Water Soluble Cationic Poly(3,4-Ethylenedioxythiophene) PEDOT-N as a Versatile Conducting Polymer for Bioelectronics

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Poly(3,4-ethylenedioxythiophene) (PEDOT) is the most popular conducting polymer in the emerging field of bioelectronics. Besides its excellent properties and commercial availability, its success is due to the aqueous processability of its anionically stabilized solutions or dispersions. In this work, a water soluble version of PEDOT is shown, which is cationically stabilized. This work reports the chemical oxidative (co)polymerization of EDOT-ammonium derivative leading to PEDOT-N (co)polymers. PEDOT-N shows the typical features of PEDOT such as UV absorbance, bipolaron band, electrical conductivity, electrochemical behavior, and film formation ability. Furthermore, the PEDOT-N films show good biocompatibility in the presence of the human embryonic kidney-293 cell line. The water solubility of PEDOT-N and its cationic nature allows its processability in the form of thin films obtained by the layer-by-layer technique or as conducting hydrogels.

1. Introduction

Poly(3,4-ethylenedioxythiophene) known as PEDOT is nowadays the most popular conducting polymer in several commercial applications ranging from antistatic coatings, touch screens, optoelectronics (OLEDs, OPVs) or electronic inks to name some examples. Furthermore, PEDOT is also the conducting polymer with the biggest prospects in the emerging field of bioelectronics due to their combination of characteristics (mixed conductivity, stability, transparency, and biocompatibility).[1] In this emerging field, PEDOT is currently being investigated in a number of new applications such as conducting hydrogels, biosensors, organic electrochemical transistors, selective detachment of cells, scaffolds for tissue engineering, electrodes for electrophysiology, implantable electrodes, stimulation of neuronal cells, electronic skin, or pan-bio electronics.[2] The most used PEDOT version is the commercially available aqueous dispersion poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT: PSS) which is usually processed by casting or spin-coating. However, in order to well connect the two fields of biology and electronics,[3] PEDOT:PSS presents some limitations associated with the low (bio)functionality of the PEDOT polymer and acidity/toxicity of the PSS stabilizer.[4–7]

For this reason, in the last few years a number of functional EDOT monomer derivatives has been synthesized including hydroxymethyl,[8] chloromethyl,[9] azidomethyl,[10] carboxylic

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Among the different functional monomers, EDOT-sulfonate has been the most popular one since it led to anionic forms of the conducting polymer. PEDOT sulfonate known as PEDOT-S has been very successful due to its water solubility and high electronic conductivity without the need of PSS scaffold. The anionic sulfonate group allows the polymer self-dope and stabilize in water being used in a great number of applications ranging from cell detachment, biopolymer conjugation, or electronic plants. However, similarly to the PEDOT:PSS case the overall charge of the conducting polymer is anionic, which limits for example its conjugation and interaction with biomolecules anionically charged like DNA/RNA, most proteins at physiological pHs and cells. In this sense, a cationic water-soluble version of PEDOT would be of interest for a number of applications.

Among the different EDOT monomers which have been synthesized, there have been some examples of cationic EDOT monomer derivatives having ammonium or imidazolium or pyridinium groups. However, these monomers have been only used to tune the properties of polymer layers obtained by electropolymerization including superoleophobicity, anion switchability or electro responsiveness of surfaces. In this paper, we show the chemical synthesis of a cationic ammonium EDOT monomer and its chemical (co)polymerization, physicochemical, and electrochemical characterization. This paper shows a cationic PEDOT derivative which is soluble in water and electronically conducting with great potential in bioelectronics applications. Furthermore, its electrochemical properties as well as its biocompatibility were investigated. To end up, the versatility of PEDOT-N was demonstrated by processing in two different ways. First, the article shows PEDOT thin films obtained by layer-by-layer (LbL) assembly of polyelectrolytes and second the easy preparation of conducting hydrogels.

