Biochemically Controlled Release of Dexamethasone Covalently Bound to PEDOT

Stefano Carli,* Claudio Trapella, Andrea Armirotti, Anna Fantinati, Giuliana Ottonello, Alice Scarpellini, Mirko Prato, Luciano Fadiga, Davide Ricci

Abstract: PEDOT is one of the most promising electrode materials for biomedical application like neural recording and stimulation thanks to its enhanced biocompatibility and electronic properties. Drug delivery by PEDOT is typically achieved by incorporating drugs as dopants during the electrodeposition procedure and a subsequent release can be promoted by applying a cathodic trigger that reduces PEDOT while enabling the drug to diffuse. This approach has several disadvantages including, for instance, the release of contaminants mainly due to PEDOT decomposition during electrochemical release. Herein we describe a new strategy based on the formation of a chemical linkage between the drug and the conductive polymer. In particular, dexamethasone was successfully integrated into a new electropolymerized Pedot-Dex composite, leading to a *self-adjusting* drug release system based on an enzymatically hydrolysable bond between Dexamethasone and PEDOT.

Conductive polymers are a very promising class of multifunctional materials their usage ranges from electrochromic devices and organic photovoltaics to biomedical applications.[1] One of the most challenging application of conductive polymers is the release of anti-inflammatory drugs for the treatment of glial scar, which leads to the encapsulation of neural probes after few weeks post implantation, causing the loss of electrical signals.[2] In this contest, poly(3,4-ethylenedioxythiophene) (PEDOT) represents one of the best candidates thanks to its very high stability and biocompatibility.[3] Dexamethasone (Dex) is an extremely potent anti-inflammatory and immunosuppressive corticosteroid and represents one of the most used drugs within the field of neural implants.[4] Unfortunately, peripheral and systemic administration of Dex faces several disadvantages, including side effects in multiple organs due to Dex overdosing. There is therefore a great need of more localized forms of delivery. The water-soluble prodrug Dexamethasone sodium phosphate (Dex-P) has been incorporated within PEDOT films coating neural microelectrodes in order to reduce the adverse reaction of the surrounding tissue.[5] Typically, the negatively charged Dex-P can be actively incorporated within the polymeric film as a dopant during the electrodeposition procedure, thereby counterbalancing the positively charged oxidized state of PEDOT. Thus, an electrochemically controlled drug release can be promoted by applying a cathodic trigger that brings PEDOT to its reduced and neutral state while enabling the free diffusion of Dex to the bulk.[6] Unfortunately, this approach meets some issues that still need to be overcome. First of all, the inclusion of Dex, or bulky dopants in general, has been reported to negatively affect both electrochemical properties and stability of PEDOT coatings.[7] Furthermore, it has been reported that PEDOT doped with Dex-P tends to release EDOT monomers and/or oligomers when applying the electrochemical stimulus that should be used to release the drug. [8] Finally, passive release due to ion exchange in interstitial media cannot be totally avoided, although it occurs within a longer timeframe with respect to the electrochemically controlled release.[8] To the best of our knowledge, the possibility of chemically link Dex to the surface of PEDOT in order to promote a release mechanism based on the chemical/biochemical cleavage of the covalent bond between Dex and PEDOT has not been explored hitherto.[9] This approach would account for a self-adjusting release system where the delivery of the drug is promoted by local changes in the biological environment, thereby avoiding the above mentioned issues due to electrochemically controlled release. In this study, for the first time, the anti-inflammatory drug Dex was chemically anchored to the surface of PEDOT, thereby enabling the drug release upon the hydrolysis of the Dex-PEDOT chemical bond. In particular, a new functionalized monomer EDOT-Dex (**2**) has been prepared by covalently grafting Dex to the hydroxyl group of the commercially available hydroxymethyl-EDOT, through a succinic spacer, as outlined in Scheme 1. The *two step* synthetic pathway consists on the preparation of the intermediate succinyl-Dex (**1**), by the reaction between Dex and succinic anhydride; the intermediate (**1**), after isolation, can be used in the next step with no further purification to give (**2**).[10] The new EDOT-Dex monomer was characterized by ^{1}H and ^{13}C NMR and ESI mass spectrometry, and the purity was evaluated to be higher than 98% by HPLC.

