ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Matrix Addressing of an Electronic Surface Switch Based on a Conjugated Polyelectrolyte for Cell Sorting

*Kristin M. Persson, Susanna Lönnqvist, Klas Tybrandt, Roger Gabrielsson, David Nilsson, Gunnar Kratz, and Magnus Berggren**

Spatial control of cell detachment is potentially of great interest when selecting cells for clonal expansion and in order to obtain a homogeneous starting population of cells aimed for tissue engineering purposes. Here, selective detachment and cell sorting of human primary keratinocytes and fibroblasts is achieved using thin films of a conjugated polymer. Upon electrochemical oxidation, the polymer film swells, cracks, and finally detaches taking cells cultured on top along with it. The polymer can be patterned using standard photolithography to fabricate a cross-point matrix with polymer pixels that can be individually addressed and thus detached. Detachment occurs above a well-defined threshold of +0.7 V versus Ag/AgCl, allowing the use of a relatively simple and easily manufactured passive matrix-addressing configuration, based on a resistor network, to control the cell-sorting device.

1. Introduction

When culturing cells aimed for tissue engineering applications in vessels and chambers, it is important to try to mimic the complex and dynamic environment that cells experience in vivo.[1] One attempt to actively control the cell environment and various cell culture events, such as adhesion and detachment, is to employ smart materials and surfaces with switchable properties as cell culture substrates.[2–4] Spatial control of the switchable properties of these surfaces can be achieved through microfabrication techniques,[5] making it possible to selectively target and impact only a few, or a cluster of, cells while leaving the rest unaffected.[6–9]

Spatially controlled detachment of cells[10–13] is of importance for example when selecting cells for clonal expansion and to obtain a homogeneous starting population of cells aimed for tissue engineering.[14] One area where selective cell-sorting methods are desired and are of particular interest is for the preparation of primary keratinocytes and fibroblasts aimed for autologous transplantation. Today, keratinocytes are routinely prepared from autologous skin biopsies and expanded to be transplanted back to burn wound patients.[15] Even though being located in separate skin compartments, fibroblast contamination of the keratinocyte preparation often occurs. Spatial control of detachment would allow for morphological observations of the primary cultures in combination with detachment of preferred cells as an initial step in the cell expansion directly after cell preparation to ensure a homogenous population. A transparent cell-sorting device would be beneficial not only to remove contaminating cells but also to establish clonal populations.

We have previously presented a method for cell detachment based on a conjugated polymer.[16] Conjugated polymers have been increasingly explored to regulate and record cell systems[17] using smart surfaces that rely on the fact that the polymers are electrochemically active so that surface properties can be controlled via electronic addressing[18–20] to control for example cell adhesion.[21,22] In addition, frequently used polymers such as polypyrrole and poly(3,4-ethylenedioxythiophene), PEDOT, are soft, flexible, and biocompatible, making them ideal substrates for cell cultures.[17] We have realized cell detachment using a thin film of a self-doped and water-soluble derivative of PEDOT, poly(4-(2,3-dihydrothieno[3,4-b]-[1,4]-dioxin-2-yl-methoxy)-1-butanesulfonic acid (PEDOT–S:H), that detaches when electrochemically oxidized above a threshold value (**Figure 1**).[16] The detachment is caused mainly by swelling due to intake of charge compensating ions and supporting electrolyte,[23] and any cells cultured on top will detach along with the film. In addition, PEDOT–S:H can be patterned

Dr. K. M. Persson, Dr. K. Tybrandt,
Dr. R. Gabrielsson, Prof. M. Berggren
Department of Science and Technology
Linköping University
601 74 Norrköping, Sweden
E-mail: magnus.berggren@liu.se
S. Lönnqvist, Prof. G. Kratz
Department of Clinical and Experimental Medicine
Linköping University
581 85 Linköping, Sweden
Dr. K. Tybrandt
Laboratory of Biosensors and Bioelectronics
Institute for Biomedical Engineering
ETH Zurich
Zurich CH-8092, Switzerland
Dr. R. Gabrielsson
Department of Physics, Chemistry and Biology
Linköping University
581 83 Linköping, Sweden
Dr. D. Nilsson
Department of Printed Electronics
Acreo Swedish ICT AB
601 17 Norrköping, Sweden
Prof. G. Kratz
Department of Hand and Plastic Surgery
Linköping University
581 85 Linköping, Sweden

DOI: 10.1002/adfm.201503542

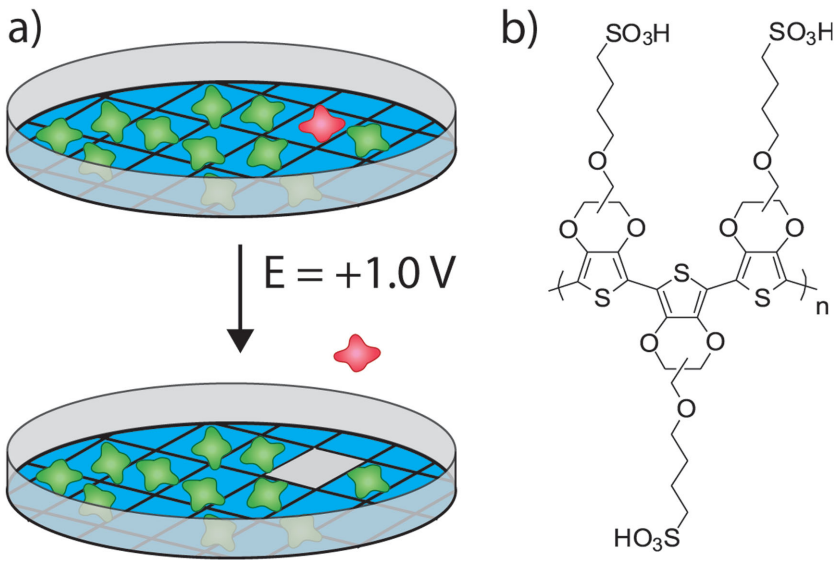

Figure 1. a) Selective cell detachment with PEDOT–S:H; one cell (red) cultured on a patterned PEDOT–S:H substrate has been selected and is detached by applying a potential of +1.0 V to the corresponding part of the substrate. b) Chemical structure of PEDOT–S:H.

