Manipulating location, polarity, and outgrowth length of neuron-like pheochromocytoma (PC-12) cells on patterned organic electrode arrays†

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In this manuscript, we describe a biocompatible organic electrode system, comprising poly(3,4-ethylenedioxythiophene) (PEDOT) microelectrode arrays on indium tin oxide (ITO) glass, that can be used to regulate the neuron type, location, polarity, and outgrown length of neuron-like cells (PC-12). We fabricated a PEDOT microelectrode array with four different sizes (flat; 20, 50, and 100 μm) through electrochemical polymerization. Extracellular matrix proteins absorbed well on these organic electrodes; cells absorbed selectively on the organic electrodes when we used polyethylene oxide/polypropylene oxide/polyethylene oxide triblock copolymers (PEO/PPO/PEO; Pluronic™ F108) as the anti-adhesive coating. In this system, the neurite polarities and neuron types could be manipulated by varying the width of the PEDOT microelectrode arrays. On the unpatterned PEDOT electrode, PC-12 cells were randomly polarized, with approximately 80% having multi-polar cell types. In contrast, when we cultured PC-12 cells on the 20 μm wide PEDOT line array, the neurites aligned along the direction of the organic electrodes, with the percentage of uni- and bipolar PC-12 cells increasing to greater than 90%. The outgrowth of neurites on the microelectrodes was promoted by ~60% with an applied electrical stimulation. Therefore, these electroactive PEDOT microelectrode arrays have potential for use in tissue engineering related to the development and regeneration of mammalian nervous systems.

Introduction

Manipulation of cells through variations of their microenvironments (e.g., using various advanced techniques to physically or chemically modify the surfaces of substrates for cell culturing) is an emerging field of research.1–5 Especially in the field of cell therapies, the mechanical or biochemical properties of microenvironments have been manipulated to regulate the neural stem cell behavior, which could further help to rebuild damaged tissue.6,7 In recent developments in neural tissue engineering, for example, both physical and chemical cues have been used to promote neuron adhesion and neurite outgrowth.8–16 Surface topography provides a molecular pathway for controlling the response of the neurite differentiation using artificially designed extracellular patterns.8–10 Cells bind to the molecules in the extracellular matrix (ECM) on the substrate surfaces through transmembrane protein integrins, providing anchorage points for the cells to sense their microenvironments. Therefore, it is a common practice to regulate the behavior of cells through the patterning of ECM molecules. When designing cell-based devices, ECM patterns are attractive coatings because they promote the adhesion of cells onto specific areas of the devices. Nevertheless, this approach also requires repulsive (e.g., anti-adhesive) coatings to ensure precise control over the locations of the cells on the devices. Various microfabrication processes such as photolithography, microfluidic patterning platforms, and μ-contact printing allow micropatterns of biocompatible polymers [e.g., poly(ε-lysine) (PLL)] or ECM proteins to be used as attraction coatings for cell proliferation and differentiation studies.11–16 In the development of contact repulsion coatings, polyethylene oxide/polypropylene oxide/polyethylene oxide triblock copolymers (PEO/PPO/PEO; e.g., Pluronic™ F108) have been introduced as anti-adhesive coatings in which the PPO domains bind strongly to hydrophobic surfaces via hydrophobic–hydrophobic interactions. Consequently, the hydrophilic PEO brushes are free to repel ECM proteins from the cell culture medium. This cellular repulsion force can be used to restrict cell adhesion and migration to specific areas of devices.17–20 In addition to controlling the spatial location of cells on chips, an important aspect of biosensing or neuroprosthetics is the ability to address or stimulate cells electrically. To perform cell manipulation and electrical stimulation, integrated devices based on conducting polymers are promising candidates for guiding the growth of cells and providing electromagnetic stimulation at the same time.21–35 In addition, because the architecture of tissues is

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neither planar nor static, the softness and flexibility of conducting polymers are suitable for implant applications, ranging from the electrode to the bioelectronics.29

