Laser Patterning of Self-Assembled Monolayers on PEDOT:PSS Films for Controlled Cell Adhesion

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Conducting polymers have shown great potential as a means to interface electronics with living tissues, toward a plethora of different biological applications ranging from in vitro to in vivo systems. However, the development of effective functionalization approaches to render this interface biomimetic still remains rather challenging, due to the lack of inherent surface functionalities in such polymers. Here, a straightforward and versatile modification strategy of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) surfaces is demonstrated for preferential and spatially confined cell adhesion and growth. By combining three simple surface modification steps, including chemical modification using self-assembled monolayers and their selective laser ablation, this study is able to design either cell-adhesive or cell-repulsive patterns of various shapes on PEDOT:PSS films. Studies using Madin–Darby canine kidney II epithelial cells reveal preferential cell adhesion and growth with good precision following the preformed patterns. The proposed surface modification approach can be extended to encompass a variety of polymeric biomaterials, without affecting their bulk properties.

A key feature in the rational design of biomaterial surfaces used to interface with cells is to mimic as much as possible the naturally occurring nanotopographical and physicochemical cues of the extracellular matrix (ECM) in order to favor and control the cell–biomaterial as well as the cell–cell interactions. Moreover, cell organization over predetermined patterns, where the pattern geometry, the number of cells, and the distance between the cells can be controlled, represents a powerful tool for biological studies to recapitulate efficient communication between the cells and mimic more efficiently in vivo processes. Among the various materials used as effective substrates for cell attachment and biological assays, conducting polymers (CPs) have received considerable attention in the past decades as promising candidates. This is due to their unique set of properties, including the ability to conduct both ionic and electronic charges, the good, and easy to tailor mechanical properties, the ease of processability and, last but not least, the excellent biocompatibility, for interfacing with both biomolecules and cells.

In the particular case of electrogenic cells (i.e., neurons and muscle cells) conducting substrates can directly regulate or induce several biological functions with the application of an electrical stimulus. Svennersten et al., and later Wan et al., demonstrated electrically controlled protein conformation in poly(3,4-ethylenedioxythiophene) (PEDOT)-based conducting polymers toward the control of the cellular functions of non-electroactive cells depending on the oxidation state of the polymer. Moreover, PEDOT-based electronic devices (i.e., electrodes and transistors) have been successfully used for cell-based assays to monitor barrier tissue properties, the cell response to specific biomolecules, as well as for electroactive cell recordings, highlighting the potential of CPs to serve both as effective cell substrates and as biological signal transducers. However, a major challenge in the latter assays lies in recording within the whole range of the electrophysiological repertoire, which not only requires good and spatially confined cell–cell communication but also sub-micrometer featured electrodes to improve the electrical coupling with the individual cells. It is well known that improving cell adhesion and reducing cleft resistance can enhance electrophysiological recordings of electrogenic cells.

Various surface modification methods have been used to date for microscale patterning of cell cultures in several substrates, including photolithography, microcontact printing, microfluidics, and laser-based techniques. Apart from controlling initial cell adhesion, patterning methods can be also useful for controlling key cell behaviors including proliferation, differentiation, and growth. However, surface modification methods become rather challenging in such cases since they need to be nondestructive or minimally destructive in order not to affect drastically the materials properties, in this case, mainly the conductivity (i.e., ionic and electronic).

Laser techniques have been widely used for the modification and the micro/nanostructuring of materials or surfaces for biomedical applications. Among a wide variety of laser-processing methods, laser ablation is a very efficient approach for material structuration due to the inherent noncontact capabilities and the high spatial resolution it offers. Specifically,
ultrafast laser ablation offers excellent compatibility with molecular and soft materials, (i.e., polymers, membranes, and tissues) as it minimizes the heat diffusion, resulting in high-quality and high-precision structures. To date, there are several reports investigating cell behavior (i.e., migration, adhesion, and differentiation) on laser-modified surfaces.[29,30] A commonly used strategy for facilitating preferential adhesion and growth to the cells is the formation of topographical micro-/nanostructures (i.e., craters, nanoripples, channels, and ribbon-like structures).[31] The same can also be achieved by altering the surface energy and the chemistry of the biological substrates. A representative example is the laser patterning of self-assembled monolayers (SAMs) such as thiol- or silane-based monolayers,[32–35] which can be effectively patterned to design a chemical template capable of repelling or attracting cell layers.[36] Indeed, current CP films lack amenable functional groups at their surface, ruling out direct covalent linkage with biomolecules in the pristine state. To overcome this issue, several strategies have emerged in the last years aiming to render CPs and, in particular, PEDOT:polystyrene sulfonate (PEDOT:PSS) film surfaces functional for use in biointegrated electronics.[37] Despite the extensive research on the electrodeposition of CPs on prepatterned substrates,[38–41] there are only a few examples in the literature focusing on the direct patterning on conducting polymers[42] for the spatial confinement and subsequent control of cells activity.[43–45]