using standard photolithography, which is of importance when preparing substrates for selective cell detachment.[16]

An ideal substrate for selective cell detachment should have a pattern with responsive areas (referred to as pixels) corresponding in size to individual cells or the coverage of colonies (Figure 1a). To minimize the risk of crosstalk and short circuits in an electroactive material, electrically conducting paths between the pixels must be supressed. Due to the common electrolyte, an ionic crosstalk will however always be present. The distance between the pixels should be kept as small as possible, i.e., the fill factor should be large, to minimize the number of cells adhering outside a specific pixel and subsequently be impossible to detach (Figure S1, Supporting Information).

The simplest strategy of addressing individual pixels in a matrix device is to have each pixel connected to an addressing wire. However, as the number of pixels increases, the amount of wiring required becomes difficult to manage in a practical manner. Instead, multiplexed matrix-addressing schemes, where all pixels along a column or row are connected to the same line, are often employed. This reduces the amount of wiring necessary for addressing from $m \times n$ to $m + n$ for a matrix with m columns and n rows. There are two common matrix-addressing schemes, both originating from the development of displays and memories, referred to as passive[24] and active.[25] Most displays employ active matrix addressing, in which active elements such as transistors, included in each crossing, are used to control the state of each pixel in the matrix. This provides excellent control, high resolution, and typically no crosstalk, but the manufacturing can be rather difficult, as at least one transistor is needed for each pixel. If feasible, passive matrix addressing may be preferable due to its lower complexity. However, for passive addressing to work, the pixels need some degree of bistability and robustness to crosstalk. Fortunately, the fact that PEDOT–S:H detaches only when oxidized at potentials above a threshold potential of +0.7 V (vs Ag/AgCl)[16] makes the pixels resistant to crosstalk and therefore a variant of passive addressing, based on a resistor network, can be used (**Figure 2**a). The employed resistor network is based on voltage division between the crossing addressing lines, resulting in only a chosen subpopulation of the pixels experiencing potentials above the threshold. This approach ensures a relatively simpler manufacturing protocol as compared to what is required for an actively addressed matrix, and at the same time provides sufficient control of individual pixel states.

Here we demonstrate for the first time a cell detachment matrix based on a conjugated polymer and addressed by a resistor network. The cell-sorting device has been evaluated using human primary fibroblasts and keratinocytes, both cell types showed a sufficiently good viability on the device prior to detachment, and cells could also be selectively detached.

2. Results and Discussion

2.1. Device Fabrication

We created a matrix consisting of 64 equally sized PEDOT–S:H pixels (300 µm × 300 µm) placed 20 µm apart in columns and rows for cell sorting. A resistor network, defined within an encapsulated backplane to ensure a large fill factor, is employed to control the potential at each pixel. The pixels are connected to the backplane circuitry at a point between two resistors, one connected to a column and the other to a row (Figure 2a). All resistors, having equal resistance, along one column or row are connected to each other and will thus experience the same potential. The potential actually reaching a pixel will be the average value of the two applied ones (Figure 2b), assuming that the line resistance is negligible relative to the resistance of the pixel.

The cell detachment matrix (Figure 2e) was fabricated on a glass wafer through subsequent steps of evaporation, coating, photolithographic patterning, and etching. At the crossings between the gold conductors, an insulating pad of photopolymerized SU-8 was patterned on top of the first gold conductor (Figure 2c). A second layer of gold was then patterned on top of the pad to close the circuit. PEDOT:PSS was chosen as the resistor material, the resistance in one pixel was estimated by applying +1.0 V between a column and a row while measuring the current, and was found to be roughly 0.5 MΩ. A second layer of insulating SU-8 was added to provide the encapsulation of the backplane circuitry. The top layer consisted of PEDOT:PSS and PEDOT–S:H creating the pixels, and was connected to the backplane through via-connections within the encapsulation layer (Figure 2d). A layer of PEDOT:PSS underneath the PEDOT–S:H film is needed to ensure proper conductivity in the entire pixel area during the triggering and completion of detachment. In order to improve the conductivity, 0.1% diethylene glycol was added to PEDOT:PSS as a secondary dopant.[26] A nonoptimized