Recent advances in the development of electrically conducting polymers have overcome several interfacial problems in the field of organic bioelectronics, including the preparation of tissue-electrode interfaces for long-term electroactivity operations for nerve regeneration,21–23 appropriate surface functionalization for biorecognition,20–23 and the preparation of polymer–electrode interfaces of high adaptability for multifunctional neuron probes.24–27 Biocompatible electrodes [e.g., polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT)] have been proposed as biointerfaces to enhance cell–electrode communication, especially in nervous systems for their combined stimulation and recording functionalities. Conducting polymers are readily tailored for specific applications, such as controlled drug release systems by the incorporation of drugs into the polymeric matrices,24,25 through chemical oxidative or electrochemical polymerization, by surface modification or doping with ionic compounds ranging from small salt ions to macromolecules [e.g., polystyrene sulfonate (PSS), polylactic acid (PLA), proteins, and peptides].24–35 Several promising results have been demonstrated recently using conducting polymer-based materials for on-chip cell manipulation. For example, PEDOT:PSS blends with promising in vivo biocompatibility have been developed to overcome the problems of cell–electrode communication at the device level.21–23,27 Conducting polymer-coated polymer nanofibers have also been used to investigate the electrical stimulation of neurite extension on the nanofibers; here, aligned nanofibers promoted neurite extension to a greater extent than did random nanofibers.34,35 Nevertheless, how to effectively manipulate the spreading morphology of individual cells has not yet been fully investigated.

In this study, we developed a PEDOT:PSS microelectrode array with different sizes (flat; 100, 50, and 20 μm) to systematically investigate the neuron-like cell spreading morphologies of PC-12 pheochromocytoma cells. Combining standard photolithography and electropolymerization, we fabricated electroactive PEDOT:PSS microelectrode arrays on indium tin oxide (ITO) glass and used them for the studies of electrical stimulation. We used Pluronic F108 as the anti-adhesive coating to control the location of the PC-12 cells. In addition, we investigated the polarities, morphologies, and neurite lengths of PC-12 cells on the differently sized PEDOT electrodes, as well as the effects of electrical stimulation.

Methods and experimental setup

Fabrication of micropatterned organic electrode systems

Commercially available ITO-coated glass (<10 Ω sq−1, RiTdisplay Corporation) was used as the substrate. A standard photolithography process, with positive resist (S-1813, Shipley) and development (MF-319, Shipley), was used to fabricate S-1813 micro-array patterns of four different sizes (flat; 100, 50, and 20 μm) on the ITO glass. Thin layers of PEDOT were then deposited electrochemically—from a bath solution containing 0.01 M 3,4-ethylene dioxythiophene (EDOT) (Sigma-Aldrich), 0.02 M sodium salt of PSS (Mw 70 000; Sigma-Aldrich), and 5 mM potassium phosphate-buffered saline (PBS)—using the three-electrode system of an Autolab PGSTAT-12 (EcoChemie, Utrecht, Netherlands) electrochemical analyzer. A constant potential of 1 V (vs. Ag/AgCl) was used to produce PEDOT layers. The thickness of the electrodeposited PEDOT:PSS films depended on the charge density passed during polymerization. When 20 mC cm−2 was used, the film thickness was measured to be ca. 150 nm (Fig. S1, ESI†). The S-1813 photoresist was removed by washing with acetone, leaving the surfaces of the ITO glasses covered with PEDOT microelectrode arrays. The solution of the anti-adhesive material Pluronic™ F108 (Sigma-Aldrich, 2.5 mg mL−1) in water was prepared, then dropped on the device surface to treat the remaining open areas on the ITO glasses for 30 min. Finally, the unbound Pluronic F108 was washed away with deionized water three times (Fig. S2, ESI†). The Pluronic F108-modified PEDOT:PSS micro-line devices are denoted herein as “PEDOT-X,” where X refers to the width of the PEDOT:PSS electrodes and the separation distance between the electrodes (in micrometres). Unpatterned PEDOT electrodes are denoted as “PEDOT-Flat.” The schematic and optical images of our device are shown in Fig. S3(a) and (b)†, respectively.

Cell culture and biocompatibility test

The rat adrenal pheochromocytoma cell line, PC-12, was obtained from the American type culture collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco’s modified Eagle’s medium containing 10% horse serum and 5% fetal bovine serum. PC-12 cells were incubated in an atmosphere containing 5% CO2 at 37 °C for 48 h to allow cell attachment; the original medium was then exchanged with the serum-free medium supplemented with 50 ng mL−1 NGF (Cat # N-240, Alamone Laboratories), and then the cells were incubated for an additional 48 h for neurite differentiation. PC-12 cells were seeded on the devices (Pluronic F108-modified PEDOT/ITO glass with PDMS reservoir) at two different seeding densities (ca. 6000 cells per cm2 for biocompatibility tests; ca. 3000 cells per cm2 for statistical quantification). Each condition was repeated at least four times. For the electrical stimulation (ES) experiments, an electric field of 120 mV mm−1 was applied through two external ITO electrodes located outside the PDMS reservoirs of the PEDOT-Flat-ES and PEDOT-20-ES devices after supplying the system with NGF in serum-free medium, and the direction of the electrical field was parallel to the PEDOT microelectrodes (Fig. S3(a)†). The biocompatibility of the PEDOT:PSS electrode systems was evaluated using two different methods: a trypan blue assay, which was performed prior to neurite differentiation, and a live/dead assay (Molecular Probes Invitrogen), which was conducted after neurite differentiation.