### 2. Results and Discussion

#### 2.1. (Co)Polymerization and Characterization of Water Soluble Cationic PEDOTs

PEDOT-N homopolymer and random copolymers PEDOT-co-PEDOT-N were synthesized by chemical oxidative polymerization, using (NH₄)₂S₂O₈ as oxidant, catalyzed by FeSO₄·7H₂O in acidic environment. The polymerization reaction for the preparation of water-soluble PEDOT-N derivatives is shown in Scheme 1 and proceeds through a well-known oxidative polymerization mechanism. The initially monomer/oxidant brown solution after 4 h reaction became dark blue which is the typical color of PEDOT. The product was extracted with dichloromethane and obtained as dark blue powder after drying. PEDOT-N homopolymer was synthesized according to Scheme 1a. In addition, different random copolymer compositions named as PEDOT-co-PEDOT-N were synthesized according to the Scheme 1b. Two different copolymer compositions were synthesized having 50 mol% and 75 mol% commercial EDOT comonomer, which corresponds to 50 mol% and 25 mol% of 2-((2,3-dihydrothieno[3,4-b][1,4]dioxin-2-yl)methoxy)-N,N,N-trimethylethan-1-aminium iodide (EDOT-TMEAI), respectively. Interestingly, the PEDOT-N (co)polymers could be solubilized/dispersed in water leading to typical PEDOT blue solutions/dispersions. Zeta potential measurements show that the z potential of the material is positive and it increases as the percentage of cationic PEDOT increases in the sample (Table 1 SI).

This demonstrates that the charge density of the surface, as well as the surface properties, could be modified with the amount of PEDOT-N in the chemical polymerization, and proves the initial goal of obtaining a water soluble or dispersible PEDOT derivative stabilized with cationic charges. The magnitude of the zeta potential allows predicting the possible aggregation behavior of the PEDOT-N derivatives. A higher surface

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**Scheme 1.** Synthesis of a) PEDOT-N homopolymer and b) PEDOT-co-PEDOT-N copolymers.
charge at 100% PEDOT-N (20 mV) will indicate a tendency to avoid electrostatic repulsion aggregates and improve polymer wettability. A lower surface charge (such as those obtained for PEDOT-co-PEDOT-N) will be insufficient to overcome the van der Waals forces between the polymer particles and will lead to a certain degree of aggregation of them.

Figure 1a (Figure S1, Supporting Information) shows the UV–vis–NIR spectra of a water solution/dispersion of the three polymers (1 mM). All polymers show characteristic strong signals at 300 nm corresponding either to the organic backbone or to iodine species.[32] The broadband from 650 nm with a tail extending into the near-infrared region consistent with the absorbance of neutral PEDOT (650 nm), the polaron (900 nm), and the bipolaron bands (after 1100 nm), which are characteristic of oxidized PEDOT.[33,34] The intensity ratio of the polaron and bipolaron band of the homopolymer is higher than the copolymers, suggesting that the homopolymer PEDOT-N has the highest doping level. The spectroelectrochemical plot is showing the electrochromic behavior of PEDOT-N. (Figure S2, Supporting Information)

The ATR-FTIR spectra (Figure 1b) of the PEDOT-N (co) polymers display the characteristic bands for polythiophene PEDOT.[35] The mentioned bands at 1529 and 1315 cm⁻¹ were attributed to C=C and C=C stretching vibrations of the thiophene ring, respectively. The C=O–C stretching vibration in the ethylenedioxy group was assigned at 1126 cm⁻¹. The N–C stretching vibration appears at 1200 cm⁻¹. The characteristic peaks in the spectrum at 988, 850, and 660 cm⁻¹ corresponding to C=S–C stretching vibrations. Moreover, the absence of bands at 3100–3050 cm⁻¹ assigned C–H stretching in the monomers of the thiophene ring confirmed the formation of a polymer. All in all, both UV and Fourier transform infrared (FTIR) spectroscopies confirm the chemical nature of the conjugated polythiophene PEDOT-N copolymer.

As mentioned before, PEDOT-N homopolymer and both copolymer compositions could be easily solubilized in water giving dark blue homogeneous dispersions. With the purpose of analyze if we have obtained a real solution of the polymers or a typical PEDOT particle dispersions, transmission electronic microscopy (TEM) was carried out, casting the three diluted dispersions directly on the TEM grids (Figure 2). As it can be seen in the TEM images, the PEDOT-co-PEDOT-N copolymers show nanoparticle morphology characteristic of PEDOT type polymers, similar to other PEDOT dispersions.

Figure 1. a) UV–vis–NIR water solution spectra and b) ATR-FTIR spectra of 25% PEDOT-N, 50% PEDOT-N, and 100% PEDOT-N. Inset: cuvette images of 100% PEDOT-N water solution.

