. Shafique and A. Zeb, *Int. J. Nanomed*. 2017, **12**, 7291–7309.