In the present work, we introduce a simple two-step functionalization method of PEDOT:PSS films followed by selective laser ablation in order to induce preferential cell attachment and growth on the predefined patterns. Given the strict requirements for conducting materials to maintain performance after surface modification, we investigate the effect of each modification step on the properties of the active material. Additionally, we study and compare two distinct approaches for selective cell patterning, one using a cell-attracting layer on top of the film and one using a cell-repelling layer and their selective removal to create cell-attracting or cell-repelling domains, respectively. Finally, a study on the effect of the laser parameters on the patterned film is performed as well. Overall, the proposed platform represents a versatile and easy-to-use surface modification approach for selective cell patterning on a broad variety of polymeric materials.

The use of SAMs has proven to be a very robust and versatile approach in the field of organic electronics as a means to fine tune or even induce certain functionalities in the active material surface.[46] Figure 1a shows a schematic representation of the functionalization steps on the PEDOT:PSS film using two different types of SAMs: for the cell-repelling surface coating a hydrophobic FDTS was used, while for the cell-attracting coating an aminosilane (APTES) was used. b) Effect of the plasma power on the conductivity and thickness of the PEDOT:PSS films. The plasma time was 1 min. c) Effect of the different SAM functionalization on the conductivity and the swelling capacity of PEDOT:PSS. In part (b) error bars represent the standard deviation from the mean of five samples. In part (c), error bars represent the standard deviation from the mean of three samples and $p > 0.05$. 

Figure 1. PEDOT:PSS surface modification steps toward the formation of cell-attracting and cell-repelling areas. a) A schematic of the functionalization steps on the PEDOT:PSS film using two different types of SAMs: for the cell-repelling surface coating a hydrophobic FDTS was used, while for the cell-attracting coating an aminosilane (APTES) was used. b) Effect of the plasma power on the conductivity and thickness of the PEDOT:PSS films. The plasma time was 1 min. c) Effect of the different SAM functionalization on the conductivity and the swelling capacity of PEDOT:PSS. In part (b) error bars represent the standard deviation from the mean of five samples. In part (c), error bars represent the standard deviation from the mean of three samples and $p > 0.05$. 

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of the biofunctionalization approach that was followed in the present work. The CP films were prepared by spin coating the PEDOT:PSS formulation for film thicknesses ranging between 100 and 400 nm, followed by thermal annealing. Two types of SAMs were introduced on the PEDOT:PSS surface by using silane precursors with different terminating groups, namely (3-aminopropyl)triethoxysilane (APTES) and perfluorodecyl-trichlorosilane (FDTS). The former silane precursor results in a highly hydrophilic −NH₂ terminated film that favors biological interactions (i.e., cell attachment),[47,48] while the latter results in a bio-repelling surface due to its highly hydrophobic nature.[49] For the SAM formation, the CP film was modified by introducing hydroxyl groups at the surface via an O₂ plasma treatment. A thorough study on the effect of the O₂ plasma treatment power was conducted with respect to the conductivity as well as the thickness of the film to assess its integrity after modification. As can be seen in Figure 1b, the conductivity decreases only slightly up to a power of 10 W and drops substantially at higher power values for a treatment time of t = 1 min. The thickness was found to remain nearly unchanged until 60 W, indicating that no etching occurs. An increase in the plasma treatment time (t = 3 min) was found to have more pronounced effects on the conductivity and the thickness of the films (Figure S1, Supporting Information). One possible explanation for the changes in the CP conductivity and, in particular, its decrease with increased plasma power and time, could be a plasma-induced breaking of the electrostatic bonding between PEDOT and PSS, as well as the modification of the conjugated chain that can lead to a decrease in the surface carrier mobility and an increase in the hopping distance. Such effects were observed in a recent study, using N₂ plasma treatment on PEDOT:PSS films.[50] The water contact angle (C.A.) of the plasma-modified films was found to be below 5° for all the plasma treatment conditions revealing their adequate induced hydrophilicity arising from the −OH groups at the polymer surface. We thus chose to use for the remainder of the study the mildest plasma power (P = 10 W). This induced hydrophilicity seemed to recover only after a period of 6 days (Figure S2a, Supporting Information) reaching the initial PEDOT:PSS film contact angle of ≈45°. Following the silane (APTES and FDTS) deposition, the contact angle of the resulting films was found to be ≈65° and ≈110°, respectively (Figure S2b, Supporting Information), values that are consistent with the literature, confirming the efficient surface coating.[51,52]