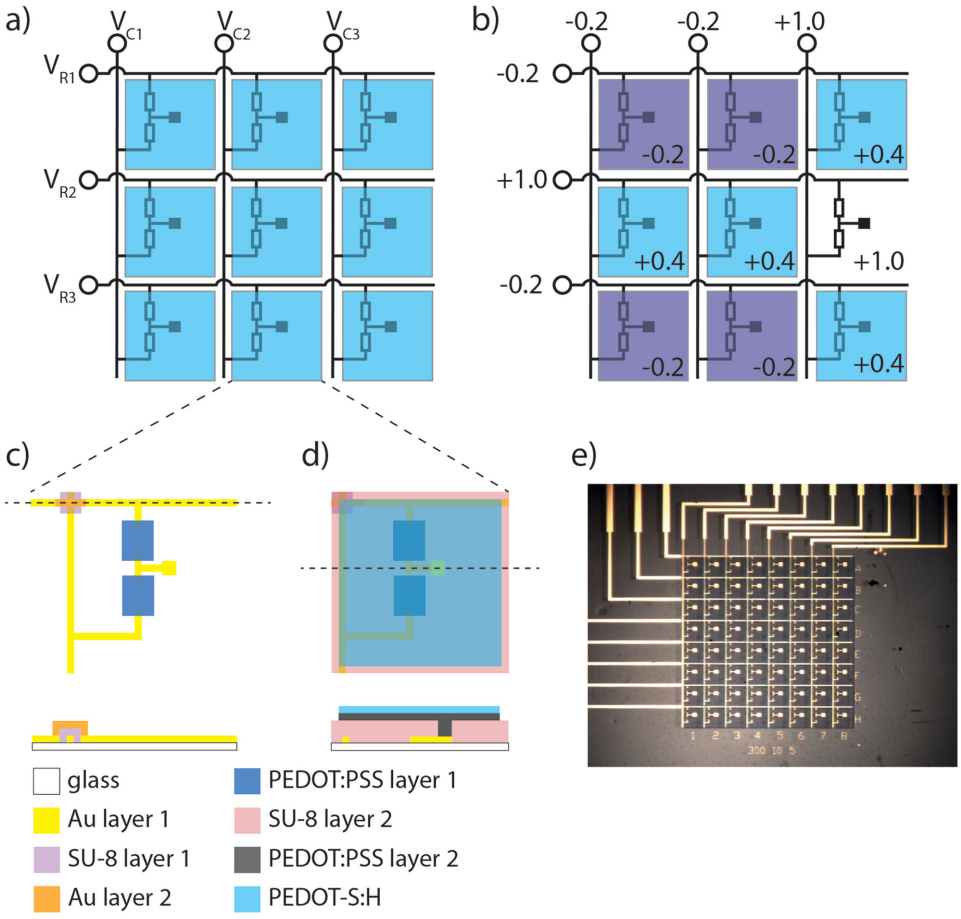

Figure 2. a) A 3 × 3 matrix based on a resistor network with pixels in blue, all circuitry in black, and the gold pad providing contact between the pixels and the backplane circuitry as a black square (one for each pixel). b) Different potentials have been applied along the columns and rows; the pixel potential will be an average of the potentials at the corresponding column and row and is displayed in the lower right corner of each pixel. One pixel (middle, right) has reached the potential for detachment and has thus detached; other pixels are either oxidized (light blue) or neutral (dark blue). c) Enlargement of one pixel showing the backplane circuitry and a cross-section (along the dashed line) of the intersection of the two gold layers. d) Enlargement of one pixel showing the encapsulation and final polymer layers constituting the active pixel with a cross-section (along the dashed line) showing the via-connection between the backplane and the pixel. e) An actual 8 × 8 matrix, with a pixel size of 300 × 300 μm^2, gold lines 10 μm wide, and a space between pixels of 20 μm.

manufacturing protocol and the large number of pixels had implications on the resulting yield of working pixels within the final matrix structure and it was hard to obtain a yield of 64 working pixels in one matrix. As it was difficult to achieve a yield of 100%, matrices with a yield above 75% were considered suitable to use for cell experiments, whereas for characterization purposes, only matrices with a yield above 90% were used. However, the resistor network is still easier to fabricate than a detachment matrix also including transistors needed in active matrix addressing, and we are confident that the processing can be much further improved, to increase the yield of functional pixels. The total number of pixels in the matrix can be increased without interfering with the performance of the resistor network if the proper addressing circuitry is available.

2.2. Characterization of the Cell Detachment Matrix

The selective switching and potential distribution within the matrix in the dry state was characterized by probing the surface of each pixel directly while measuring the potential. One pixel (6D) was oxidized by applying a potential of +1.0 V to column 6 and row D while −0.5 V was applied to all other columns and rows, the calculated potential distribution is shown in **Figure 3**a. When performing the measurements, pixel 6D did have the expected potential of +1.0 V. The potentials at the other pixels were displaying the correct polarity even if the magnitude was not always agreeing with the calculated values (Figure 3b; Table S1, Supporting Information). The recorded deviations in the dry state are most likely due to variations in the resistance values or due to remains of PEDOT:PSS or PEDOT–S:H in between pixels due to insufficient etching. Disruption of the gold lines at the intersections was identified as a common process-induced problem, which will lead to a change in the potential distribution within the matrix and is yet another possible explanation for deviations from calculated values. However, as only one pixel in the matrix reached the critical potential for detachment and all other pixels in the matrix both displayed potentials of the correct polarity and remained at potential values below the threshold, the

