Enhanced Cell–Chip Coupling by Rapid Femtosecond Laser Patterning of Soft PEDOT:PSS Biointerfaces

Francesca Santoro,†‡§ Yoeri van de Burgt,†⊥∥ Scott Tom Keene,†⊥∥ Bianxiao Cui,*‡¶ and Alberto Salleo*†⊥∥

†Department of Chemistry and ‡Department of Material Science and Engineering, Stanford University, Stanford, California 94305, United States
§Center for Advanced Biomaterials for Healthcare, Istituto Italiano di Tecnologia, Naples 80125, Italy
∥Department of Mechanical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven 5612 AZ, The Netherlands

Supporting Information

ABSTRACT: Interfacing soft materials with biological systems holds considerable promise for both biosensors and recording live cells. However, the interface between cells and organic substrates is not well studied, despite its crucial role in the effectiveness of the device. Furthermore, well-known cell adhesion enhancers, such as microgrooves, have not been implemented on these surfaces. Here, we present a nanoscale characterization of the cell–substrate interface for 3D laser-patterned organic electrodes by combining electrochemical impedance spectroscopy (EIS) and scanning electron microscopy/focused ion beam (SEM/FIB). We demonstrate that introducing 3D micropatterned grooves on organic surfaces enhances the cell adhesion of electrogenic cells.

KEYWORDS: organic bioelectronics, PEDOT:PSS, biointerface, scanning electron microscopy/focused ion beam, electrochemical impedance spectroscopy, femtosecond laser

In the last decades, many biomedical devices such as prosthetics, implants, and biosensors have been developed,1 while new biomaterials are constantly being designed to improve the organ–device interface.2,3 In particular for implantable biosensors, the biotic/abiotic interface has to be mechanically, chemically, and electrically stable. For both in vivo and in vitro biosensors, the adhesion between cells and the electrode surface determines the sensing/stimulating efficiency of the device.4 A recent approach is to replace traditional conducting and semiconducting materials in bioelectronics applications, which have Young’s moduli in the range of MPa, with soft organic semiconductors (i.e., conjugated polymers). These materials offer biocompatibility,5,6 attractive mechanical properties (low Young’s modulus),7 as well as high conductivity.8 Devices based on organic semiconductor materials can be adopted for the detection or the control of various biomolecular/biological processes such as binding of specific ligands,9 cell proliferation and migration,10,11 and recording/stimulation of action potentials in neuronal and cardiac cells.12 Despite demonstrating a wide range of biomedical applications, current devices have been designed primarily on 2D planar interfaces while it has been recently proposed that 3D topologies are preferable for cell–chip coupling in bioelectronics.13 In fact, one crucial parameter is the interfacial cleft between cells and substrates that determines the effective coupling. Reducing the cleft is essential in enhancing signal strength and reducing noise and could be achieved by implementing 3D topologies in the interface material. However, systematic 3D nano- and microstructuring and patterning of organic semiconductors can involve complex and time-consuming processes due to the sensitivity of conjugated polymers to traditional cleanroom-based patterning methods.14,15 Furthermore, the characterization of the interface between those 3D electroactive materials and cells requires investigations at the nanoscale of soft interfaces, which presents significant challenges.

Here, we demonstrate a rapid patterning technique to produce 3D micropatterns in poly(ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS) organic electrodes based on femtosecond laser direct-writing. Femtosecond laser ablation of polymer materials has been studied widely17

Received: August 16, 2017
Accepted: October 30, 2017
Published: October 30, 2017

DOI: 10.1021/acsmi.7b12308
and various reports on laser patterning for cell control demonstrate a range of applications, such as directing cell migration and preferential cell adhesion by laser ablation of self-assembled monolayers. However, in this work, we demonstrate for the first time a rapid out-of-cleanroom direct-write laser patterning method to increase the adhesion at the conducting polymer/cell interface.