Microscopy and data analysis

After a total of 96 h of incubation, the differentiated PC-12 cells were directly monitored through a confocal microscope (Leica TCS SPS) using differential interference contrast (DIC) or fluorescence imaging. To confirm the adsorption of Pluronic F108 on the ITO surfaces, X-ray photoelectron spectra were recorded using a PHI 5000 VersaProbe spectrometer (ULVAC-PHI, Chigasaki, Japan) operated in the constant analyzer energy mode.
with Mg-Kz (1253.6 eV) radiation as the excitation source. Furthermore, fibronectin (Fn) labeled with tetramethylrhodamine-5-maleimide (TMR) was employed to mimic the adsorption of ECM proteins on the organic PEDOT electrodes. Polar charts of neurite lengths and orientations (r, θ) on the different surfaces (Control, PEDOT-Flat, PEDOT-100, PEDOT-50, and PEDOT-20) were used for statistical analysis of PC-12 differentiation. The neurite length measurement for each neurite was the straight-line distance between the end of the neurite and cell body (Fig. S3(c)). The control study was conducted using tissue culture polystyrene (TCPs) dishes. The PC-12 cells were labeled with the FAK100 staining kit (Millipore, USA) to identify the location of adhesion sites “focal adhesion”.

Results and discussion

Organic PEDOT materials are promising biocompatible electrodes because they provide strong interfacial interactions between the electronic and biological components. 34–36 For example, the conducting polymer PEDOT:PSS can be used as a material for neural communication, with enhanced electrical recording and stimulating properties. Using conducting polymers as electrodes for electrical stimulation allows the regulation of the orientation and spreading morphologies during neurite differentiation processes. 34–36 To further understand how to precisely control the growth of neurites, in terms of neuronal location, polarity, and outgrowth length, on electrodes made of conducting polymers, we fabricated four PEDOT:PSS micro-electrode arrays with various line widths (PEDOT-Flat, PEDOT-100, PEDOT-50, and PEDOT-20). We also investigated the behavior of PC-12 cells on the PEDOT electrodes under electrical stimulation.

Normally, stimulation of PC-12 cells with NGF induces one or more neurites with random-polarity multipolar neuron types on TCPs dishes as shown in Fig. S2(a). Fig. S2(b) depicts the three possible PC-12 cell morphologies: uni-, bi-, and multipolar. To control the growth of PC-12 cells on the PEDOT electrodes, we fabricated PEDOT microelectrodes using a combination of standard photolithography, PEDOT electropolymerization, and sequential coating of anti-adhesive Pluronic F108 (Fig. S2(c)–(g)). The PC-12 cells adhered preferentially to the PEDOT:PSS electrodes and the outgrowth of neurites was restricted to the regions of the PEDOT:PSS electrodes (Fig. S2(h)); these PC-12 cells exhibited multipolar-rich cell types, similar to those on the TCPs dishes. In the absence of the anti-adhesive Pluronic F108 coating, the PEDOT patterns were attractive for cell attachment, with the extending neurites readily striding over two patterns (Fig. 1(a)). We measured the thickness of the PEDOT:PSS microelectrodes by AFM (Fig. S1). The thickness of PEDOT:PSS was ca. 150 nm, which was not thick enough to have any confinement effect. Therefore, we concluded the chemical repulsion coating of Pluronic F108 on the ITO surfaces was the active ingredient to confine PC-12 cells to the PEDOT electrode; presumably, its hydrophobic PPO domains bound to the ITO surface, leaving the hydrophilic PEO chains free in solution to repel proteins and other adsorbents from the surface through steric repulsion. 37–39 To verify the presence of Pluronic F108 on the surfaces, we used X-ray photoelectron spectroscopy (XPS) to monitor the surface element composition. XPS O 1s spectra indicated that Pluronic F108 preferred to adsorb on the ITO surface (Fig. 1(b)). Furthermore, we used Fn labeled with TMR dye to mimic the adsorption of ECM proteins on these organic electrodes. Fig. 1(c) displays a fluorescence image of Fn-TMR on our device. The image reveals that the ECM molecules, which promote cell adsorption on surfaces, had adsorbed preferentially on the organic electrodes. The cell viability on the organic electrodes is another important issue influencing the development of organic electrodes for biological applications. We tested the viability of undifferentiated PC-12 cells on the TCPS, ITO, and PEDOT:PSS surfaces through trypan blue exclusion, 48 h after seeding. Fig. 1(d) indicates that more than 80% of the PC-12 cells remained viable 48 h after incubation on all of the tested devices.