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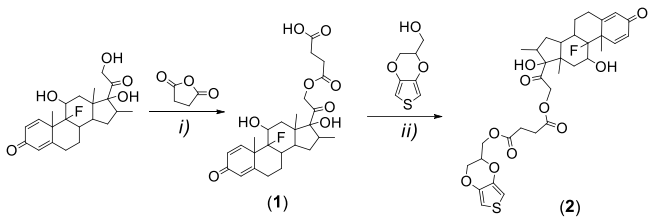

Scheme 1. Synthetic route to EDOT-DEX (**2**): *i*) DMAP, TEA, CH_2Cl_2, r.t. overnight ; *ii*) HOBT, WSC, N-methylmorpholine, DMF, r.t. overnight.

Figure 1 compares the absorption spectra of of (**2**), Dex and EDOT in acetonitrile. Pristine Dexamethasone exhibits a strong absorption at 234 nm and a shoulder at 260 nm likely ascribed to the electronic transitions of the α,β-unsaturated carbonyl group, while the absorption of EDOT shows a weak feature at 239 nm and stronger absorption at 257 nm that could be attributed to the π–π* electronic transitions. As expected, the spectrum of EDOT-Dex exhibits all the above mentioned features, being EDOT and Dex units electronically decoupled: in particular the two bands at 234 nm and 254 nm, ascribed to the electronic absorption Dex and EDOT units, respectively, are observed.

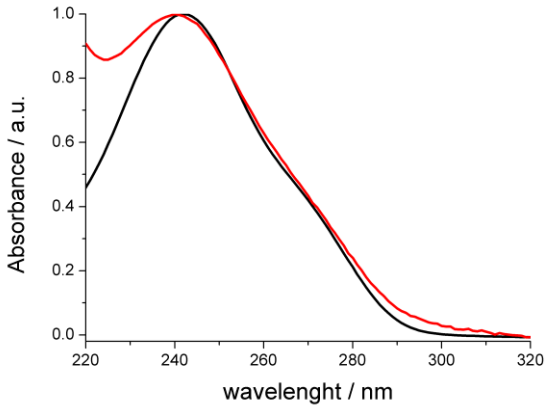

Figure 1. UV spectra of EDOT-Dex(**2**), EDOT and Dexamethasone in acetonitrile.

The modified EDOT-Dex monomer (**2**) was used during the electrodeposition of Dex-functionalized PEDOT films (PEDOT-Dex). Among other electrodeposition methods, e.g. galvanostatic and potentiostatic, the potentiodynamic mode was preferred in this study since it provides highly reproducible films with improved porosity and electrochemical properties.[11] By the analysis of the first deposition cycle from a solution containing the monomer EDOT or EDOT-Dex (Figure 2), respectively, it can be observed that the typical trace crossing on the reverse sweep, which is ascribed to the initial stages of polymer nucleation and growth on the electrode surface, is present only in the case of pristine monomer.[12] This suggests that the oxidation of (**2**) at the electrode interface does not lead to the growth of the conductive polymer film.

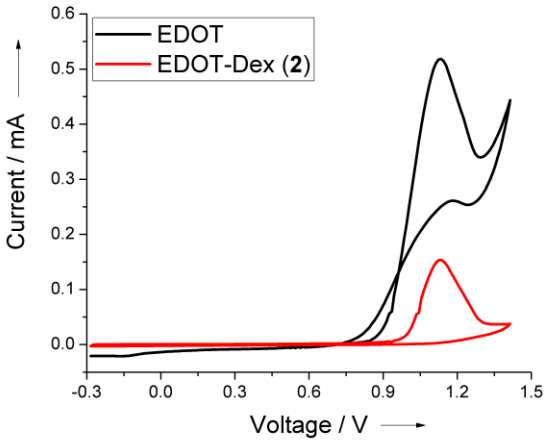

Figure 2. Cyclic voltammetry of 0.1N EDOT or EDOT-Dex (**2**) in 0.1N $LiClO_4$/acetonitrile.

