Laser Printing Mediated Cell Patterning

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Materials and Methods:

Materials. A4 laser and copier transparencies (210×297 mm) were from Supplies Team (West Yorkshire UK). The HP toner composition was: Styrene-acrylate copolymer (75-85% et), wax (5-10% wt), pigment (1-5% wt).

Four-well rectangular plates made from PS and with a non-treated surface and a volume of 22 ml/well, were from Nunc (Langenselbold, GE). All other materials were purchased from Sigma-Aldrich. Mouse embryonic stem cells (mES-Oct4) were kindly provided by Dr Josh Brickman, Institute for Stem Cell Research (ISCR), University of Edinburgh.

Contact angle measurement: Contact angle analysis was carried out using a KRUSS G10 contact angle goniometer. The angles were measured by dropping three drops (~10μl) of distilled water at three positions on a coated coverslip at 20ºC in air. Three coverslips for each toner were tested to take the average value.

Cell cultivation and staining. HeLa cells were grown in RPMI 1640 medium, supplemented with heat inactivated fetal calf serum 10% v/v, penicillin (100 units/mL), streptomycin (100mg/mL) and L-glutamine (4.0mM) at 37 °C with 5% CO_2 (Gibco). mES cells were grown using GMEM growth medium supplemented

with heat inactivated fetal calf serum 10% v/v, penicillin (100units/mL), streptomycin (100units/mL) and L-glutamine (2.0mM), Sodium pyruvate (2.0mM), 2-mercaptoethanol (0.1mM), LIF (0.18 units/ml), puromycin (1µg/ml). The patterned slides were placed into a 4-rectangular well-plate and sterilized for 20 min under UV irradiation prior to the addition of cells (7×10^5 cells per slide with 7mL of complete media) into the well-plate and incubation for 48 hours with the media changed after 24h, prior to fixing, staining (Hoechst 33342) and analysis.

Cells staining and fixing. Media in the well plate was removed and the slides were cleaned with PBS three times. Cells were fixed using para-formaldehyde (4%w/v in PBS) for 30 min, stained using Hoechst 33342 (0.50 µg/ml) for 15 min and washed with distilled water.

Cellular binding and viability on toner coated coverslips. HeLa cells were cultured on toner coated coverslips (19mm in diameter) and blank coverslips as controls for 24h. Any cells on the back of the coverslips were removed, before being washed with PBS and being placed in the well of a 12-well plate. Trypsination (200 µl trypsin solution (0.25%) and 200 µl PBS) was carried out for 5 min in at 37ºC, 5% CO_2. The detached cells were diluted in 1ml of complemented RPMI and 10 µl were taken and mixed with a solution of trypan blue (40 µl, 0.4% in 0.81% sodium chloride, 0.06% potassium phosphate) followed by cell counting using a hemocytometer (n = 3).

Visualisation of patterned slides. Patterned fixed cells were analysed using a Nikon microscope controlled by Pathfinder (Imstar, France) using a 10×/0.30 objective and a DAPI filter set. Bright field phase images were taken using the same microscope. The microscope imaged areas (1234µm×943µm) were combined to give a mosaic of the whole slide. Cell numbers were determined automatically using Pathfinder. Living cell images were taken with a Leica microscope using a 5×/0.12 objective. Overviews

of the slides were performed using a BioAnalizer 4F/4S white light scanner using a DAPI filter.

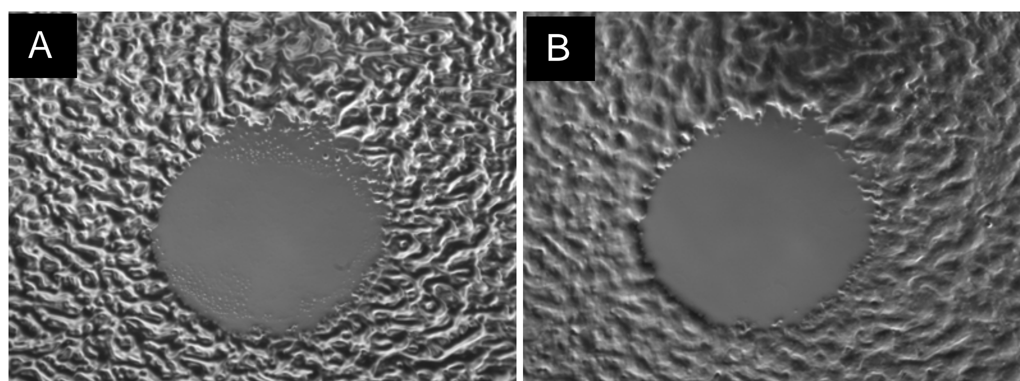

Figure S1. Influence of annealing on printed images: (**A**) pre-annealing; (**B**) post-annealing (200 °C, 10 min.).