We compare the functionality and the morphology of 3D devices with those of a planar analogue using electrochemical impedance spectroscopy (EIS) and scanning electron microscopy/focused ion beam (SEM/FIB) to investigate the effective cell spreading of electrogenic cells and discriminate optimal cell adhesion conditions. EIS is known to be an appropriate technique to evaluate the adhesion of individual and confluent cells on conductive electrodes based on impedance models (in terms of resistance and capacitance). However, in this work, we report on the effective use of EIS constitutive parameters to evaluate the adhesion of cardiomycocytes-like cells on 2D planar vs 3D patterned PEDOT:PSS devices. In parallel, we characterize the cell-organic material interface with a unique electron microscopy technique, which allows the visualization of the adhesion area between cell membrane and 3D patterned surface for the first time at nanoscale level. The combination of results obtained by EIS and SEM/FIB lead to the identification of a range of dimensions for which cell coupling conditions on 3D grooved organic substrates are improved compared to 2D organic surfaces.

Planar PEDOT:PSS films have been prepared as described in the Supporting Information. A femtosecond laser was used to locally etch material from the planar PEDOT:PSS film to produce microgrooves with pitches of 10, 20, and 40 μm between grooves having widths of 4–5 μm. The groove depth is ca. 3−5 μm, comparable to the thickness of the deposited PEDOT:PSS films. Figure 1A shows a schematic of the fabrication process and Figure S1A shows a photograph of the complete device. The pitch distances were chosen considering the average diameter of the cells cultured on the films. Considering that cardiomycocytes typically spread over a 20 μm diameter, we chose pitches that are smaller, comparable and larger than the cell reference diameter. Figure 1B and Figure S2 show a typical SEM image of a 3D-grooved PEDOT:PSS device. We first investigated the local roughness of the fabricated structures to ensure that possible redeposition of material due to laser ablation would not introduce features at a relevant length scale for cells to sense. It is known that surface roughness above ~100 nm is typically considered the length scale for cells to be affected by nanotopographical cues while grooved-topographies in the micrometer range can influence cell adhesion and directionality. From atomic force microscopy (AFM), we could further evaluate the groove depth and surface roughness introduced on the PEDOT:PSS surface, shown in Figure 1C–E (for reference, AFM of unpatterned PEDOT is shown in Figure S1B). After patterning, the measured RMS surface roughness of the PEDOT:PSS film increased from ca. 10 nm to ca. 50 nm on the groove plateau.

In general, the laser etches away material from the surface of the PEDOT:PSS electrode to a depth of ca. 3−5 μm, which leaves a thin layer of PEDOT:PSS on the gold electrode surface in the groove trenches. After laser ablation, the films are rinsed and blown with compressed air to remove any residue from the electrode surface. After this, the films are washed three times with distilled water, one time with 70% ethanol (for sterilization) and three times with sterile distilled water under a laminar flow-hood. The surface functionalization was done...
with fibronectine-gelatin. A final wash with sterile distilled water was done before cells plating. Cells were labeled to evaluate their survival rate. After staining, fluorescence images were acquired scanning through the patterned and flat unpatterned areas. Low density HL-1 cultures (2 × 10^3 cells/mm²) showed similar behavior for grooves with 10 and 20 μm pitches (Figure 2A, B), where cells tend to elongate parallel to the main direction of the groove. In contrast, HL-1 showed a rounder morphology on 40 μm grooves (Figure 2C), similar to cells on unpatterned areas as shown in Figure S3. Cells were counted (ImageJ, NIH) and average percentage of live and dead cells were plotted as a function of the surface type (Figure 2G). On average, ca. 80–85% of cells survived on both planar and grooved PEDOT:PSS after 2 DIV. Cells were then prepared for SEM/FIB as previously described. A brief description of the ultrathin plasticization embedding can be found in the Supporting Information. As shown in Figure 2D–F and Figure S4, the ultrathin layer plasticization was successfully carried out for HL-1 cells grown on PEDOT:PSS grooved devices. From the scanning electron beam imaging, we could identify areas where cells adhered and spread over the grooves (Figure 2D–F). It is clear from the images that membrane protrusions such as filopodia/lamellipodia, which are known to be responsible for the cell outgrowth on nano- and microstructured materials, are extended to sense the substrate.