Figure 2. Transmission electronic microscopy micrographs of PEDOT-N homopolymer, 50% PEDOT-N, and 100% PEDOT-N.
It can also be clearly seen how the size and the interconnectivity of the particles increase as the amount of PEDOT-N units decreases in the copolymer. Being 35 nm for 50% PEDOT-N and 85 nm for 25% PEDOT-N the average values of particle size for both copolymers. This is expected since the N-cationic ammonium group is responsible for the dispersion and solubility of the polythiophene backbone. Interestingly, in the case of the PEDOT-N homopolymer, the image only shows very small dots or particles of PEDOT-N, which may be an indication of its solubility as individual chains. All in all, these images demonstrate that the cationic group plays an important role in the dispersibility or even solubility of the polythiophene polymer in water.

Interestingly, as in the case of the well-known PEDOT:PSS, PEDOT-N thin films could be obtained by casting from the aqueous dispersions onto a number of different surfaces. First, we analyzed the morphology and the homogeneity of the PEDOT-N films by SEM (Figure 3). As it can be seen, the surface morphology of the PEDOT-N homopolymer film was smoother and regular without showing any surface roughness. However, the surface morphology of PEDOT-co-PEDOT-N films was more irregular showing increasing roughness by increasing the amount of PEDOT or decreasing the amount of PEDOT-N solubilizing unit. This behavior is aligned with the previous TEM images of the water solutions.

### 2.2. Electro(Chemical) Characterization of PEDOT-N

It is well known that the most important characteristic of PEDOT is its electronic conductivity. The electronic conductivity value of the three copolymers shows values of 0.03 S cm$^{-1}$ for PEDOT-N homopolymer and 0.06 and 0.12 S cm$^{-1}$ for PEDOT-co-PEDOT-N copolymers (25% and 50% PEDOT-N), respectively, as measured by the four-points probe method. Although these conductivity values are relatively low as compared to the most optimized versions of anionically stabilized PEDOT, it is in fact comparable with initial works in the development of PEDOT:PSS or PEDOT:S.[30] Interestingly, in the copolymers it is observed how the electronic conductivity increases, by increasing the amount of PEDOT. On the other hand, PEDOT-N homopolymer is a highly hygroscopic material and the thin films soften after some hours in the open-air by effect of the adsorbed water. To conclude this section, it can be said that the increment of the cationic PEDOT-N monomer ratio is favorable to increase the water solubility of the material but has a detrimental effect on the electronic conductivity and hygroscopicity of the films.

The electrochemical properties of the PEDOT-N polymers were investigated by cyclic voltammetry (CV) in a three-electrode system. Measurements were carried out in water solution as well as acetonitrile solution, using LiClO$_4$ as electrolyte and a platinum disk as working electrode. Figure S3, Supporting Information, shows the voltammograms corresponding to 3 mm aqueous solution of the three polymers. It is clearly distinguishable a redox wave for the iodide/iodine redox couples, with an oxidation potential of 1 V (vs Ag/AgCl). Iodide is oxidized to molecular iodine, the redox behavior corresponds to the one-electron oxidation of either the iodide (Figure S3, Supporting Information, Equation (1)).[36]

On the same way, the cyclic voltammetry (CV) for all polymers in organic medium (acetonitrile/LiClO$_4$) showed two oxidation peaks (Figure S4, Supporting Information) at 0.09 and 0.39V vs Ag/Ag$^+$, attributed to two oxidation processes of iodide species $I^-/I_3^{-}$ and $I_1^-/I_2^{-}$, respectively. This redox behavior is corresponding to iodide/iodine redox couples (Figure S3, Supporting Information, Equation (1)), equilibrium between iodine and iodide (Figure S3, Supporting Information, Equation (2)), and oxidation of triiodide complex (Figure S3, Supporting Information, Equation (3)).[37] In both media, it is possible to observe that the current peak decreases as the percent of PEDOT-N units in the polymer reduces, in agreement with the decrease in the amount of iodine that is the electroactive group present in the samples.