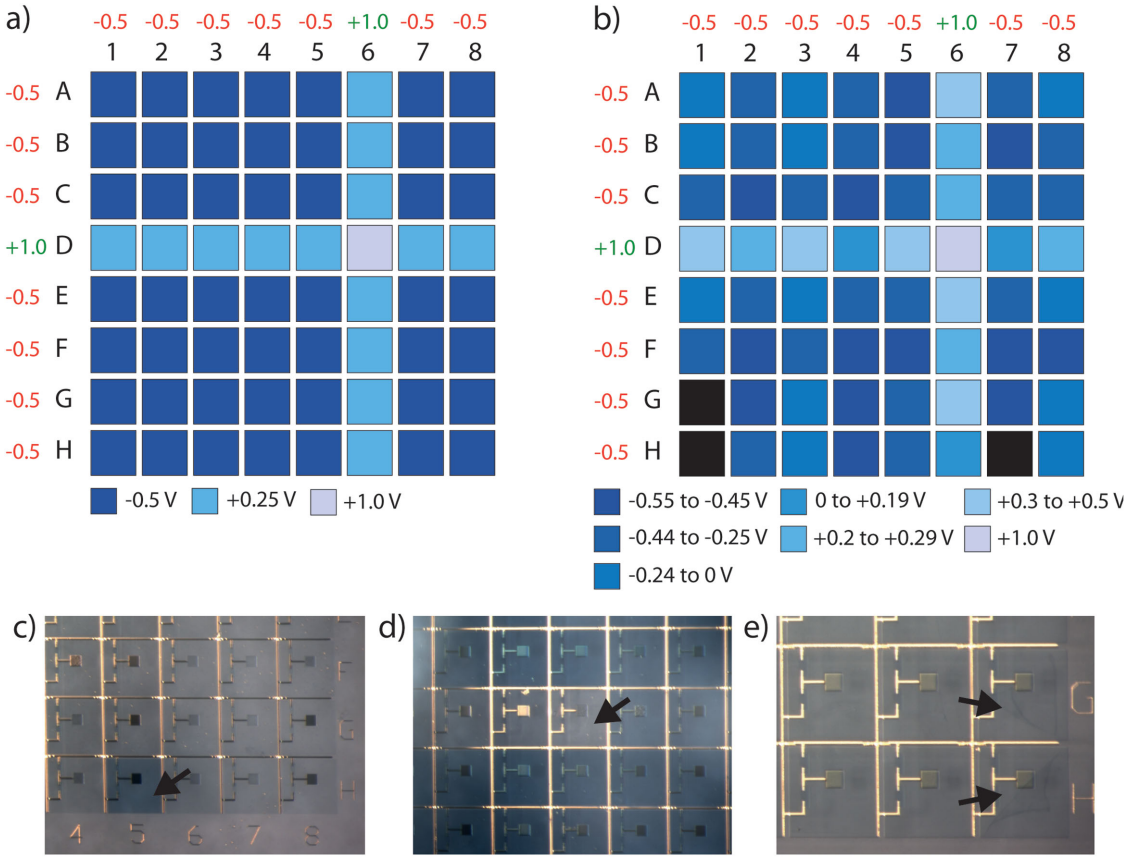

Figure 3. a) The calculated pixel potentials when applying +1.0 V to column 6 and row D and –0.5 V to all other columns and rows. b) Depiction of the measured pixel potentials in the dry state when applying +1.0 V to column 6 and row D and –0.5 V to all other columns and rows. Different shades of blue indicate the redox state, ranging from fully reduced to fully oxidized state; black pixels could not be measured. c) Selective reduction of the pixel indicated by an arrow, –2.0 V was used for reduction and +1.0 V was applied to all other columns and rows. d) Selective oxidation of the pixel indicated by an arrow, +0.8 V was used for oxidation, and –2.0 V was applied to all other columns and rows. e) Detachment of two pixels in the matrix as indicated by arrows, +1.2 V was applied for 20 min. c–e) Microscope images obtained in electrolyte (0.1 M NaCl, aq) using Ag/AgCl as the counter electrode.

resistance network provides sufficient control for addressing and triggering detachment.

The selectivity of the matrix was further visualized in the wet state by microscopy using the fact that PEDOT–S:H is electrochromic and appears dark blue in its neutral state and sky blue, close to completely transparent, in its oxidized state.[27] In the wet state, the common electrolyte is a possible source of crosstalk, however its potential should be dictated by the Ag/AgCl counter electrode. To the contrary, the effective pixel potential in the wet state may deviate slightly from the assigned value due to potential drops over the conducting polymer layers.

It was possible to selectively reduce or oxidize pixels in the matrix by applying either a negative (–2.0 V) or positive (+0.8 V) potential to one column and one row and a potential of opposite polarity (+1.0 or –2.0 V respectively) to all others (Figure 3c,d). Detachment of the thin PEDOT–S:H film is apparent upon oxidation (+1.2 V, 20 min) in two pixels in Figure 3e, proving that detachment of the patterned PEDOT–S:H pixels is possible. It is also possible to detach more than one pixel at the same time. All pixels in a rectangular shape within the matrix, or any number of pixels along the same column or row can be detached simultaneously. Alternatively, the matrix can be scanned one line at a time, detaching the selected pixels on each line before moving on to the next line.

In Figure 3d,e, it is evident that also other pixels than the ones selected for detachment are relatively lighter in colour and thus partially oxidized. However, as only the selected pixels detached, the potential of the others evidently remained below the threshold, again showing that the addressing scheme employed is sufficient to control the pixel state.

Another concern arising when using the matrix in an electrolyte is the risk of overoxidation, a process known to terminate conductivity that may appear at elevated positive potentials.[28] When characterizing the matrix, oxidized pixels were subsequently reduced to ensure that they were still conductive and thus not overoxidized. In all experiments discussed above, the oxidized pixels could be reduced after oxidation proving that overoxidation had not occurred.

2.3. Cell Viability

To evaluate the performance of the PEDOT–S:H matrix as a cell culture substrate prior to detachment, keratinocytes and fibroblasts were cultured at the device for various periods of times.