Table S1. Contact angles of water drops on the four toner coated coverslips (n=9).

	Toner printed coverslips				Blank coverslip
	Yellow	Magenta	Cyan	Black	
Contact angle	108	104	107	103	63
STDV	3	3	1	1	1

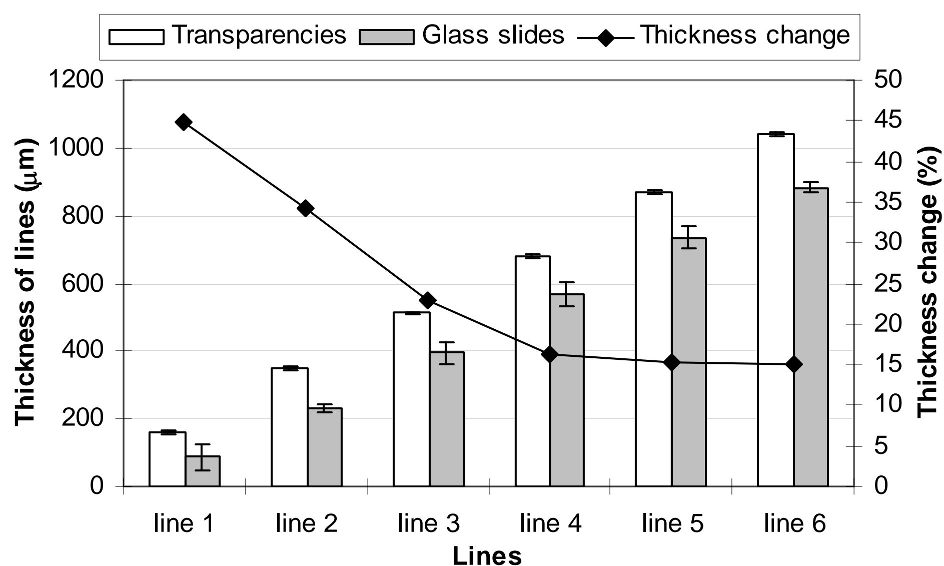

Figure S2. Thickness change of heat-transfer printed lines. White bars show average thickness of the laser printer printed lines on transparencies (line 1 with the line weight of 1pt in PowerPoint software, line 2-2pt, line 3-3pt and so on for other lines). Gray bars show the heat-transfer printed lines on the glass slides. The line shows the % thickness change of the lines following the heat-transfer process.

