DEVICE, Volume *4*

Supplemental information

Microfabrication of cell culture microenvironments with spatially controlled oxygen levels

Maila Becconi, Marco Malferrari, Andreas Lesch, Daniela Salvatore, Isabella Zironi, Maria Conte, Giorgia Simonetti, Pier Giuseppe Pelicci, Hubert H. Girault, Stefano Salvioli, Gastone Castellani, Francesco Zerbetto, and Stefania Rapino

Supplemental information

Table of Content

Note S1: Height profiles of inkjet-printed hydrogels

Height profiles of inkjet-printed hydrogel bands were recorded with the LSM after washing with DI water. The image focus was made on one printed band that was generated by three consecutive nozzles in one and two printing passes for each ink. 1 IJPL cross-linker ink and 1 IJPL protein ink after washing resulted in a hydrogel pattern with a general thickness of ~120 nm (**Figure S1a**). The higher parts on the boarder of the printed lines are a result of the well-known coffee ring phenomenon where solid material accumulates at the substrate-liquid-air interface during drying of the printed bands. The height in this confined region can reach 850 nm. Printing the double amount of material enhances the coffee ring effect and results on a height profile with slightly larger variations (**Figure S1b**). The center of the printed bands shows a height of ~250 nm while the border of the bands can reach a height of ~2.7 µm.

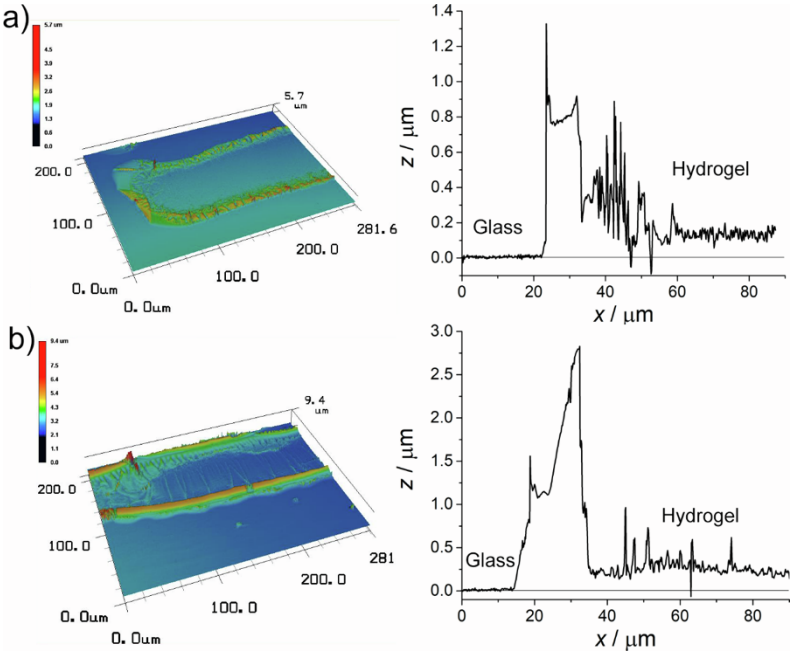

Figure S1. Height profiles of inkjet-printed hydrogels. (**a**) 1 IJPL cross-linker ink and 1 IJPL protein ink. (**b**) 2 IJPLs cross-linker ink and 2 IJPLs protein ink.