In fact, several attempts of electrodeposition in multiple scan mode from a solution of EDOT-Dex did not provide any evidence of polymer formation, presumably due to steric hindrance of (**2**) and/or a slow charge transfer kinetic at the electrode|solution interface (Figure S1). As expected, mass-transport limitations for EDOT-Dex, due to its large size, was confirmed by the lower peak current observed in the first deposition cycle (Figure 2).[13] Electrochemical copolymerization of EDOT with different monomers is a well-established procedure to modify the properties of PEDOT films.[14] Therefore, electrodeposition was carried out from an equimolar solution of both EDOT and the new EDOT-Dex monomers, in 0.1N $LiClO_4$/acetonitrile.

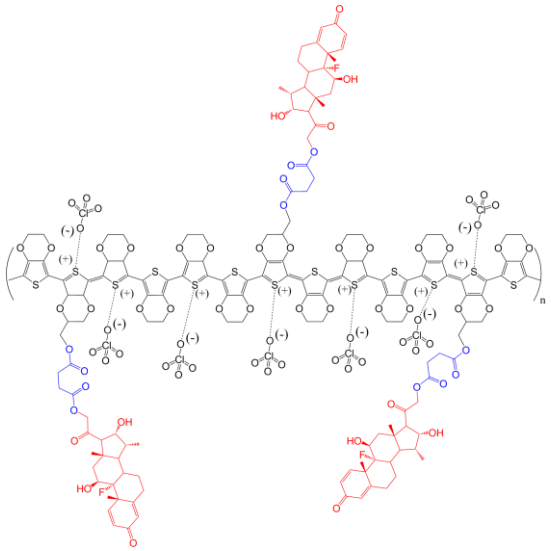

Figure 3. Schematic representation of PEDOT-Dex structure.

As depicted in Figure 3 the new PEDOT-Dex composite is expected to be composed of a mixture of EDOT and EDOT-Dex units in a well-defined ratio.

X-ray photoelectron spectroscopy (XPS) analysis (Figure 4) was performed in order to assess the presence of PEDOT-Dex chemical links on the surface of the new composite film, and the same procedure was adopted for PEDOT based coating, for comparison. The covalent incorporation of Dex within the new PEDOT-Dex composite was confirmed by the signal at the binding energy of 686 eV which is ascribed to the fluorine atoms at the position C-9 of Dexamethasone moiety (Figure 3).

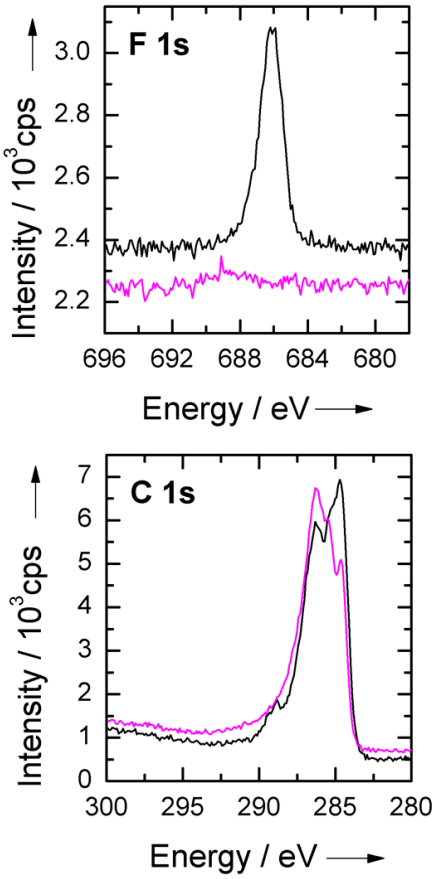

Figure 4. XPS fluorine (1s) (top panel) and carbon (1s) (low panel) spectra of PEDOT-DEX (black lines) in comparison to PEDOT (purple lines).