In the presence of the contact attractive and repulsive coatings, the attachment of PC-12 cells was effectively restricted to the PEDOT microelectrodes of different sizes (PEDOT-100, PEDOT-50, and PEDOT-20) at both high and low seeding densities (Fig. 2). The ECM proteins (e.g., Fn-TMR) adsorbed preferentially on the PEDOT:PSS surfaces (Fig. 1(c)). We expected nerve growth factor (NGF), an important protein for neurite outgrowth, to adsorb preferably on the PEDOT surfaces and, therefore, further induce neurite extension along the lengths of the PEDOT patterns. 34–37 Because the widths of the PEDOT-100 and PEDOT-50 electrodes were much larger than the sizes of undifferentiated PC-12 cells (ca. 20 μm), no cell spreading occurred over two electrodes after we had added NGF to these devices at high seeding density. In contrast, the width and separation between electrodes of the PEDOT-20 electrodes were
similar to the dimensions of the undifferentiated PC-12 cells, suggesting that it would be more likely for PC-12 cells to spread over the electrodes. Indeed, it was difficult to control the spreading morphology of the PC-12 cells on the PEDOT-20 electrodes. Optimal control of cell spreading on microelectrodes has been achieved by using wider spacing. Shown in Fig. S4(b) and S5† are PC-12 cells on the 20 μm wide microelectrodes and networks with 100 μm spacing. It can be seen that the PC-12 cells are well confined on the microelectrodes.

To further characterize the neurite outgrowth and spreading morphologies of the differentiated PC-12 cells on the PEDOT:PSS electrodes, we also conducted the NGF stimulation experiments on different PEDOT micro electrode arrays (PEDOT-Flat, PEDOT-100, PEDOT-50, and PEDOT-20) at low seeding density (Fig. 2(d)–(f)). The cell viability after NGF stimulation on the PEDOT electrodes was evaluated by a live/dead assay. In Fig. 3(a)–(d), with live cells labeled green and dead cells labeled red, it is clear that the majority of PC-12 cells remained alive after NGF stimulation on all of our tested PEDOT:PSS microelectrodes. Because of the anti-adhesive Pluronic F108 coating, the outgrowth of the neurites was confined to the electrodes. Interestingly, the PEDOT:PSS electrodes of narrower width appeared to promote alignment of the cells along the direction of the electrodes and the distribution of the cell spreading morphology changed from multipolar to bipolar or unipolar. Fig. 3(e)–(h) provide graphic representations of the morphologies of the PC-12 cells on the microelectrodes with various widths. To understand the molecular nature of the morphological difference of PC-12 cells on the PEDOT-Flat and PEDOT-20 devices in detail, we have used immunostaining to evaluate the location of integrin, focal adhesion and F-actin. It is assumed that our devices first promoted the adsorption of ECM molecules on the

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**Fig. 2** DIC images of PC-12 cells on the various PEDOT:PSS electrodes with Pluronic F108 patterns. Cell seeding densities: (a–c) 6000 cells per cm²; (d–f) 3000 cells per cm². PEDOT:PSS electrode arrays: (a and d) PEDOT-100, (b and e) PEDOT-50, (c and f) PEDOT-20. Scale bars 100 μm.