With the goal exchange of avoiding the redox-activity of the iodine counter-anion and be able to analyze the redox activity of the PEDOT backbone, the iodine counter-ion was exchanged by a chloride anion by dialysis as shown in the scheme in Figure 4a. As shown in scheme, the homopolymer was dissolved in water and, sited into the dialysis membrane, the exchange was done using a solution of KCl 1 m and HCl 0.1 m. The solution was renewed every 4 h for 2 days, to be sure to push the equilibrium and, after that, the polymer was dried under vacuum overnight. Then, the electrochemical CV behavior of PEDOT-NMe$_3^+$Cl$^-$ was compared with the electrochemical behavior of PEDOT-N in Figure 4b. The redox characteristics of PEDOT-NMe$_3^+$Cl$^-$ was studied in acetonitrile by cycling the potential between −0.5 and 0.5 V at 25 mV·s$^{-1}$ scan rate. The waves corresponding to the oxidation of the different iodine species disappear and the polymer shows a quasirectangular shape without any prominent redox wave, indicating the significant contribution of electrical double-layer capacitance and low internal resistance. The scan rate was then tuned, in order to show a diffusional process, visible in the inset in Figure 4b.
2.3. Biological Characterization of PEDOT-N

The compatibility of both PEDOT-N homopolymers with iodine and chloride counter-anions with cell proliferation was preliminarily investigated by employing secondary line cell models, namely human embryonic kidney (HEK-293) cells. Different dispersion concentrations were delivered to the cell cultures 2 h after deposition, and left within the cell growth medium for 24 h. Afterward, the cell medium was washed out and replaced with untreated medium. Cell viability tests were carried out at three different time points (24 h, 48 h, 120 h), until cell confluence was reached, by using the consolidated alamarBlue assay. The latter provides a reliable indication of the metabolic activity of cultured cells, either exposed to the conducting polymers and in control, untreated samples, and is a direct proof of the cell proliferation capability, observed at subsequent times after plating.

Results obtained for the cationic PEDOT-N and PEDOT-NMe3⁺Cl⁻ polymer dispersions, endowed either with a Cl⁻ or I⁻ counter-ion, are shown in Figure 5. Cell cultures treated with PEDOT polymer with chlorine as a counter-ion show a proliferation rate fully comparable to the one of control, untreated samples, in the whole range of tested concentrations (10–50 μg mL⁻¹), up to 5 days. The PEDOT-N polymer with iodine as the counter-ion shows good cell proliferation upon delivery of polymer dispersion up to 20 μg·mL⁻¹ concentration; conversely, at 50 μg mL⁻¹ there is a drastic cell viability decrease, which is reduced at 26% 5 days after. This may be ascribed to different effects. First of all, the two considered dispersions may show different aggregation dynamics in the intracellular fluid over time. The positive values of the zeta potential indicate a good stability over time, nevertheless different ionic strength may lead to different evolution over time in the biological medium. Second, the presence of the Cl⁻/I⁻ anions may be responsible for a different interaction with the cell membrane, thus leading to a different internalization efficiency of particles within the cell cytosol. Finally, the presence of the I⁻ anion may result in intrinsic intracellular toxicity. The detailed understanding of these processes deserves a dedicated analysis, beyond the goal of this work, and it will be the object of further studies. Nevertheless, cell proliferation data show very good biocompatibility of cationic PEDOT dispersions at concentration lower than 20 μg mL⁻¹, and open the way to the use of PEDOT-N for several biotechnology applications.
2.4. Processability of PEDOT-N in Thin Films by the Layer-by-Layer Technique

The processability of the PEDOT-N materials represents an important parameter for successful implementation. As indicated before, thin conductive polymer films could be obtained by simple casting from the aqueous solution. The films showed electrical conductivity levels similar to other commercially available PEDOT:PSS materials. However, the PEDOT-N homopolymer films were very hygroscopic. A popular method to obtain, stable thin film is the LbL assembly of polyelectrolytes. In this method, a polycation is alternately deposited with a polyanion onto a charged surface. The method is simple and robust, requiring only the alternately immersion of the charged substrate in solutions of polyanions and polycations with washings in between, to remove excess of polymers that could remain on the surfaces. Polyelectrolyte multilayers (PEMs) with nanoscale controlled thickness and vertical composition are produced in short time by the LbL technique, making it an appealing tool for the engineering of surface and the integration of different components non covalently.[38,39] LbL films have been used for integrating charged conductive polymers.[40] Indeed, PEDOT has already been used to produce LbL films but always in combination with a non-conductive polycation. So, we evaluated here the capacity of our PEDOT-N to form LbL layers in combination with PEDOT:PSS (polystyrene sulfonate), which, bearing an excess of PSS, result in an anionic version of PEDOT-mixture, as shown schematically in Figure 6a. In this way the PEMs would be composed of only conductive polymer mixtures, which could facilitate their integration in electrical devices.