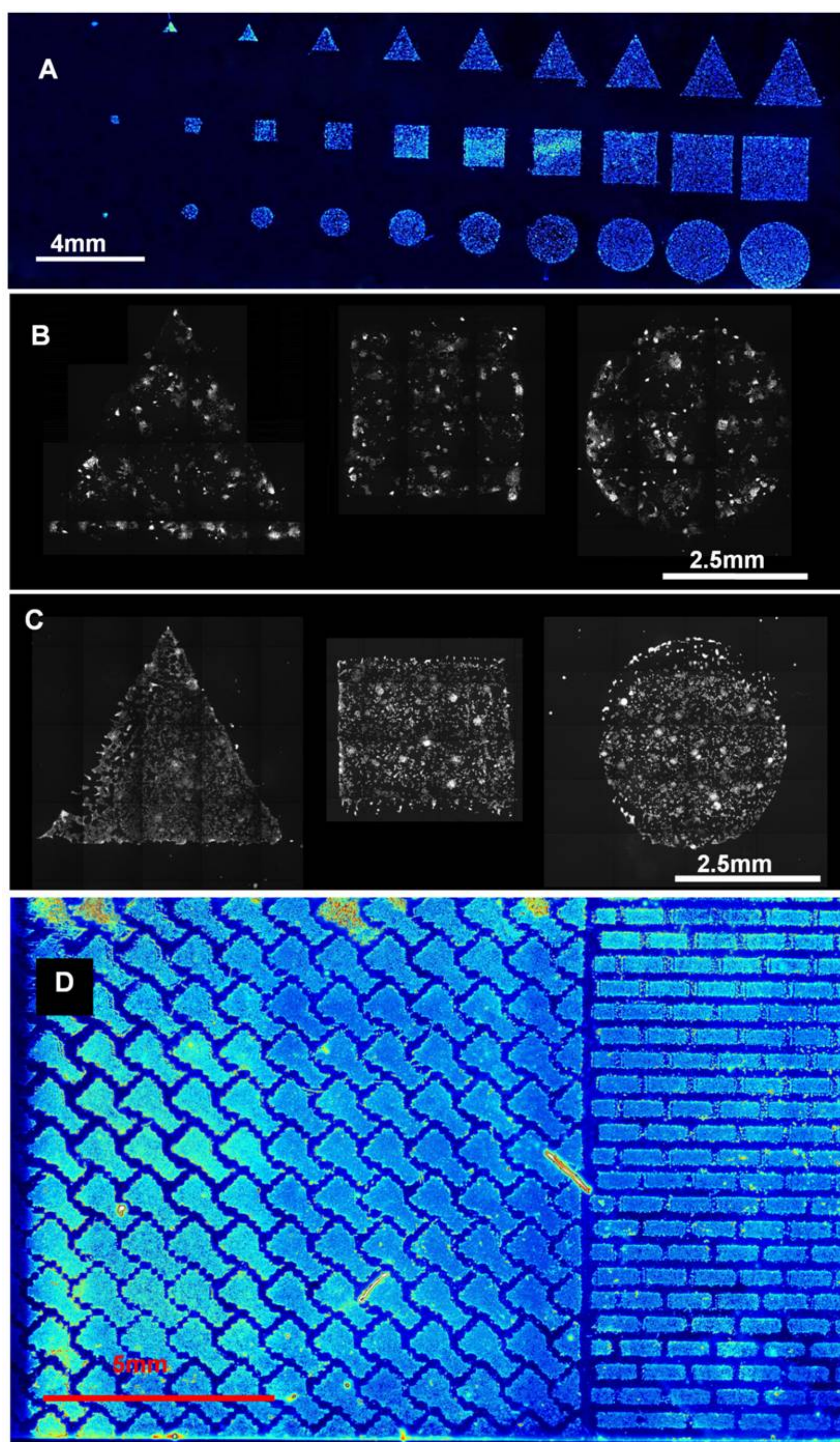
A
4mm
B
2.5mm
C
2.5mm
D
5mm

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Figure S3. Images of patterned cells on heat transfer printed glass slides.
(**A**) A fluorescent image of mES cells patterned on various sized shapes patterned on a glass slide; (**B**) Expanded images of mES cells on defined shapes; (**C**) Expanded images of HeLa cells on defined shapes and; (**D**) An image of a patterned HeLa cell array; (**E**) HeLa cells patterned words ‘cellular patterning’ on a glass slide (2 cm x 5 cm). In all cases cells were fixed and stained with Hoeshest 33342 (nuclei dye). Images were captured using a CCD–based fluorescent Bioanalyser (LaVision Biotec) with a DAPI filter (for images **A** and **D**) or a Nikon microscope controlled by Pathfinder (Imstar, France) using a 10x/0.30 objective and a DAPI filter set (for images **B**, **C** and **E**).