Quantitative analysis of XPS results was performed in order to determine the amount of Edot-Dex units incorporated within the PEDOT-Dex film. Taken into account that (**2**) has a F:S atomic ratio of 1, whereas PEDOT-Dex has a F:S ratio of 0.9:4.7, as reported in Table 1, the EDOT:EDOT-Dex(**2**) ratio was estimated in the order of 5:1. This is consistent with a higher concentration of pristine EDOT units within the backbone of PEDOT-Dex. Moreover, despite the signals related to the polymeric matrix of PEDOT dominate both the C 1s and S 2p XPS spectra of PEDOT-Dex and PEDOT coatings, in the case of PEDOT-Dex a much more intense signal at 284 eV of the C 1s can be observed: this signal has been attributed to the sp^2 C=C component and is clearly related to the unsaturated carbons of the Dex structure.[15]

Table 1. Quantitative XPS analysis: atomic %.

element	F 1s	C 1s	S 2p	O 1s
Pedot-DEX	0.9	72.4	4.7	20.8
Pedot	0	71.5	7.8	19.1

It is known that the morphology of PEDOT films is strongly affected by the electrodeposition method, the size of the dopant as well as by the nature of the underlying electrodic material.[1b,16]

Scanning Electron Microscopy (SEM) analysis of PEDOT-Dex and the control PEDOT films reported in Figure 5 are.

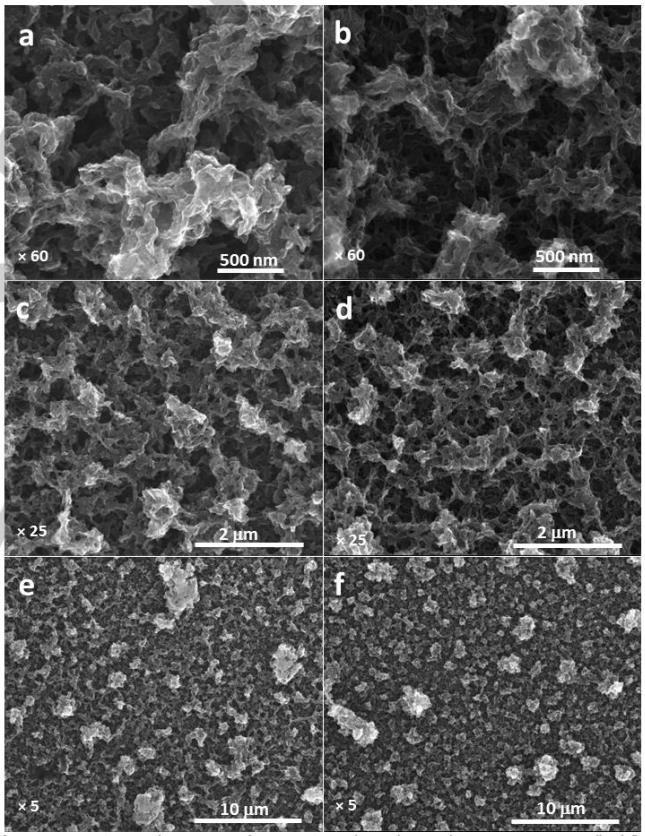

Figure 5. SEM images of PEDOT (a,c,e) and PEDOT-Dex (b,d,f) electrodeposited onto GC-plates.