To monitor the assembly of PEDOT-N and PEDOT:PSS by the LbL technique, the PEM was assembled in a quartz microbalance with dissipation (QCMD) and followed in real time.[41,42] The assembly was performed on quartz crystals coated with gold. Figure 6b shows the frequency changes during the assembly of PEDOT-N and PEDOT:PSS on a QCM gold crystal, and the mass associated with the frequency changes, calculated after each layer deposition with the Sauerbrey equation. We first assembled a PEDOT-N layer on the gold coated crystal. The assembly took place through the sulphur atoms in the EDOT monomer that form a thiol bond with gold. PEMs assemble and growth in a continuous way up to the 9th, 10th layers. There is a large shift in frequency when the 1st PEDOT:PSS layer is assembled, around half of the whole frequency change associated to the assembly of the 10th polymer layers, which hints that PEDOT:PSS is forming particles or aggregates in solution and deposits as such. A polyelectrolyte layer usually induces a shift of 15 Hz, while the first PEDOT:PSS layer deposited after the PEDOT-N layer results in 30 to 60 Hz. After this layer there is a more moderate growth for each layer assembled. Frequency seems to decrease as the
layer number increases but still the mass of film growths continuously as can be appreciated from Figure 6c. The mass of the first layer of PEDOT-N is significantly smaller than the following PEDOT:PSS layer. This can be explained by the fact that the first layer deposited in a LbL assembly does not result in a continuous coating of the surface. Moreover, in this case the direct assembly of PEDOT-N thorough the sulphur atoms in the EDOT monomer relies on the availability of these groups to interact with gold in water, which may be limited as the non-charged parts of the polymers will be less exposed to water.

The characteristics of the PEDOT-N/PEDOT:PSS assembly hint that the PEDOT:PSS deposits as particles, with a large increase in mass in the first layer of PEDOT:PSS. The layer of PEDOT-N shows a larger frequency shift than for an average polyelectrolyte layer but significantly smaller than the PEDOT:PSS layer. This is possible because if the PEDOT:PSS assembles as a particle the area for the deposition of the next PEDOT-N layer is larger. However, the second PEDOT:PSS layer results in a smaller frequency shift than the first PEDOT:PSS layers, and this indicates that the PEDOT:PSS cannot arrange well on the PEDOT-N, that is, not all positive charges are accessible for the assembly or the PEDOT:PSS particles are sterically hindered. Then, also the amount of PEDOT-N deposited in next layer is also smaller. However, the assembly continues up to the 9th–10th layer still. To resume, the LbL assembly between positively charged PEDOT and PEDOT:PSS takes place, the film growths continuously increasing the number of layers deposited, and the growth kinetics reflects the fact that PEDOT PSS assembles as particles. Atomic force microscopy (AFM) micrographs (Figure 6d) show that the after 10 layers assembled the PEDOT-N/PEDOT:PSS is continuous over the surface. AFM images of the deposition of one PEDOT-N/PEDOT:PSS double layer show a less homogenous film with large discrete aggregates that could come from the large PEDOT:PSS particles (Figure S5, Supporting Information). After assembling 5 polymer layers the film is more homogeneous over the surface. The root-mean-square (rms) surface roughness of the film increases however with increasing number of layers assembled, being 5.4 nm for 2 layers, 9.9 nm for 5 layers, and 11.9 nm for 10 layers.

2.5. PEDOT-N Semi-Interpenetrating Polymer Network Hydrogels

Conducting polymer hydrogels combining both electronic and ionic conductivity possess high interest for applications in energy and bioelectronics. Hence, we wanted to assess whether the PEDOT-N could be further processed and integrated into hydrogel materials. For this purpose, different amounts of PEDOT-N homopolymer were dissolved in water in the presence of PEGDA diacrylate and a photoinitiator. By fast UV-photopolymerization hydrogels with different amounts of PEDOT-N and compositions could be easily prepared, as illustrated in the images in Figure 7 and described in the experimental section. This simple experiment shows the versatility of PEDOT-N to be processed in the form of conducting hydrogels of potential interest in applications such as tissue engineering.