We performed EIS measurements to model and compare the cell adhesion for 3D-grooved and planar PEDOT:PSS devices when cells reached monolayer confluence. Previous reports have shown that cell adhesion can be extracted from frequency dependent impedance measurements on organic and inorganic electrodes. The ions that constitute the current in EIS flow either around the cell (paracellular) or through the cell (transcellular). The paracellular pathway consists of two constricted pathways for ion motion: the first is vertical motion through tight cell junctions (R_B) and the second is lateral motion in the cell cleft, which is the interface layer between the cells and device surface (R_Cleft). The transcellular pathway consists of two cellular membranes which behave as a capacitor (C_M) and a resistor (R_M) in parallel. Figure 3A depicts a simplified circuit model by combining R_B and R_Cleft into a single resistance (R_Para) for the paracellular pathway, and combining the two C_M values to into a single capacitance (C_Trans) for the transcellular pathway. R_M is neglected because it is in parallel with R_Para which is much smaller and dominates the resistive behavior of the cell layer (Supporting Information). As the cell adhesion to the device is enhanced, the separation between the cells and substrate is reduced, further restricting lateral ion motion in the cell cleft, thereby increasing R_Cleft and therefore R_Para. We investigated the contributions of each ionic pathway by varying frequency (f_{AC}); at high f_{AC}, the entire ionic current contributes to charging C_Trans whereas at low f_{AC}, C_Trans is charged within the oscillation time and the ionic current travels entirely through R_Para.

We obtained values for R_Para and C_Trans from the Bode representation of the EIS measurement for the planar, 10, 20, and 40 μm devices shown in Figure 3D, summarized in Table 1. By comparing the EIS spectra before and after plating cells, we find a clear increase in impedance between ca. 10 kHz and 1 Hz.
due to the plated cell layer. The cell layer impedance is higher for both the 10 and 20 μm pitches, which indicates a significantly higher $R_{\text{Cleft}}$ than that of the 40 μm pitch and planar geometries. This measurement conforms to 95% confidence bounds for the fitting for one sample. For the cleft measurements from SEM/FIB cross sections images, the analysis was performed by placing 10 equidistant measuring points then taking the average of all measured distances. The error reported is standard deviation of the mean.  

Table 1. Correspondence between EIS Measurements and SEM/FIB Cleft Measurements

<table>
<thead>
<tr>
<th>sample</th>
<th>$R_{\text{Para}}$ (Ω·cm²)</th>
<th>$C_{\text{Trans}}$ (μF·cm⁻²)</th>
<th>avg distance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μm</td>
<td>19.5 ± 0.6</td>
<td>14 ± 1.3</td>
<td>50 ± 20</td>
</tr>
<tr>
<td>20 μm</td>
<td>65.6 ± 0.9</td>
<td>7.4 ± 0.3</td>
<td>30 ± 15</td>
</tr>
<tr>
<td>40 μm</td>
<td>3.3 ± 0.18</td>
<td>9.8 ± 0.7</td>
<td>70 ± 20</td>
</tr>
<tr>
<td>planar</td>
<td>5.2 ± 0.7</td>
<td>10 ± 3.6</td>
<td>89 ± 79</td>
</tr>
</tbody>
</table>

$R_{\text{Para}}$ and $C_{\text{Trans}}$ values are fit from EIS data using the equivalent circuit model in Figure 3A, and the error corresponds to 95% confidence bounds for the fitting for one sample. For the cleft measurements from SEM/FIB cross sections images, the analysis was performed by placing 10 equidistant measuring points then taking the average of all measured distances. The error reported is standard deviation of the mean.  

Due to the plated cell layer. The cell layer impedance is higher for both the 10 and 20 μm pitches, which indicates a significantly higher $R_{\text{Cleft}}$ than that of the 40 μm pitch and planar geometries. This measurement confirms that there is an optimal 3D geometry of the organic surface leading to enhanced physical adhesion and electrical coupling of the cells. It is well-known that some cell layers can have high cleft resistances of up to 2000 Ω·cm². However, these values not only depend on coverage and geometry but are also heavily affected by the barrier properties of the tight-junctions.  

The relative trend presented in Table 1 should therefore be viewed as qualitative, confirming the hypothesis that cells tend to adhere significantly better to substrate microstructures with a similar dimension range as the cells’ size, resulting from the increased surface area and related anchoring points for the cells to adhere to. This means that cell-size dependent microstructuring can enhance cell-chip coupling in bioelectronics.