In order to investigate the effect of the silane coatings on the CP surface, the conductivity as well as the swelling of PEDOT:PSS was measured and compared to unmodified samples, as shown in Figure 1c. The conductivity decreased after the silane coatings; however, the values remain high enough for studies that rely on high film conductivity. The observed decrease in the film’s conductivity could be partially attributed to the plasma treatment conditions as already shown and also to the silane entering the film during the coating, possibly affecting as well to some extent the charge carrier transport. Moreover, the swelling of the material was slightly affected due to the increase in the ionic resistance as a result of the additional layers, thus impeding ion penetration to some extent, especially in the case of FDTS treatment (~30% loss of swelling). Overall the CP seems to maintain its properties after surface modification (p > 0.05).

In this work, a femtosecond laser (500 fs) with a wavelength of 343 nm was used for the effective patterning of SAM-modified PEDOT:PSS films, as shown in the schematic of Figure 2a. The experimental configuration resulted in a beam diameter (defined at 1/e² of the maximum intensity) of 30 μm at 343 nm, capable of creating high-resolution patterns at the microscale. An example of the beam profile taken with a CCD sensor in the sample plane is given in the inset of Figure 2b. For a laser beam having a Gaussian beam profile, the ablation spot diameter (d) depends on the laser fluence according to the following equation[55]

\[ d^2 = 2w_0^2 \ln \left( \frac{F}{F_{th}} \right) \]  

where w₀ is the 1/e² Gaussian beam radius, F is the laser fluence, and F_{th} is the ablation threshold fluence. The laser fluence F is related to the pulse energy E_p and is given by

\[ F = \frac{2E_p}{\pi w_0^2} \]  

In order to calculate the F_{th} value and further adjust the effect of the laser fluence on the PEDOT:PSS films, single-shot and multi-shot ablation experiments were conducted as shown in Figure 2b. Taking into account the spot diameter values, the energy can be converted to fluence using Equation (2) while the F_{th} is determined from the d versus F plot by extrapolating the curve to the zero spot diameter. Accordingly, a decrease of the ablation threshold values with increasing the number of pulses from 1 to 10 can be observed. More specifically, a threshold of about 0.34 J cm⁻² was estimated for the single-pulse experiments, while in the case of 10 and 100 pulses, the threshold was approximately the same, with a value of 0.21 J cm⁻². These values are consistent with previous studies on picosecond ablation of PEDOT:PSS films.[54] High-quality PEDOT:PSS structures with a resolution of the order of 30 μm were realized on the glass substrates using the sub-picosecond laser at an optimal fluence of 0.45 cm⁻² V⁻¹ s⁻¹ and a beam overlap of more than 80%, as shown in the Figure 2c. Additionally, clear and fine ablation patterns can be observed since there is no evidence of heat-affected area or debris redeposition due to the high fluence applied and the ultrafast pulse patterns. This indicates that the dominant factors governing the ablation process are the photophysical/photocchemical effects.[55] In Figure S3 (Supporting Information) we show additional images of different patterns, shapes, and aspect ratios of laser-patterned PEDOT:PSS films. Further experiments were performed at low laser fluence, close to the threshold ablation value. In this regime, it is feasible to partially remove, reconstruct, or even nanostructure the irradiated layer. Figure 2d shows a PEDOT:PSS film patterned at a fluence of 0.35 J cm⁻². It can be observed qualitatively that the polymer is partially decomposed or ablated at the irradiated areas resulting in a biphasic surface comprising of SAM-modified and SAM-free PEDOT:PSS domains.