3. Conclusions

To conclude, in this paper, we show the synthesis of a new water soluble and processable cationic PEDOT-N polymer. The PEDOT-N materials show the typical features of PEDOT such as UV absorbance and bipolaron band, electrical conductivity, electrochemical behavior and can be processed into films. By modifying the EDOT-N and EDOT ratio different copolymers were obtained. The PEDOT-N homopolymer shows the higher solubility in water and best quality films. By increasing the amount of EDOT, PEDOT nanoparticle aqueous dispersions were obtained which lead to more irregular films but with slightly higher conductivity values. The PEDOT-N materials show good biocompatibility in the presence of HEK-293 cells. The water solubility of PEDOT-N and its cationic nature allows its processability also in the form thin films obtained by LbL or

![Figure 7](image-url) Pictures of hydrogels containing a decreasing amount of PEDOT-N polymer.
hydrogels. Future work will be devoted to investigate the applications of those materials in bioelectronics.

4. Experimental Section

**Materials:** Dichloromethane, 2-hydroxy-2-methylpropionophenone (DAROCUR), polyethylene glycol diacrylate (PEGDA), hydroxymethyl-EDOT, acetonitrile, and LiClO₄ were purchased from Sigma-Aldrich. FeSO₄·7H₂O was obtained from Merck. Deuterated solvents were purchased from Eurisotop. (NH₄)₂S₂O₈ and 3,4-ethylenedioxythiophene (EDOT) were purchased from Acros. NaH was used at 60% m–m dichloromethane and freeze-dried for 3 days. The solid obtained were collected from 4000 to 250 cm⁻¹ were followed by quartz crystal microbalance with dissipation (QCM-D). The QCM-D measurements were carried out using a Q-Sense E4 quartz crystal microbalance (Q-Sense, Göteborg, Sweden) and Au (50 nm)-coated quartz crystals (5 MHz) (QSY-301, Q-Sense) previously treated with a UV-ozone cleaning protocol. As first layer PEDOT-N was assembled in the substrate. In between layer deposition the QCMD chamber was washed with water to remove non electrostatically bound polyelectrolyte from the crystal. Data are shown for the third overtone. Frequency was converted in mass applying the Sauerbrey equation that links frequency shifts and adsorbed masses per unit area in a very simple way: with the mass sensitivity constant C₁₈M⁻¹Ω⁻¹, the total frequency shift is calculated as

\[ \Delta f = \frac{C_1}{M} \]

where \( \delta \) is the mass sensitivity constant 18.06 ± 0.15 ng cm⁻² Hz⁻¹ for sensors with a resonance frequency of 4.93 ± 0.02 MHz and the overtone number \( i \). The normalized frequency shifts, \( Df = \delta f/\delta \), for the 3rd overtone was employed to determine the mass deposited (\( \delta M = -\Delta fi/C_1 \)).

**Atomic Force Microscopy:** LbL films were imaged in the tapping mode in air at room temperature with a Multimode AFM (Veeco) connected to a Nanoscope V controller. Antimony (n) doped Si cantilevers (Bruker) with a K = 42 N m⁻¹ were used. Images were taken for films with 2, 5, and 10 layers of polyelectrolytes.

**Preparation of PEDOT-N Hydrogels:** The hydrogels were formulated with an aqueous solution of PEDOT-N and 10 wt% of PEGDA, as a photocrosslinker, containing 0.5 wt% of photoinitiator DAROCUR. Subsequently, the mixture was put on a silicone scaffold and photocrosslinked via UV exposure for 60 s. To remove the extra ions and impurities, all hydrogel samples were washed 3 times for 5 min in MilliQ water.

**Characterization Methods:** The ultraviolet-visible-near-infrared (UV–vis–NIR) spectra were done using a Perkin-Elmer lambda 950 spectrophotometer.

FTIR spectra were measured on a Bruker Alpha II spectrophotometer employing Platinum ATR module with diamond window. Spectra were collected from 4000 to 250 cm⁻¹ with the following settings: 42 scans per sample and spectral resolution: 4 cm⁻¹. The conductivity was tested using The four-point probe system, Ossila. The solid films were done by coating of glass substrate with water solution and dried under vacuum for 2 days.

TEM. TEM images were collected using a JEOL JEM-2100F model EM-20014, which features a 200 kV field emission gun (Schottky) “FEG” and an ultra high resolution pole piece “UHR”. First, 0.1 mg of the studied sample was solubilized in 1 mL of deionized water. The sample was sonicated during 15 min. Then 5 μL of the solution was deposited into a TEM grid and evaporated at room temperature.