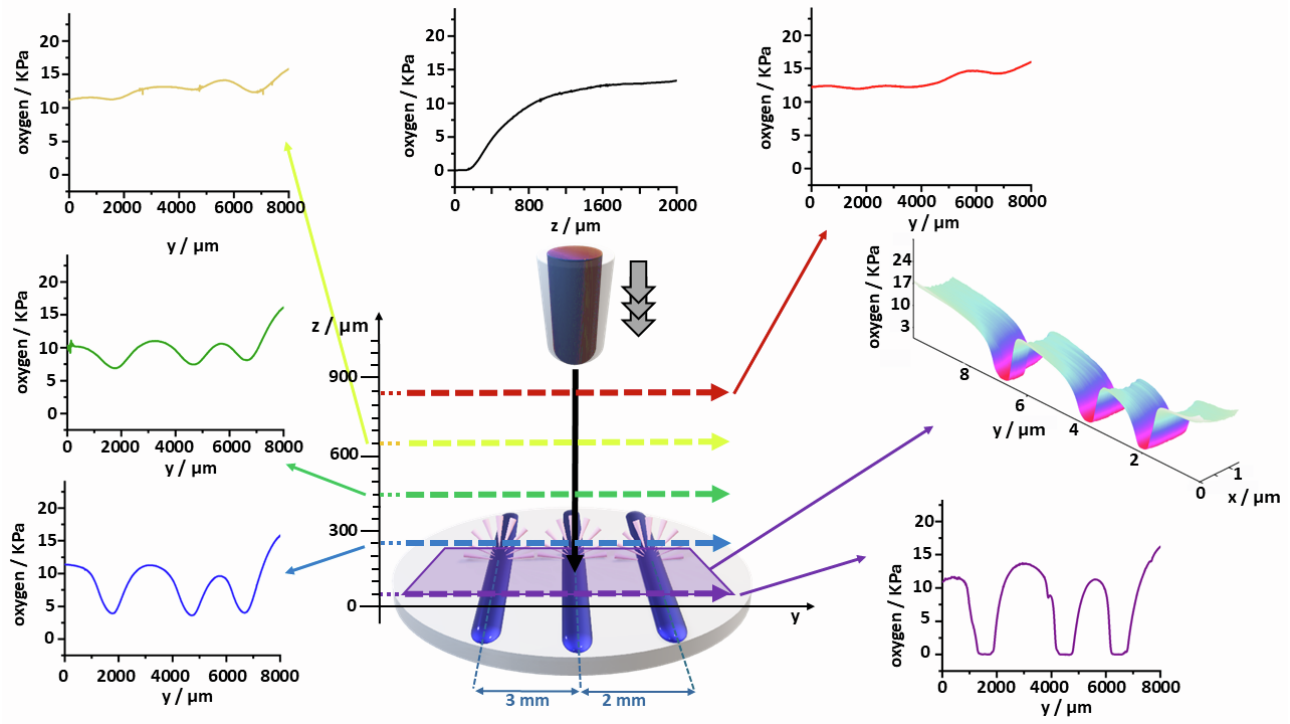

Figure S2. SECM determination of the three-dimensional oxygen gradients generated by the device. SECM scans were performed parallel to the surface of the hydrogel bands at different heights above the dish surface (50, 250, 450, 650, and 850 µm, shown in purple, blue, green, yellow, and red, respectively). Experimental data are reported as continuous lines in separate plots, while the corresponding SECM scan paths are indicated as dashed arrows in the schematic. The vertical black line in the schematic represents a probe scan curve performed while approaching the dish surface above an active band, enabling determination of the vertical (*z*-axis) oxygen gradient over the active material; the corresponding experimental measurement is reported above the electrode schematic. The three-dimensional SECM surface shown in the middle-right panel represents the oxygen gradient measured in the *xy*-plane at a height of 50 µm, corresponding to the oxygen landscape likely experienced by adherent cells under culture conditions. The experimental setup is the same described in the Scanning Electrochemical Microscopy paragraph of the Methods section in the main manuscript.