Nanometer-scale structures, abundant in the ECM, highly affect basic cell function as well as cell–cell communication
in almost all types of cells, a phenomenon known as contact guidance.[1] To gain an insight into the micro- and nanoscale characteristics of our laser-modified structures, topographical characterization was carried out. In particular, single pulse lines were scribed using two different fluence conditions (0.38 and 0.35 J cm\(^{-2}\)) that both result in partial ablation of the film as shown in the schematic of Figure 3a. It should be noted that working close to the threshold ablation regime provides only a small optimization window for the control of the ablated surface topography. This means that a small change in the laser fluence can result in significant surface alterations. Indeed, for the lower value of 0.35 J cm\(^{-2}\), the remaining PEDOT:PSS layer was measured to be \(\approx 170\) nm, while by slightly increasing the fluence to 0.38 J cm\(^{-2}\) only a thin layer of about 32 nm remains on top of the substrate as shown in the profilometry profile of a cross section of the two contiguous lines in Figure 3a. As shown by both scanning electron microscopy (SEM) and atomic force microscopy (AFM) topographical characterization studies in Figures 3b–e, both films exhibit distinct ablation marks on their surface, indicating that the film is sufficiently modified or decomposed. Comparing the two cases by AFM, we observe that in the lower fluence regime the crater rim demonstrates a smaller and clearer profile, while the step height values indicate that the PEDOT:PSS is well preserved, and only a part has been removed from the film surface. AFM measurements inside the engraved domains allow the identification of nanomorphological effects caused by ultrafast laser processing. Indeed, from the topography of Figure 3f, which corresponds to the low-fluence-irradiated sample, periodic structures, known as laser-induced periodic surface structures, can be seen decorating the irradiated area. Such nanoripple formations are typically observed near the ablation threshold and are attributed to the interference between the incident and the reflected/scattered light. Sakabe et al. showed that the spacing of such structures
for femtosecond processing on metals is expected to be shorter than the laser wavelength.\[^{[56]}\] This is in accordance with our findings where an interspace of about 260 nm between two consecutive waves was measured. To the best of our knowledge, this is the first work that presents self-formed PEDOT:PSS nanostructures by femtosecond laser. We assume that the formation of biphasic PEDOT:PSS regions along with the self-nanostructuring of the irradiated domains can provide a complex environment suitable for preferential cell attachment and growth. For the remainder of the study we chose to use low-fluence (0.35 J cm\(^{-2}\)) conditions as they maintain the integrity of the PEDOT:PSS film while simultaneously providing good surface properties. Concerning the effects of laser ablation on the CP conductivity, we assume that the laser energy deposition in the material can possibly induce some heat transfer to the CP. This could result in local changes of morphology that can affect the charge transport depending on the temperature induced. However, we note that conductivity of PEDOT:PSS is known to improve after annealing (100–140 °C) and studies have shown that it can undergo high temperatures without dramatic effect on its electronic properties.\[^{[57]}\]

To further evaluate the effect of the laser surface patterning on the biofunctionalized films, Madin–Darby canine kidney (MDCK) II kidney epithelial cells were grown on top of the substrates. As can be seen in Figure 4a, the FDTS patterned samples resulted in an excellent and high-precision cell confinement on the ablated areas which was evidenced by the initial well-defined cell attachment in the first 30 min as well as 24 h after cell seeding (Figure 4b). This, we believe, is attributed to the formation of biphasic patterns consisting of alternating highly hydrophobic FDTS and hydrophilic PEDOT:PSS ablated domains. In the case of the APTES-patterned samples, the cells did not show a strong tendency to remain confined at the nonablated (APTES-rich) areas as expected due to the hydrophilic nature of the SAM. This is most probably due to the equally attractive areas inside and outside the patterns, arising from the affinity with the APTES as well as with the laser-microstructured PEDOT:PSS domains, thus favoring in both cases the attachment and growth (Figure 4c,d). This is further confirmed by the contact angle measurements in Figure S2 (Supporting Information) of the SAM-treated samples indicating that PEDOT:PSS is even more hydrophilic (C.A. ≈ 45°) than the APTES (C.A. ≈ 65°) modified sample. A large area image of the FDTS-treated samples patterning (using a different pattern based on “honeycomb grids”) is given in Figure 4e highlighting the accuracy of the patterning over multiple mm\(^2\). Overall, our results indicate that cells can be precisely patterned on top of our CP film following the induced physicochemical cues especially during their initial attachment. We believe that FDTS acts as an initial cell-repellent cue that leads to the microconfinement of cells outside the hydrophobic patterns whereas the microstructured areas potentially allow for the subsequent cell spreading and growth as evidenced by previous studies.\[^{[43]}\]