To verify the enhanced adhesion of the cell layer on 3D patterned devices, transversal sectioning with FIB etching was performed on the plated cells embedded in resin on top of the PEDOT:PSS grooved devices and then imaged with SEM to observe the cell substrate interface at the nanoscale. The ion beam etches through the cells and the material underneath leaving their relative positions unaltered. A low current etching process (Supporting Information) was necessary to limit redeposition of etched material and to avoid curtaining artifacts which can occur in correspondence of 3D cell-device features. The etching ion beam was placed perpendicularly to the substrate as a reference direction while the imaging was carried out with the scanning electron beam at a 52° angle. Cross-sections were created for cells growing on planar and grooved PEDOT:PSS (Figure 4A–D and Figures S5 and S6). The effective adhesion of cells on the PEDOT device was evaluated by measuring the vertical distance between the plasma membrane and the edge of the organic semiconductor as similarly presented in studies where the cleft was analyzed by TEM or SEM cross sections. The analysis was performed by placing 10 equidistant measuring points. We considered the average of all measured distances and the standard deviation of the mean. Micrographs show increased cellular membrane deformation and smaller cell substrate separation in the presence of 10 and 20 μm spaced grooves when compared to planar films. By measuring $N \approx 4$ samples for each pitch, we find that the average cleft values ranging from 70 to 89 nm for 40 μm pitched and planar samples is reduced to 50 and 30 nm for 10 and 20 μm pitched samples, respectively. The average distance between the plasma membrane and the organic semiconductor surface along with the EIS cell adhesion...
modeling parameters are summarized in Table 1. The data in the table confirms that 10−20 μm grooves are the optimal microstructural length scale in the organic semiconductor to improve the electrical coupling between the cell and the semiconductor, as characterized by the reduction in the measured cleft. Furthermore, this length-scale corresponds to the cell size (~25 μm average) which confirms the common assumption that cells tend to spread and adhere onto microstructures with similar dimensions of roughly their size.23−25

In this work, we have demonstrated a rapid femtosecond laser patterning method to significantly enhance cell substrate coupling, by creating 3D microgrooves in organic semiconductor materials. Generally, these inherently soft materials are difficult to pattern because of their sensitivity to traditional lithographic techniques, but the extremely short pulse length of a femtosecond laser allows for well-defined local ablation resulting in a range of controllable 3D microstructures and geometries. We additionally found that the interaction of the femtosecond laser with the organic substrates neither affects the electrical properties of the material (Figure S7) nor created surface modifications which could induce toxicity effects for cells as proven by our biocompatibility assay. From the EIS measurements we could model the interface resistance, and found that grooves with pitches in the range of 10−20 μm promoted cell adhesion and directionality compared with grooves with 40 μm pitch and planar surfaces. This enhanced cell adhesion was further validated by nanoscale characterization of the adhesion interface via a unique SEM/FIB imaging method. The unique combination of EIS and SEM/FIB allows for the characterization of the cell−chip interface provides a quantitative morphological characterization of the effective coupling of chip-based devices and cells. Finally, this method can be further adopted to investigate the effect of other geometrical parameters such as groove depth on cell adhesion or even characterize the cell−chip coupling in the presence of more complex patterned surfaces to optimize the cell−electrode interface.

■ ASSOCIATED CONTENT

 Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.7b12308.

Experimental details, morphological and electrical PEDOT:PSS characterization, cross-sectioning procedure, cleft measurement, cells on planar PEDOT:PSS, details of electrical equivalent circuit (PDF)

■ AUTHOR INFORMATION

Corresponding Authors
* E-mail: asalleo@stanford.edu.
* E-mail: bcui@stanford.edu.

ORCID
Yoeri van de Burgt: 0000-0003-3472-0148
Scott Tom Keene: 0000-0002-6635-670X
Bianxiao Cui: 0000-0002-8044-5629

Author Contributions

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Part of this work was performed at the Stanford Nano Shared Facilities (SNSF), supported by the National Science Foundation under award ECCS-1542152. The authors thank Juliet Jamtgaard and Richard Chin for the useful discussions. The authors also acknowledge the Heart Rhythm Society for F.S.’s research fellowship, the National Science Foundation for the grants NSF 105511, NSF 1344302, and the Neurofab at Stanford. S.T.K. was supported by the Stanford Graduate
Fellowship fund. A.S. gratefully acknowledges financial support from the National Science Foundation (Award DMR1507826).

**REFERENCES**