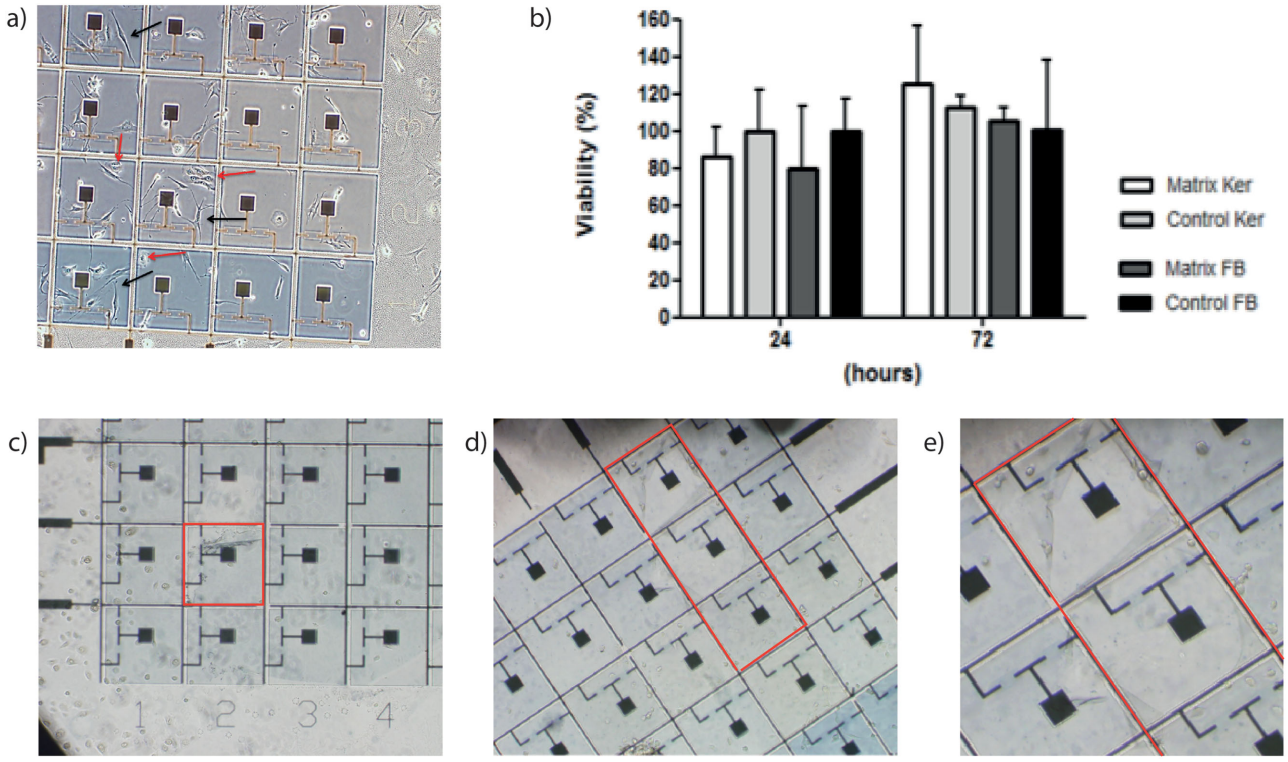

Figure 4. a) Fibroblasts (black arrows) and keratinocytes (red arrows) cultured on the PEDOT–S:H matrix obtaining normal morphology. b) Viability of keratinocytes and fibroblasts on the PEDOT–S:H matrix compared to control cell culture polystyrene after 24 and 72 h of cell culture, assessed with the MTT assay. Results are presented in percentage relative to control cells ± SD. Asterisk (*) indicates significance ($p < 0.05$, $n = 4$), Ker = keratinocytes, and FB = fibroblasts. c) Detachment of keratinocytes in the pixel indicated in red. d) Detachment of fibroblast in the pixels indicated in red. e) Enlargement of the two detaching pixels from (d). Detachment was performed by applying +1.5 V to the selected pixels and −1.0 V to all other pixels for 20 min; an Ag/AgCl counter electrode was used.

Viability was investigated after 24 and 72 h compared to control cells on polystyrene cell culture substrates (**Figure 4**b). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay showed reduced viability of both cell types when cultured on the PEDOT–S:H matrix as compared to the control cells after 24 h. Viability of keratinocytes relative to control cells was 86.5% ± 7.9% and fibroblasts exhibited a viability of 80.0% ± 19.0%, with no significant reduction ($p < 0.05$). After cell culture for 72 h, the reduction of viability was no longer present (keratinocytes 125.7% ± 17.8%, fibroblasts 105.3% ± 4.1%) indicating that the initial cell adhesion to the substrate was lower for both cell types than compared to the polystyrene control substrate, but that proliferation was not affected. Both cell types presented normal morphologies (Figure 4a) when cultured on the matrix in respective maintenance media and confluent cultures could be achieved (Figure S2, Supporting Information). The viability of 80% for fibroblasts and 86% for keratinocytes as compared to controls was considered acceptable for subsequent detachment experiments. The viability study shows that the extensive processing required during fabrication of the cell detachment matrix did not have a detrimental effect on the biocompatibility characteristics of PEDOT–S:H and that no harmful residues from the processing remained. As both the polymers and the encapsulation layers used in the matrix fabrication are transparent, visual inspection of cells cultured on top of the matrix was easily achieved with common microscopy techniques, and the conducting gold lines did not interfere with the transparency.