**Fig. 3** (a–d) Fluorescence images of differentiated PC-12 cells grown on (a) PEDOT-Flat, (b) PEDOT-100, (c) PEDOT-50, and (d) PEDOT-20 microelectrode arrays, labeled with a live/dead assay (live cells: green; dead cells: red). Scale bars 100 μm. (e–h) Schematic representations of the PC-12 cell spreading morphologies on the various electrodes.
PEDOT-100 substrates. In contrast, on the PEDOT-patterned substrates, we observed distinct orientations of all of the neurites on the various devices; Table 1 summarizes the morphologies and the median neurite lengths on these surfaces. Comparing the distributions of neurite lengths on the differently sized PEDOT microelectrodes (PEDOT-100, PEDOT-50, and PEDOT-20) with that of the unpatterned PEDOT surface (PEDOT-Flat), we find that the distribution of neurite lengths shifted to shorter lengths, with the percentage of neurites longer than 100 μm decreasing from 49.1% to 22.6, 33.9, and 46.1%, respectively. The width of the PEDOT electrode influenced not only the orientations and lengths of the neurites but also their morphologies. Table 1 reveals that 80% of the PC-12 cells on the PEDOT-20 devices possessed multipolar morphologies, with median neurite lengths of 91 and 98 μm, respectively. As the width of the electrodes decreased from 100 to 20 μm, the percentage of multipolar morphologies decreased from 52% to only 4%, whereas the percentage of bipolar morphologies increased from 22 to 72%. In other words, controlling the width of PEDOT electrodes allows regulation of the lengths, orientations, and morphologies of PC-12 cells.

One of the most important features of our devices is that the growth of neurites could be manipulated through electrical stimulation (ES). An electrical field of 120 mV mm⁻¹ was applied to the PEDOT-Flat and PEDOT-20 devices during the PC-12 cell differentiation experiment. The order parameters measured on PEDOT-Flat-ES and PEDOT-20-ES (Fig. 5(f)) were similar to those measured on the same devices without electrical stimulation, but the percentage of neurites longer than 100 μm on these devices increased from 49.1% to 68.0% (PEDOT-Flat-ES) and 73.9% (PEDOT-20-ES), respectively (Fig. S6(f) and (g) in the ESI†), and the median neurite length increased from 98 μm to 119 and 143 μm, respectively (Table 1). The effects of electrical stimulation for promoting neurite outgrowth through the PEDOT-Flat-ES and PEDOT-20-ES electrode systems could be attributed to two possible factors: One was the redistribution of ion homeostasis or polarized receptors at the cell surface and the other was by increasing the degree of protein (e.g., Fn and NGF) adsorption on the PEDOT surfaces. The results of electrical stimulation on our devices were similar to those grown on the aligned nanofibers, where the electrical stimulation only promotes neurite extension without changing the alignment along the lengths of the PEDOT microelectrode arrays, we used two-dimensional (2D) polar order parameters to represent the asymmetry behavior of neurites related to the PEDOT patterns (Fig. 5(f)). We obtained individual polar order parameters in each device using the first Legendre polynomials:

\[
S = 2(\cos^2 \theta) - 1
\]

The value of \(S\) was 1 for neurite outgrowth with perfect alignment along the length of the patterns, close to 0 for random orientations, and \(-1\) for antipolar spreading. Fig. 5(f) reveals that the order parameters for the PC-12 cells on the PEDOT-50 samples were very close to zero, indicating that their orientations on these samples were random. In contrast, the order parameters of the PC-12 cells on the patterned electrode surfaces increased from 0.22 on PEDOT-100 to 0.97 (very close to perfect alignment) on PEDOT-20, that is, upon decreasing the pattern width from 100 to 20 μm. Fig. S6† presents histograms of the lengths of the neurites on the various devices; Table 1 summarizes the morphologies and the median neurite lengths on these surfaces. Comparing the distributions of neurite lengths on the PEDOT electrode influenced not only the orientations and lengths of the neurites but also their morphologies. Table 1 reveals that 80% of the PC-12 cells on the PEDOT-20 devices possessed multipolar morphologies, with median neurite lengths of 91 and 98 μm, respectively. As the width of the electrodes decreased from 100 to 20 μm, the percentage of multipolar morphologies decreased from 52% to only 4%, whereas the percentage of bipolar morphologies increased from 22 to 72%. In other words, controlling the width of PEDOT electrodes allows regulation of the lengths, orientations, and morphologies of PC-12 cells.
In our devices, the order parameters of PEDOT-Flat-ES and PEDOT-20-ES are \((/C0 \approx 0.07, /C6 \approx 0.36, 0.97)\) in the presence and \((/C0 \approx 0.08, /C6 \approx 0.36, 0.97)\) in the absence of electrical stimulation, respectively, which did not change with the electrical stimulation. Therefore, we postulate that the enhanced neurite outgrowth was promoted by increased protein adsorption during electrical stimulation. To prove the mechanism of the electrical stimulation for promoting the outgrowth of neurites, we used Fn-TMR to mimic the degree of protein adsorbed on PEDOT surfaces. Comparing the fluorescence intensity of protein adsorbed on the PEDOT-Flat and PEDOT-Flat-ES devices, it was found that the fluorescence intensity increased about 2.7 times after electrical stimulation indicating that the electrical stimulation would increase the amount of protein adsorbed on PEDOT surfaces (Fig. S7 in the ESI†). We therefore conclude that the electrical stimulation would promote the adsorption of protein (e.g., Fn and NGF) while the asymmetry behavior of neurites was mainly regulated through the width of PEDOT patterns in our devices.