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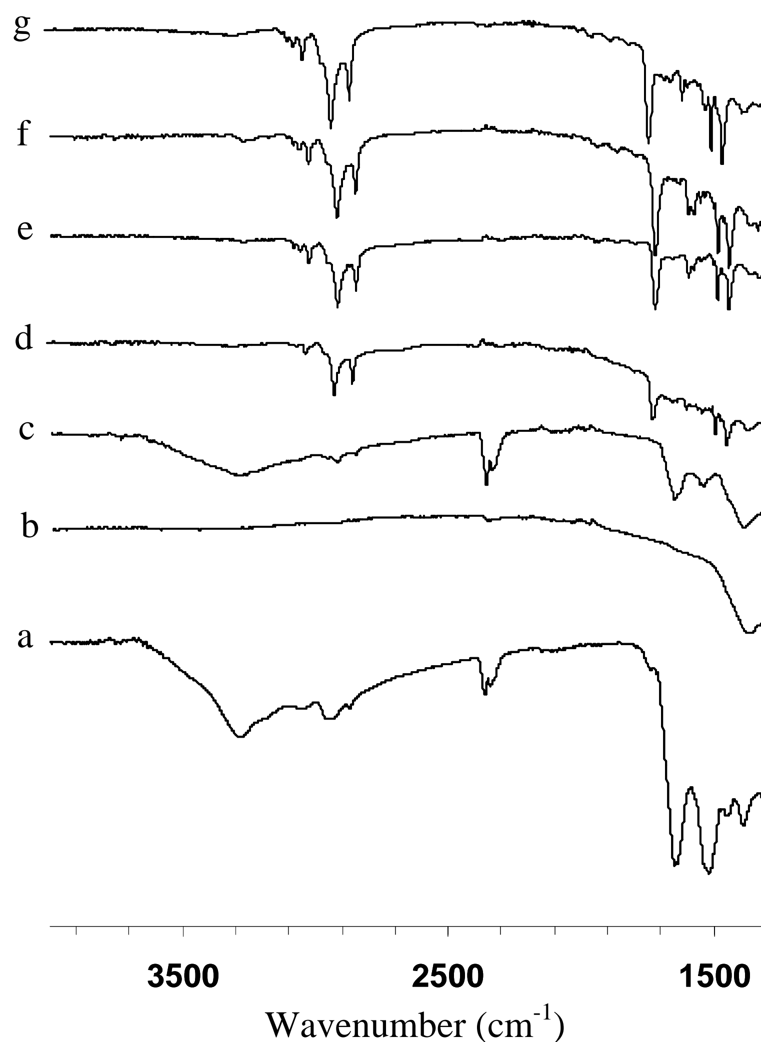

Figure S4. FTIR results of coverslips (19mm in diameter) obtained on a Bruker Tensor 27 FITR (ATR).

a. dried bovine serum albumin (BSA).
b. blank coverslip.
c. coverslip incubated with complemented RPMI.
d. black toner printed coverslip with complemented RPMI.
e. cyan toner printed coverslip with complemented RPMI.
f. magenta toner printed coverslip with complemented RPMI.
g. yellow toner printed coverslip with complemented RPMI.

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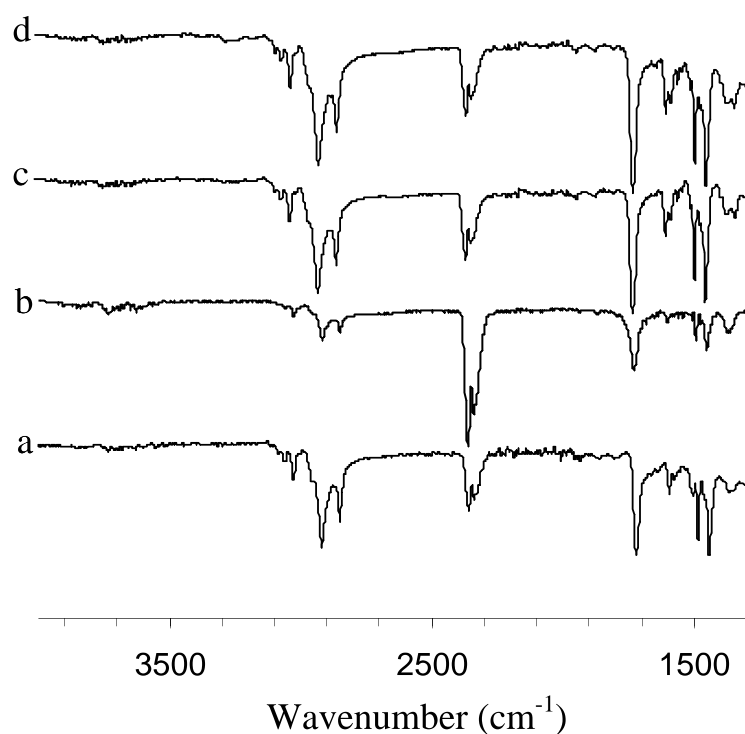

Figure S5. FTIR results of toner printed coverslips (19mm in diameter) obtained on a Bruker Tensor 27 FITR (ATR). The coverslips were heat-transfer printed with four toners on with

a: black toner,
b: yellow toner,
c: cyan tonner,
d: magenta toner and.