The new PEDOT-Dex composite material exhibit a highly porous structure morphology very similar to the nanostructured porosity observed for the control PEDOT. This is the typical “sponge-like” porous structure exhibited be electrodeposited PEDOT films in conjunction with small dopants like perchlorate.[1b] Thus, this suggests that the co-deposition of the new monomer EDOT-Dex(**2**) and EDOT accounts for the efficient and covalent incorporation of Dex within the conductive polymer backbone, while preserving a porous and homogeneous morphology. It is

worth noting that high surface porosity is an extremely important goal for such applications, like neural sensing and stimulation, that need to increase the electroactive surface area in order to significantly reduce the total impedance of coated microelectrodes.[17]

To further confirm the presence of the covalent bond between Dex and the polymeric surface, a PEDOT-Dex electrode was soaked in phosphate buffer (PBS, pH=7.4) at room temperature for five days. Moreover, as detailed in the experimental section, electrodeposited PEDOT-Dex electrodes were treated in acetonitrile for five hours in order to remove any traces of *not-bound* EDOT and/or EDOT-Dex monomers, as confirmed by UV analysis (see Figure S2). The drug release was monitored by evaluating the absorbance at 242 nm assuming a value of the molar extinction coefficient of 13300 L mol^{-1} cm^{-1}, in accordance with literature.[5] After five days, the release was forced by dipping the electrode in an aqueous solution of sodium hydroxide (0.1N, pH =13). This strong basic treatment promoted a fast and complete release of the remaining part of incorporated Dex within five hours, as depicted in Figure S3. The increased rate of release at higher pH is consistent with the ester bond hydrolysis and clearly states the presence of a covalent bond between the drug and the conductive polymer matrix.

A total average amount of chemically linked Dex was estimated in the order of 235.39 ± 12.15 μg cm^{-2}, by treatment of PEDOT-Dex films electrodeposited onto GC electrodes with 0.1N sodium hydroxide for five hours (see Figure S4 and Table S5). Thus, the amount of covalently incorporated Dex is much higher if compared with the incorporation of Dex-P as a dopant of PEDOT, that have been previously estimated in the order of 140 μg cm^{-2}.[5b,d] It should be noted that a therapeutic bioactive concentration of Dex in the range of 0.2 μM was estimated, and that a release of 0.5 μg cm^{-2} of Dex has been calculated to correspond to a concentration of 1 μM within a 500-μm radius from the neural microelectrode.[18] This means that even a small fraction of released Dex from the PEDOT-Dex composite coating should provide a bioactive release of drug.

Having confirmed the covalent linkage between Dex and the conductive polymer surface, the next step of this study was to understand whether PEDOT-Dex is able to release the drug in its active form as a direct consequence of a biological trigger. PEDOT-Dex has the rationale to realize enzymatically hydrolysable bond, between Dex and the succinic spacer, and between the spacer and PEDOT backbone as well. Therefore, the drug release pathway is expected to be strongly influenced by the presence of hydrolytic enzymes including, for instance, carboxylesterases. It is known that the implantation of neural microelectrodes as well as their chronic persistence within the nervous tissue lead to the activation of the inflammatory tissue response thereby upregulating the production of lytic enzymes to aid in foreign body degradation.[19] Moreover, this class of enzymes are typically localized in many biological fluids and tissues, including human brain, and they are involved in the bioconversion of ester-based prodrugs, as in the case of PEDOT-Dex drug release system.[20] PEDOT-Dex films were incubated at 37°C in PBS (pH=7.4) and in PBS/esterase (porcine liver), respectively, and the released drug was monitored during 20 days by HPLC analysis. It is worth noting that hydrolysis can occur at the Dex-linker (succinate) site or at the EDOT-succinate site, respectively (see Scheme 1). In Figure 6a we report the release profile of PEDOT-Dex in PBS in absence and in presence of esterase, respectively. Results clearly outlined how the presence of the hydrolytic enzyme lead to a faster release of Dex in its biologically active form. More importantly, as outlined in Figure 6b, the pro-drug Dex-succinate (**1**) is predominantly released in absence of esterase whereas it was not detected when the experiment was conducted in presence of the enzyme. These results suggest that esterase accounts for a quick and selective hydrolysis of the ester bond between the drug and the spacer. Moreover, in the absence of esterase the release of Dexamethasone from PEDOT-Dex reached its plateau after ten days, when the release becomes due to the only prodrug Dexamethasone-succinate (Figure 6b). This particular behavior suggests that the main contribute of esterase is likely due to the quick hydrolysis of Dexamethasone-succinate released from the PEDOT-Dex coating. Similar results were reported for a Dexamethasone functionalized poly-aspartamide confirming the selectivity of esterase toward the hydrolysis at the spacer-drug ester bond.[21]