To conclude, we have demonstrated a straightforward three-step modification strategy for the conducting polymer PEDOT:PSS, aiming to introduce spatially confined cell attachment and growth. We investigated the effect of chemically grafted self-assembled-monolayers carrying different functional groups, with subsequent selective laser ablation to induce cell-attracting or cell-repelling domains. By tuning the laser fluence, we were able to control the effect of the laser ablation and induce biphasic domains (SAM-treated and SAM-free). Interestingly, under ablative conditions the PEDOT:PSS films exhibited periodic nanometer-scale structures, which could act as topographical features and can potentially promote cells adhesion and growth. MDCK II cells were found to preferentially adhere and grow on the predetermined patterns, especially in the case of the negatively patterned films bearing the hydrophobic moieties. This could be attributed to the dominant effect of the cell-repelling nature of those moieties and to a certain extent to the nanotopographical features formed by the laser beam which is a side effect. In the case of the positively patterned surfaces (domains carrying a cell-attracting moiety), the cells...
Figure 4. Cell adhesion and growth in the laser-patterned areas. Optical microscope images of MDCK II cells as imaged after 30 min and 24 h after seeding on the laser patterned a,b) FDTS- and c,d) APTES-modified PEDOT:PSS films, respectively. e) Macroscopic phase contrast image of the cell network on the laser-patterned FDTS/PEDOT:PSS films (after 30 min). Insets showing the honeycomb designs used for the laser patterning, with the white solid lines and fill representing the irradiated areas.

did not exhibit such a strong preference toward the nonablated patterns, which could be attributed either to the equally hydrophilic nature of PEDOT:PSS or to the laser-induced nanostructuring. The proposed approach can be extended to a large repertoire of polymers as well as adopted with several device configurations, for example, microelectrode arrays, where the spatial confinement of the cells and the electrode nanostructuring could improve the cell–cell communication and the electrical coupling respectively.

**Experimental Section**

**PEDOT:PSS Film Preparation:** For the preparation of the PEDOT:PSS films, 19 mL of aqueous PEDOT:PSS dispersion (Clevios PH-1000 from Heraeus Holding GmbH) was mixed with 1 mL of ethylene glycol (Sigma) and two drops of dodecylbenzene sulfonic acid (Sigma). The dispersion was then placed in an ultrasonic bath for 10 min and 180 µL of 3-glycidyloxypropyl trimethoxysilane (Sigma) was then added to the dispersion, followed by sonication for 20 min. After the spin-coating of the PEDOT:PSS solution, films were baked at 140 °C for 1 h under ambient atmosphere.

**Surface Activation:** The film was surface activated using an O₂ plasma under a pressure of 60 mTorr and a 50 sccm O₂ gas flow using an Oxford PlasmaLab plus reactive ion etcher. Two different film thicknesses were investigated: one thick (>400 nm) and one thin (>80 nm). The oxygen plasma power ranged between 10 and 100 W, while the exposure time was set to 1 min.

**Water Contact Angle Measurement:** Hydrophilicity of activated PEDOT:PSS films was evaluated with static water contact angle measurements using an APOLLO INSTRUMENT/OCA 200 goniometer.

**Conductivity and Etching Rate:** Conductivity of activated PEDOT:PSS films was measured using the transmission line method. Prior to measurement, gold lines were patterned on PEDOT:PSS films through a Kapton shadow mask using a metal evaporator. Conductivity was then determined with sheet resistance measurement using a Keithley multimeter and thickness measurements using an AMBIOS technology/XP-2 profilometer.