The signal at ~2350 cm^{-1} is CO_2.

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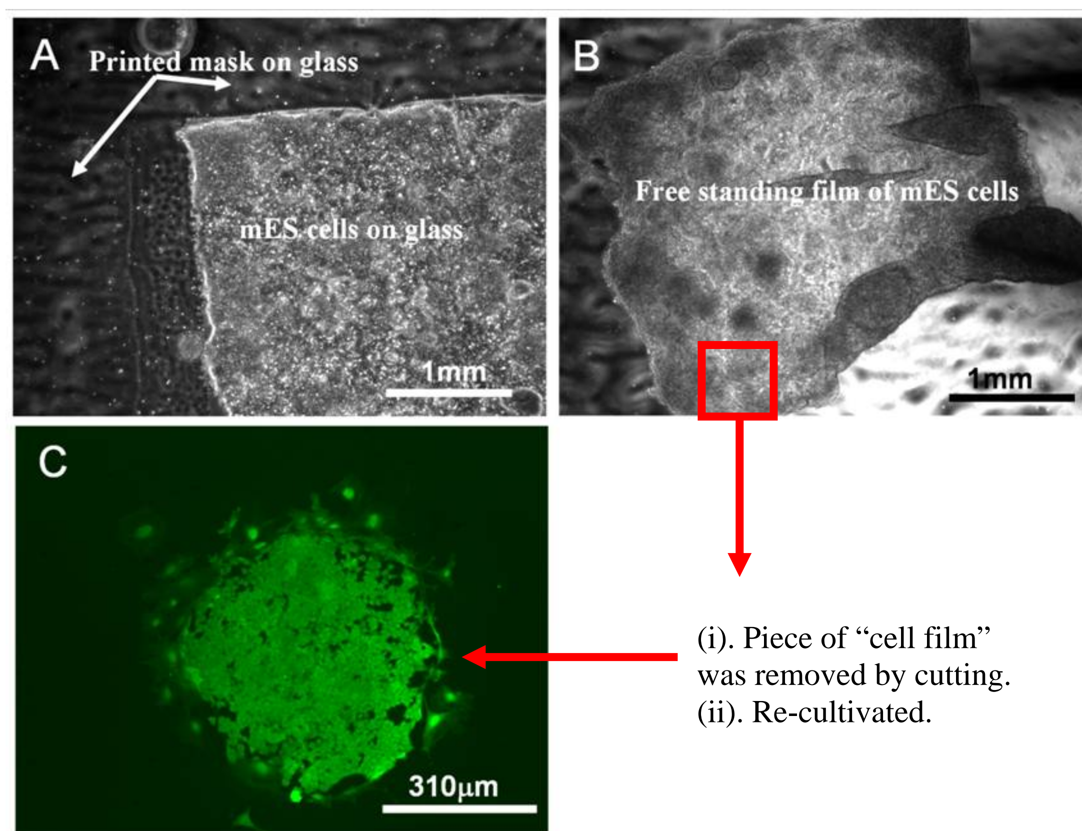

Figure S6. Preparation of free standing films of mES -Oct4 cells.[1]
(**A**) Image of mES-Oct4 cells attached and proliferating on a patterned surface; (B) Image of a free standing film of mES-Oct4 cells detached from the transfer printed glass after 72h's incubation. Images were taken using a Leica microscope with 5× objective; (**C**) A fluorescent image of mES cells after the freestanding film was peeled off from the transfer printed glass and cultivated on tissue culture plastic for 24h. The mES cells were stained using Celltracker green (Sigma-Aldrich) for 15min in PBS before images were captured using a Nikon microscope using a 10×/0.30 objective and a FITC filter set.

Reference:

1 Q. Ying, J. Nichols, E. P. Evans and A. G. Smith, *Nature*, 2002, **416**, 545-548.