2.4. Selective Detachment of Keratinocytes and Fibroblasts

Fibroblasts and keratinocytes were cultured on the matrix until a normal morphology (compared to polystyrene) was obtained (6 h) and subsequently detached. As the pixels were larger than an individual cell (10–20 µm, excluding protrusions), several (usually between 10 and 30) cells could adhere to the same pixel. Cells can adhere both to the PEDOT–S:H pixels and to the nonresponsive areas in between pixels. The ratio of pixel to nonresponsive area in the matrix is about 8:1, making it more likely that cells will end up on a pixel. In the future, it might however be possible to further guide cells toward the pixels by rendering the nonresponsive areas less likely for cell adhesion, for example by addition of polyethylene glycol. By applying +1.5 V for 20 min, the selected and addressed PEDOT–S:H pixels could be detached by aid of medium agitation, and the adhered keratinocytes or fibroblasts detached along with the polymer (Figure 4c–e). At lower applied potentials or at shorter times, it was difficult to achieve any cell detachment. The cells did not remain adhered to the polymer after detachment as the polymer film was disintegrated to small flakes. The flakes will however remain in the cell culture media but can subsequently

be removed by centrifuging. The detached cells were able to proliferate on a new culture dish indicating that they were not damaged by the detachment process, in accordance with previous results.[16]

In Figure 4d, two pixels were detached, but initially also a third pixel was selected for detachment and even though it is clearly oxidized, the pixel did not detach. Most likely this was due to overoxidation of the PEDOT:PSS electrode underneath the PEDOT–S:H layer as the pixel could not be reduced following application of the rather high oxidative potential (+1.5 V), in turn needed as the matrices used suffered from process-induced defects. Replacing PEDOT:PSS with a different transparent and conductive material that is not sensitive to overoxidation might be an option.

It should be noted that at the high potentials required for detachment, some electrolysis of water will inevitably occur, leading to—for cells—a potentially harmful local decrease in pH. However, in order to minimize electrolysis, only PEDOT–S:H and not the gold circuitry was exposed to the electrolyte. This in combination with the buffering capacity of the cell culture media was enough to prevent any noticeable harm to the cells.

By optimizing processing conditions, mainly concerning the line width and alignment error, we also aim to further downscale the matrix to having a pixel size of 20 × 20 µm, thus reaching dimensions corresponding to the size of an individual cell, and enabling single-cell detachment, which is of importance when establishing clonal populations. As the included polymers are transparent, cells cultured on top can be monitored and inspected using standard microscopy techniques, which are beneficial when selecting cells for detachment. This then opens up for an automatic detachment technology for cell sorting using electronic addressing and detachment, achieved with the reported matrix, combined with microscopy and image recognition software. This would enable detachment and sorting based on morphology and size directly without any preparation or labelling of the cells, which is pivotal in applications for handling cells that are aimed for transplantation. Additionally, the detachment is enzyme free, which ensures cells of high quality for further expansion.

In addition, the matrix can be used for other systems where threshold potentials are involved. Cell adhesion has been shown to depend on the exact redox state of PEDOT,[22,29] making it possible to guide cells on a microscale when using the matrix. If combined with the addition of bioactive molecules to the conjugated polymer,[30,31] additional control of cells is established and it will be possible to create cell sheets with multiple cell types that could be the basis for multicell constructs for tissue engineering purposes. It is also possible to obtain precise spatial stimulation of cells cultured on the matrix, by loading the pixels with bioactive molecules that are either released or exposed depending on the polymer redox state.[32–34] Cell adhesion, stimulation, and detachment are all examples of functions that are of great importance and relevance for the field of tissue engineering.

3. Conclusion

In summary, we have shown selective detachment of human primary keratinocytes and fibroblasts using a simple cross-point matrix with individually addressable pixels including the conjugated polymer PEDOT–S:H as the detachment layer. PEDOT–S:H swells, cracks, and disrupts upon electrochemical oxidation, taking with it any cells cultured on top. The addressing of the matrix is based on an encapsulated backplane containing a resistor network relying on the fact that PEDOT–S:H only detaches at values above a certain threshold potential.

We found that the resistor network proved an excellent alternative to active matrix addressing for our device, with the benefit of simpler manufacturing and a more straightforward addressing protocol. By means of visual inspection, we found that selected pixels in the matrix could be switched back and forth between the two electrochromic states of the polymer. When measuring the potential at each pixel, it was confirmed that it was possible to bias only one pixel in the matrix at a potential beyond the threshold needed for detachment. The cell detachment matrix was evaluated using human primary keratinocytes and fibroblasts and both cell types showed sufficiently good viability on the matrix, showing that no harmful residues remained from the processing. It was also possible to achieve selective detachment of cells cultured on top of the matrix, showing that the principle works.

In the future, we aim to further downscale the matrix to reach pixel sizes corresponding to individual cells, and to use it not only for detachment but also for control of other cell culture events such as adhesion. The presented matrix technology puts us one step closer to achieving an electronic cell culture dish where excellent control of all individual cells in the culture is possible.

4. Experimental Section

Polymer Synthesis: PEDOT–S:H was synthesized according to a previously published protocol.[16]