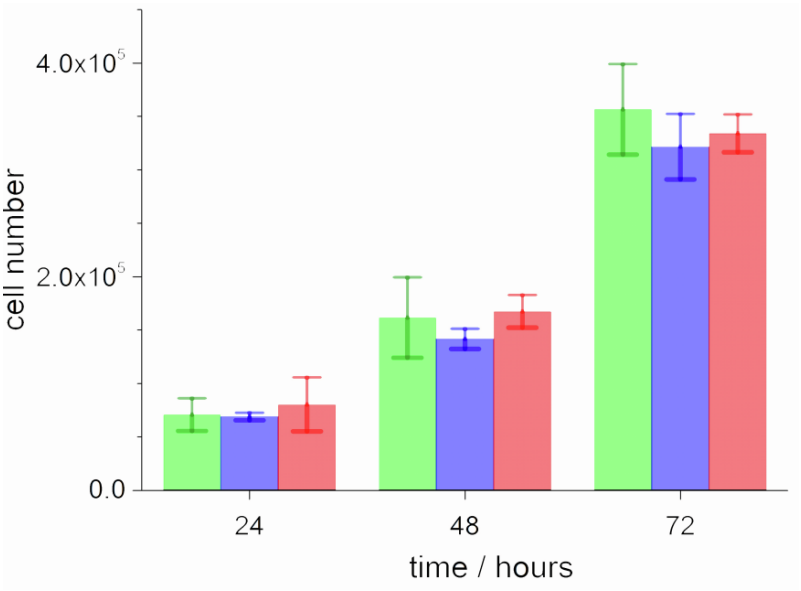

Figure S3. **Biocompatibility study of the hydrogel.** Cell growth of MCF7 in the presence of the active (red) or control (blue) functional hydrogel; these data were compared to cultures in the absence of any hydrogel (green). The reported values are the average of three independent measures, while error bar is the standard deviation. The active and control material were patterned on the dish using a design allowing to test the toxicity of the material. In average most of the culturing area was not influenced by the gradient generated by the activity of the material as we wanted to test the biocompatibility of the employed material when it is present in the culturing apparatus. The cell growth curves show that no toxic components are released by the material in the culturing environment. The experimental procedure is detailed in the Cell Cultures paragraph of the Methods section in the main manuscript.

Note S2. Protein extraction, SDS-PAGE and Western blotting

For western blots, 10 cm diameter Petri dishes were patterned with grids covering all the surface of the Petri. Grids of the active material were patterned with parallel lines at a distance of 10 mm, control grids were also patterned with a 10 mm distance between adjacent parallel lines; $1.5x10^6$ cells were seeded in each Petri dish in complete growth medium; after 24 h incubation, cells were harvested after trypsin digestion; cells were washed with ice-cold PBS and lysed in RIPA buffer (Tris Buffer pH8 50mM, NaCl 150 mM, Sodium deoxycholate 0.5 %, SDS 0.1%, Triton-X 1%, EDTA 5mM), 1 mM PMSF, protease and phosphatase inhibitors (Sigma-Aldrich). After lysis on ice for 30 min, cells were centrifuged at 12,000 g for 15 min at 4 °C, and supernatants analyzed for protein content. Protein concentration was determined by the Bradford assay (Bio-Rad). 15 µg of protein lysate were separated on Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (Trans-Blot Transfer Medium, Bio-Rad). Membranes were blocked with 5 % non-fat dry milk in Tris-buffered saline (TBS), 0.1 % Tween20 (TBS-T) and probed with 1:500 rabbit polyclonal HIF-1α (Cell Signalling) and 1:500 rabbit monoclonal 7F5 anti-p53 (Cell Signalling) antibodies in bovine serum albumin (BSA)/0.01 % TBS-T blocking solution overnight at 4 °C. Housekeeping 1:1000 mouse monoclonal anti-βtubulin (Santa Cruz) was used as a loading control. The secondary anti-rabbit and anti-mouse horseradish peroxidase-conjugated antibody (Bio-Rad) were used, followed by ECL detection (Santa Cruz Biotechnology). Densitometry analysis of bands was performed using ImageJ software from NIH Image.