**Conclusions**

We have developed a biocompatible PEDOT electrode platform that allows precise manipulation of the location, polarity, and morphology of PC-12 cells. When we employed Pluronic F108 as an anti-adhesive coating, the deposition of PC-12 cells was restricted to the regions presenting the PEDOT microarray arrays. This platform is easy to fabricate and allows fundamental studies of the behavior of the morphologies of individual cells (e.g., neuron type, neurite outgrowth orientation, and neurite

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**Table 1** Distributions of PC-12 cell spreading morphologies on the differently sized PEDOT patterns

<table>
<thead>
<tr>
<th></th>
<th>Unipolar (% of cells)</th>
<th>Bipolar (% of cells)</th>
<th>Multipolar (% of cells)</th>
<th>Average number of neurites per cell</th>
<th>Median neurite length/(\mu m)</th>
<th>Polar order parameter (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>18</td>
<td>78</td>
<td>3.7</td>
<td>91</td>
<td>(-0.05 \pm 0.36)</td>
</tr>
<tr>
<td>PEDOT-Flat</td>
<td>6</td>
<td>14</td>
<td>80</td>
<td>3.2</td>
<td>98</td>
<td>(-0.08 \pm 0.36)</td>
</tr>
<tr>
<td>PEDOT-100</td>
<td>26</td>
<td>22</td>
<td>52</td>
<td>2.7</td>
<td>60</td>
<td>(0.22 \pm 0.36)</td>
</tr>
<tr>
<td>PEDOT-50</td>
<td>8</td>
<td>54</td>
<td>38</td>
<td>2.4</td>
<td>66</td>
<td>(0.68 \pm 0.24)</td>
</tr>
<tr>
<td>PEDOT-20</td>
<td>24</td>
<td>72</td>
<td>4</td>
<td>1.8</td>
<td>88</td>
<td>(0.97 \pm 0.07)</td>
</tr>
<tr>
<td>PEDOT-Flat-ES</td>
<td>6</td>
<td>12</td>
<td>82</td>
<td>3.5</td>
<td>119</td>
<td>(-0.07 \pm 0.36)</td>
</tr>
<tr>
<td>PEDOT-20-ES</td>
<td>20</td>
<td>74</td>
<td>6</td>
<td>1.8</td>
<td>143</td>
<td>(0.97 \pm 0.06)</td>
</tr>
</tbody>
</table>

**Fig. 5** Polar plots of the orientations and lengths of the neurites of the differentiated PC-12 cells on the (a) Control, (b) PEDOT-Flat, (c) PEDOT-100, (d) PEDOT-50, and (e) PEDOT-20 devices. (f) Two-dimensional order parameters of the cell spreading morphologies, related to the different sizes of the PEDOT patterns.
length) positioned on organic electrodes. On the unpatterned PEDOT-Flat surface, the neurites were randomly polarized, with approximately 80% of them having multipolar cell types; the median length was 98 μm. In contrast, the neurites on the PEDOT-20 surface were aligned along the length of the electrode, with the total distribution of uni- and bipolar PC-12 cells increasing to greater than 90%; the median length was 88 μm. The orientation of the neurites was controlled by the width of the PEDOT electrodes; indeed, the order parameters increased from approximately 0 (random) on the unpatterned PEDOT electrodes to almost 1 (perfect alignment) on the 20 μm wide PEDOT electrodes. Furthermore, electrical stimulation across the PEDOT-20 electrode system promoted neurite outgrowth by ~60%. Therefore, controlling the widths of the contact attractive (PEDOT) and repulsive (Pluronic F108) coatings allowed us to regulate the orientations and morphologies of the neuron-like PC-12 cells. In addition, electrical stimulation allowed further manipulation of the lengths of the neurites. The PEDOT microelectrode arrays reported here could potentially be used for tissue engineering related to the development and regeneration of mammalian nervous systems. Interestingly, this platform could also be used to study the synapse junctions of neuron–neuron contacts or to develop biocompatible lab-on-a-chip systems.

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