Fabrication of the Cell Detachment Matrix: Glass wafers were cleaned in acetone and deionized water prior to thermal evaporation of titanium (50 Å) and gold (500 Å). Substrates were then dried at 140 °C for 30 min prior to patterning the first layer. First, hexamethyldisilazane primer (Shipley) and photoresist, s1805 (Shipley) were spin-coated. After exposure in a mask aligner (Suss MA/BA 6), the resist was developed using Microposit MF-319. After baking (110 °C, 90 s), the gold conductor pattern was wet-etched in an I_2/KI solution (Au) and a 2:1:1 solution of H_2O_2:NH_3:H_2O (Ti). The remaining resist was removed using Shipley 1112A. Next, the substrate was reactive ion-etched in O_2/CF_4-plasma (150 W, 30 s) and baked (140 °C, 5 min) before spin-coating a layer of diluted (14.6 g in 38 ml cyclopentanone) MicroChem SU-8 3010. After baking (95 °C, 60 s), exposure and postexposure baking (65 °C, 3 min and 95 °C, 60 s), substrates were developed using mr-Dev 600 (MicroChem) and baked (110 °C, 30 min), providing an insulating pad on top of the bottom gold layer at the crossings. Next, a second layer of Ti (50 Å) and Au (500 Å) was thermally evaporated, and patterned with photolithography and wet etching to realize gold conductors on top of the insulating pads. In the next step, PEDOT:PSS (Clevios PH-1000, Hereaus) with 0.5% 3-glycidoxypropyltrimethoxysilane (GOPS) and 0.25% dodecylbenezenesulphonic acid (DBSA) was spin-coated and baked (140 °C, 30 min). A protecting layer of poly(vinylidene fluoride-*co*-hexafluoropropylene) was added before patterning with photolithography. After patterning, the polymer was reactive-ion etched with O_2/CF_4-plasma (150 W, 30 s) to create the resistors. Next, a second layer of diluted (29.7 g in 25 mL cyclopentanone) SU-8 3010 (MicroChem) was spin-coated, baked (95 °C, 90 s), exposed, baked (65 °C, 2 min and 95 °C, 90 s) and developed, followed by baking (100 °C, 30 min) to provide an insulating

cover with holes exposing one gold pad in each pixel. Then, PEDOT:PSS with 0.5% GOPS, 0.25% DBSA, and 1% diethyleneglycol was spin-coated and baked (140 °C, 30 min), followed by a layer of PEDOT–S:H (10 mg mL^{-1}) that was spin-coated and baked (80 °C, 20 min). The polymers were patterned with photolithography, using Shipley s1813 as photoresist, and reactive-ion etched with O_2/CF_4-plasma (150 W, 60 s) to realize the pixels. A plastic ring was glued around the matrix on top of the glass to create a cell culture vessel.

Characterization and Addressing of the Matrix: Resistances were estimated by applying a potential of 1.0 V with a Keithley 2602 source meter, controlled via LabVIEW, while measuring the current. Matrix addressing was achieved using a 16-channel analog output data acquisition card (NI9264, National Instruments) controlled via LabVIEW. An Ag/AgCl counter electrode was placed in the electrolyte (0.1 M NaCl, aq). A pixel was switched by applying a potential between –2.0 and +1.5 V to the corresponding row and column. At the same time, a potential of the reverse polarity was applied to all other rows and columns. Electrochemical switching and detachment was followed in a long working distance microscope (Nikon SMZ1500). The potential at individual pixels was measured while applying –0.5 V to all columns and rows and then changing the potential to +1.0 V at one column and one row. The potential was measured by probing directly at the surface of the pixel using a probe connected to a Kiethley 2602 source meter, in turn connected to the DAQ card. When doing the potential characterization, no electrolyte was used.

Cell Viability Study: Viability of keratinocytes and fibroblasts on matrices was investigated with the aid of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Keratinocytes were cultured in keratinocyte serum free medium supplemented with bovine pituitary extract (25 μg mL^{-1}), epidermal growth factor (1 ng mL^{-1}), penicillin (50 U mL^{-1}), and streptomycin (50 μg mL^{-1}) (Gibco, Life Technologies). Fibroblasts were cultured in FM 10% medium; Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (FCS, HyClone, Thermo Scientific), penicillin (50 U mL^{-1}), and streptomycin (50 μg mL^{-1}) (Gibco, Life Technologies). Plastic rings (6 mm in diameter) were attached to substrates with silastic medical adhesive (Dow Corning) to create culture wells. Cells were seeded at 80 000 cells mL^{-1} and viability was investigated after 24 and 72 h of culture. At time-point cells, cultures were incubated with 0.3 mg mL^{-1} MTT solution (Sigma-Aldrich) for 4 h at 37 °C, after which dimethyl sulfoxide (Sigma-Aldrich) was added to solubilize the formed formazan. After 10 min of incubation at 37 °C, absorbance was measured with a VERSAMax plate reader (Molecular Devices) at 570 nm. Student's *t*-test was performed and graphs were constructed in GraphPad Prism 5.0 (GraphPad Software).

Cell Detachment Using the Matrix: Keratinocytes and fibroblasts were cultured in their normal maintenance media for up to 6 h before detachment experiments. The matrix was addressed using a 16-channel analog output data acquisition card (NI9264, National Instruments) controlled via LabVIEW. An Ag/AgCl counter electrode was placed in the culture vessel. A pixel in the matrix was visually selected using a phase-contrast microscope (Olympus IX51 light microscope) and was addressed with increasing positive voltages over the threshold voltage, up to +1.5 V, for 15 to 30 min. Surrounding pixels were maintained in a reduced to partly oxidized state over the course of the experiment. Detachment was evoked by agitation of the medium in the culture well.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This research was financed by the Swedish Foundation for Strategic Research and VINNOVA (the OBOE center 2010-00507). Additional funding was also provided by the Önnesjö foundation (Holmen) and the Swedish Government Strategic Research Area in Materials Science on Functional Materials at Linköping University (Faculty Grant SFO-Mat-LiU # 2009-00971).