**Scheme 2.** Synthesis of EDOT-DMEA and EDOT-TMEAI.

Under the same conditions, two copolymers were synthesized with different proportions of EDOT-TMEAI and EDOT monomer. Thus obtaining copolymers with 50 and 25 mol% of the cationic compound.
Scanning electron microscope (SEM): Measurements were performed on JEOL JSM-6490LV at 15 kV, running in a point by point scanning mode. Cylindrical coverslip with 11 mm diameter were introduced in isopropanol and sonicated during 15 min in order to remove impurities, they were dried at room temperature. 10 mg of PEDOT cationic or copolymers was solubilized in 1 mL of deionized water. Then, it was sonicated during 15 min and drop casted in a cylindrical glass coverslip. The water was evaporated at room temperature. The coverslip was placed on an aluminum holder with double-side carbon tape and introduced in the SEM chamber. All the samples were evaluated at different magnifications.

Zeta Potential was measured with dynamic light scattering using a Zetasizer nano series (Malvern Instrument) at 20 °C. A fraction of each polymer dispersion was diluted with deionized water for the analysis and the reported zeta potential values represent an average of three repeated measurements.

The ionic exchange of PEDOT-N was performed thorough a dialysis with standard regenerated cellulose (RC) dialysis tubing, pre-treated, 38 mm flat width, MWCO: 1 kD (1000 Daltons) by spectrum, using a solution of KCl 1 M and HCl 0.1 M for 2 days.

Electrochemical characterization was carried out by the CV technique, using an Autolab PGSTAT204 potentiostat/galvanostat and a three-electrode cell configuration, Pt Bas was employed as a working electrode (WE), platinum wire as a counter electrode (CE), Ag/AgCl (0.01 M AgNO3) as reference electrodes in aqueous and organic media, respectively. The supporting electrolyte was LiClO4 in both media, the acetonitrile was used to provide the organic environment.

Cell Cultures Preparation: HEK-293 cells were grown in Dulbecco's modified Eagles' medium (D-MEM, Sigma-Aldrich) with 10% fetal bovine serum (FBS, Sigma Aldrich), supplemented with 2 mm glutamine (Sigma-Aldrich), 100 U mL−1 streptomycin (Sigma-Aldrich), and 100 U mL−1 penicillin (Sigma-Aldrich). Cells were kept in T-75 culture flasks and maintained in the incubator at 37 °C in a humidified atmosphere with 5% CO2. After reaching 80–90% of confluence, cells were detached by incubation with 0.5% trypsin-0.2% EDTA (Sigma–Aldrich) for 5 min and plated for experiments. A layer of 2 mg mL−1 fibronectin (from bovine plasma, Sigma-Aldrich) in phosphate buffer saline (PBS, Sigma Aldrich) was deposited on the surface of the 12-well plates and incubated for 30 min to promote cell adhesion. After rinsing the fibronectin with PBS, cells were plated in their culture medium and eventually treated with PEDOT-N polymer.

Biocompatibility Tests: In order to preliminarily evaluate the PEDOT-N cytotoxicity, the alamarBlue (Invitrogen) assay was performed on HEK-293 cells. The alamarBlue reagent is a resazurin based solution that functions as an indicator of cells metabolic activity. Resazurin is a cell-permeable non-fluorescent compound that upon entering living cells, is reduced to resorufin, a highly fluorescent compound. Changes in the cell viability can be easily detected using a fluorescent-based plate reader. Cells were seeded in 12 wells plates at a density of 1 × 104 cells per well and after 2 h of incubation, the PEDOT-N derivatives were added at three different concentrations: 10, 20, and 50 μg mL−1. Each polymer concentration was added to three different wells to work in triplicate, while three wells were left without PEDOT-N as controls. Cell proliferation was evaluated 24, 48, and 120 h after incubation. For each time point, the growing medium was replaced with fresh medium without phenol red containing 100 mg mL−1 of alamarBlue. The samples were incubated again for 3 h at 37 °C in the dark. Afterward, for each well, three aliquots of culture media (100 μL) were removed and placed in black 96-well microplates, and the fluorescence was measured by a Tecan Spark microplate reader, exciting at 530 nm and recording the emission at 590 nm.

**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.

**Keywords**

biocompatibility, bioelectronics, conductive polymers, hydrogels, layer-by-layer assembly

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