**Evaluation of Surface Hydrophilicity:** Evaluation of the hydrophilicity of the surface of the PEDOT:PSS films was performed through static contact angle measurements. Average contact angle is extrapolated from numerous measurements per sample (several samples per plasma treatment conditions). Contact angle measurement was performed before and immediately after treatment, and afterward once every day until restoration of the film’s initial hydrophilic properties. The contact angle of the untreated PEDOT:PSS dry films was ~40°. Immediately after treatment, regardless of the power and the time of exposure to oxygen plasma, the contact angle drops to 0°. Determining the longevity of this hydrophilic state is important for long-term applications. First, contact angle measurements were performed every hour after surface activation by oxygen plasma treatment. This hydrophilic state (5° contact angle) is stable for at least 4 h, regardless of the plasma treatment parameters.

**Surface Topography:** The surface microtopography of the films was investigated using atomic force microscopy in tapping mode and at high resolution (AFM, Bruker Dimension Edge). The SEM characterization was carried out using a HITACH T1000.

**Laser Processing:** The system used for direct laser patterning of the samples with the ablative process was based on a sub-picosecond laser source coupled to a laser scanning head. The laser source was a femtosecond-diode-pumped ytterbium amplified laser (Amplitudes Systèmes S-Pulse HP) with a fundamental wavelength of 1030 nm, while for the present experiments the third harmonic (343 nm) was used, after frequency conversion in nonlinear crystals. The pulse duration was set to 500 fs FWHM (full width at half maximum), estimated from single-shot autocorrelation trace. The laser power was adjusted externally with a set of half wave plate and polarizer for each wavelength. The beam was focused on the sample after passing through galvomirrors (Thorlabs GVS12) and an f-Theta-lens that depends on the wavelength: focal length of 254 mm (Thorlabs FTH254-1064) for the infrared and 100 mm for the UV (63-312, Edmund Optics). For the described experiments, the repetition rate of the laser was set at 400 Hz and the galvomirrors were synchronized with the laser, meaning that only one shot per location was done in case of a single pass. A homemade software was used for the control of beam displacements on the sample for a particular pattern.
At 343 nm, the step size was 8 µm, resulting in a beam overlap of more than 80%. A calibration of the energy in the sample plane was done with a calibrated pyroelectric sensor (OPHIR PE9-C) so that the local fluence can be estimated.

**Film Conductivity and Thickness:** PEDOT; PSS film conductivity was measured using the transition line modeling method. Briefly, parallel gold lines were deposited on the substrate at defined interval length. The resistance was measured between lines, which corresponds to the sum of the contact resistance due to the contact metal, the contact resistance between the gold and the polymer film, and the resistance of the film itself. Afterwards, the evolution of the resistance as a function of the distance gave a line (if the dependence is linear) where the sheet resistance of the film can be extracted from the slope. Finally, the film conductivity σ is related to the sheet resistance R and the film thickness d by the following formula

\[ \sigma = \frac{1}{R} \times \frac{d}{R} \]

The conductivity and the thickness (using a mechanical profilometer) of each sample were also measured before and after activation.

**Cell Culture:** MDCK II cells were kindly provided by Frederic Luton (IPMC, Valbonne). These cells were transfected to obtain an expression of fluorescent actin protein. Briefly, MDCK II cells were transfected with pCMV LifeAct–TagRFP (ibidi) using Lipofectamine3000 in Opti-MEM Reduced Serum Medium (Invitrogen). MDCK II LifeAct was cultured with 5% CO₂, 5% glucose and supplemented with 10% fetal bovine serum (Invitrogen), 1000 µg mL⁻¹ of streptomycin (Invitrogen), and 50 U mL⁻¹ of penicillin (Invitrogen), 50 µg mL⁻¹ of streptomycin (Invitrogen), 50 µg mL⁻¹ of gentamicin (Invitrogen), and 500 µg mL⁻¹ of Geneticin (Sigma). After sterilization, using ethanol 70% for 30 min and then rinsing with PBS 1x, MDCK II LifeAct cells were seeded at 1 × 10⁵ Cells cm⁻² and were incubated at 37 °C, 5% CO₂, and 95% air humidified. A fluorescent microscope (Axio Observer Z1 Carl Zeiss) was used to appreciate the cell adhesion 30 min after the seeding and the proliferation at 24 h.

**Supporting Information**

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

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**Conflict of Interest**

The authors declare no conflict of interest.

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