Received: August 21, 2015
Revised: September 15, 2015
Published online: October 21, 2015

[1] J. Kim, R. C. Hayward, *Trends Biotechnol.* **2012**, *30*, 426.
[2] J. Lahann, *Science* **2003**, *299*, 371.
[3] M. A. Cole, N. H. Voelcker, H. Thissen, H. J. Griesser, *Biomaterials* **2009**, *30*, 1827.
[4] J. Robertus, W. R. Browne, B. L. Feringa, *Chem. Soc. Rev.* **2010**, *39*, 354.
[5] A. Khademhosseini, R. Langer, J. T. Borenstein, J. P. Vacanti, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2480.
[6] M. Yamoto, C. Konno, M. Utsumi, A. Kikuchi, T. Okano, *Biomaterials* **2002**, *23*, 561.
[7] A. Khademhosseini, K. Y. Suh, J. M. Yang, G. Eng, J. Yeh, S. Levenberg, R. Langer, *Biomaterials* **2004**, *25*, 3583.
[8] L. Li, J. R. Klim, R. Derda, A. H. Courtney, L. L. Kiessling, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11745.
[9] J. M. Curran, R. Stokes, E. Irvine, D. Graham, N. A. Amro, R. G. Sanedrin, H. Jamil, J. A. Hunt, *Lab Chip* **2010**, *10*, 1662.
[10] W.-S. Yeo, M. Mrksich, *Langmuir* **2006**, *22*, 10816.
[11] H. Hatakeyama, A. Kikuchi, M. Yamato, T. Okano, *Biomaterials* **2007**, *28*, 3632.
[12] S. S Shah, J. Y. Lee, S. Verkhoturov, N. Tuleuova, E. A. Schweikert, E. Ramanculov, A. Revzin, *Langmuir* **2008**, *24*, 6837.
[13] T. A. Kolesnikova, D. Kohler, A. G. Skirtach, H. Möhwald, *ACS Nano* **2012**, *6*, 9585.
[14] R. S. Tuan, G. Boland, R. Tuli, *Arthritis Res. Ther.* **2003**, *5*, 32.
[15] J. N. Mcheik, C. Barrault, G. Levard, F. Morel, F.-X. Bernard, J.-C. Lecron, *Plast. Reconstr. Surgery* **2014**, *2*, 218.
[16] K. M. Persson, R. Karlsson, K. Svennersten, S. Löffler, E. W. H. Jager, A. Richter-Dahlfors, P. Konradsson, M. Berggren, *Adv. Mater.* **2011**, *23*, 4403.
[17] R. M. Owens, G. G. Malliaras, *MRS Bull.* **2010**, *35*, 449.
[18] L. Xu, W. Chen, A. Mulchandani, Y. Yan, *Angew. Chem. Int. Ed.* **2005**, *44*, 6009.
[19] B. Garner, A. Georgevich, A. J. Hodgson, L. Liu, G. G. Wallace, *J. Biomed. Mater. Res.* **1999**, *44*, 121.
[20] M. R. Gandhi, P. Murray, G. M. Spinks, G. G. Wallace, *Synth. Met.* **1995**, *73*, 247.
[21] K. Svennersten, M. H. Bolin, E. W. H. Jager, M. Berggren, A. Richter-Dahlfors, *Biomaterials* **2009**, *30*, 6257.
[22] A. M. D. Wan, D. J. Brooks, A. Gumus, C. Fischbach, G. G. Malliaras, *Chem. Commun.* **2009**, 5278.
[23] K. M. Persson, R. Gabrielsson, A. Sawatdee, D. Nilsson, P. Konradsson, M. Berggren, *Langmuir* **2014**, *30*, 6257.
[24] J. Kawahara, P. Andersson Ersman, M. Berggren, *Org. Electron.* **2013**, *14*, 3371.
[25] P. Andersson, R. Forchheimer, P. Tehrani, M. Berggren, *Adv. Funct. Mater.* **2007**, *17*, 3074.
[26] X. Crispin, F. L. E. Jakobsson, A. Crispin, P. C. M. Grim, P. Andersson, A. Volodin, C. van Haesendonck, M. Van der Auweraer, W. R. Salaneck, M. Berggren, *Chem. Mater.* **2006**, *18*, 4354.
[27] R. H. Karlsson, A. Herland, M. Hamedi, J. A. Wigenius, A. Åslund, X. Liu, M. Fahlman, O. Inganäs, P. Konradsson, *Chem. Mater.* **2009**, *21*, 1815.
[28] X. Du, Z. Wang, *Electrochim. Acta* **2003**, *48*, 1713.

[29] M. H. Bolin, K. Svennersten, D. Nilsson, A. Sawatdee, E. W. H. Jager, A. Richter-Dahlfors, M. Berggren, *Adv. Mater.* **2009**, *21*, 4379.
[30] D. H. Kim, S. M. Richardson-Burns, J. L. Hendricks, C. Sequera, D. C. Martin, *Adv. Funct. Mater.* **2007**, *17*, 79.
[31] K. J. Gilmore, M. Kita, Y. Han, A. Gelmi, M. J. Higgins, S. E. Moulton, G. M. Clark, R. Kapsa, G. G. Wallace, *Biomaterials* **2009**, *30*, 5292.
[32] B. C. Thompson, S. E. Moulton, J. Ding, R. Richardson, A. Cameron, S. O'Leary, G. G. Wallace, G. M. Clark, *J. Controlled Release* **2006**, *116*, 285.
[33] R. Wadhwa, C. F. Lagenaur, X. T. Cui, *J. Controlled Release* **2006**, *110*, 531.
[34] A. Herland, K. M. Persson, V. Lundin, M. Fahlman, M. Berggren, E. W. H. Jager, A. I. Teixeira, *Angew. Chem. Int. Ed.* **2011**, *50*, 12529.