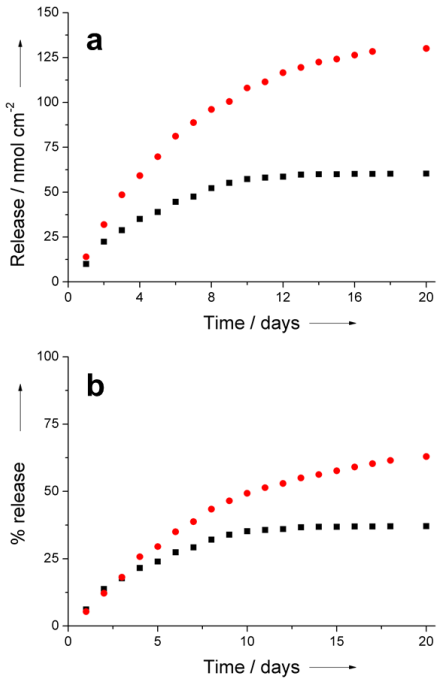

Figure 6. a) Cumulative release of Dexamethasone from PEDOT-Dex coatings in PBS buffer solution at pH 7.4 in absence (black squares) and in presence of porcine liver esterase (red circles); b) % of dexamethasone and dexamethasone succinate released in absence of esterase.

Finally, analysis of the UV spectra of the crude release solutions (Figure S6) shows that PEDOT-Dex coatings enable an efficient delivering of Dex. In fact, the ratio between the absorbance values at 242 nm and 270 nm, respectively, was calculated in the order of 2.43 for both Dex and released Dex, thereby confirming the absence of contaminants, in particular in the spectral zone typically ascribed to EDOT oligomers (270 nm).[8]

In conclusion, this work lays the foundation for a novel approach to the delivery of bioactive molecules from conductive polymers that can be used for diverse applications. It is important to note that our experiments confirm that PEDOT-Dex prolongs the drug delivery through a time window that extends over the typical timeframe of post-implantation inflammatory reaction.[2] Future investigations will be aimed to further optimize the molecular architecture in order to improve the drug release. Moreover, in vivo experiments with chronically implanted neural probes coated with PEDOT-Dex, will be performed to assess the extent of the inflammatory response and the self-adjusting activation of our new coating material.

Keywords: PEDOT • Dexamethasone •Electrochemistry • Copolymerization • Neural implants

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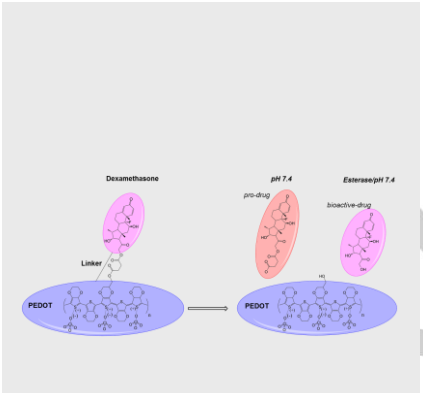
Layout 1:

COMMUNICATION

Let's make it self-releasing: For several years the delivery of bioactive molecules, including Dexamethasone, from conductive polymers has been based on the "*ionic-approach*" which needs an external electrochemical trigger. Our new protocol, based on the chemical conjugation of Dexamethasone to the PEDOT backbone, enables a biochemically controlled release of Dexamethasone in response to the inflammation of the surrounding tissue.



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Layout 2:

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