Note S3. Electrochemical measure of the residual concentration of glucose in cell culture medium

The glucose residual concentrations in aliquots of the cell culturing medium at 24 h and 48 h of incubation were determined by using an electrochemical methodology: a three electrode setup (working electrode, CHI platinum 10 μm diameter; reference electrode, Ag/AgCl KCl 3 M; counter electrode, platinum wire) was employed to monitor hydrogen peroxide produced by the oxidation of glucose which is catalysed by glucose oxidase; the hydrogen peroxide produced is directly proportional to the concentration of glucose present in the solution. We measured hydrogen peroxide by adding 50 μL of 1:2 diluted cell culturing aliquots to 4 mL of 1 mg/mL glucose oxidase solution in PBS. The working platinum microelectrode was biased at 0.6 V vs Ag/AgCl (KCl 3 M) to measure the oxidation currents which are due to the oxidation of hydrogen peroxide. The amount of glucose was quantified employing calibration curves obtained by successive addition of known concentrations of glucose in the analysis solution. The obtained residual glucose concentrations are reported in **Table S1**, reported as averages together with standard deviations obtained from three independent measurements.

Table S1 - Residual concentration of glucose in cell culture medium. Residual concentration of glucose under the same culturing conditions (e.g. medium composition, temperature, CO_2 %, humidity) used for the experiments reported in Figures 4 and 5

Time of culturing / h	[glucose] / mM
0	11 ± 0
24	10 ± 2
48	12 ± 4

Glucose concentrations are reported as average ± standard deviation of three independent measurements.

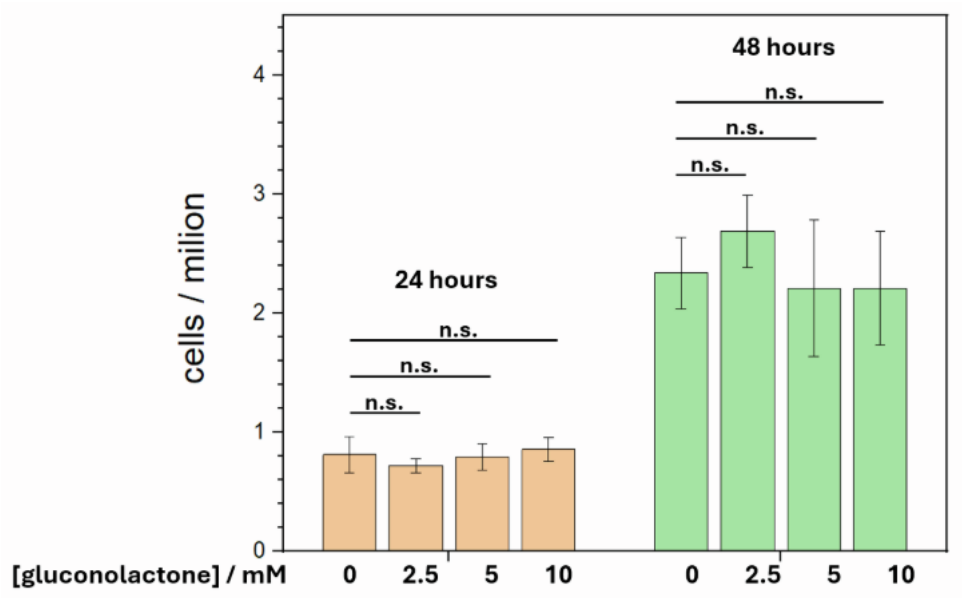

Figure S4. Effects of gluconolactone on cell growth. MCF7 cell growth curves in the presence of 2.5, 5, and 10 mM gluconolactone in the cell culture medium. Data are reported as the mean ± standard deviation of three independent measurements. Two-tailed t-tests assuming unequal variances were performed, and no statistically significant differences were observed at any time point of the 0, 2.5, 5, and 10 mM gluconolactone conditions. The experimental procedure for cell growth determination is detailed in the Cell Cultures paragraph of the Methods section; gluconolactone, at the specific concentrations listed in the figure caption, was added to the cell culture medium before plating the cells at time 0 h.