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A Simple Approach for Protein Covalent Grafting on Conducting Polymer Films

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Abstract

Covalent immobilization of biomolecules, such as proteins, on conducting polymer films is critical to organic bioelectronics to create tailored interfaces with biological systems. In this study, we propose a simple approach to graft proteins on films of the conducting polymer Poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS). PEDOT:PSS is a biocompatible and easy to process conducting polymer, widely used in bioelectronics. However, it does not possess any chemical reactive groups available for protein grafting. By mixing a commercial PEDOT:PSS suspension with the modified biopolymer carboxymethylated dextran (CMD), we obtained films displaying carboxyl (–COOH) groups allowing for covalent grafting of proteins via amide bonds, without any further functionalization step. By fine-tuning the concentration of CMD as well as those of a conductivity enhancer (glycerol) and a crosslinking agent (glycidoxypropyltrimethoxysilane, GOPS) in the film processing mixture, we were able to produce COOH-functionalized PEDOT:PSS films displaying excellent electrical conductivity and high stability in aqueous environment.

Keywords: Organic Bioelectronics; Thin Films; Conducting Polymers; PEDOT:PSS; Carboxymethylated Dextran; Biofunctionalization.

Introduction

Bioelectronics deals with the translation of electronic signals into biological signals and vice versa. Pacemakers, defibrillators and neural electrodes are a few examples of bioelectronic devices that have brought enormous benefits to our society.[1-3] Organic electronic materials, in particular conducting polymers, are emerging as the ideal candidates for bioelectronics, as they are able to sustain mixed ionic-electronic conduction, are easy to process on various substrates and can be used for controlled incorporation/release of biological species.[1-7] Conducting polymers are widely studied for several biomedical applications, such as biosensing, tissue engineering, drug delivery as well as neural electrodes for recording and stimulation.[8-11] Immobilization of biomolecules at the surface of conducting polymers is highly desirable for biosensors and bioelectronic implants, to create a specific interface with biological systems and to favor the integration with surrounding tissues.

The most common strategies to immobilize biomolecules at the surface of conducting polymers are doping/entrapment and covalent attachment. Doping and entrapment are typically achieved carrying out electrochemical polymerization in a solution containing the monomer and the biomolecule of choice. While doping is limited to charged species (typically anionic), entrapment is applicable to a wide range of neutral biological species. Peptides, drugs or proteins have already been entrapped in poly 3,4-ethylenedioxythiophene, (PEDOT) or polypyrrole films.[8, 12] However, doping and or entrapment are typically pursued when the conducting polymers are prepared by electrochemical polymerization, which is limited to conducting substrates. Moreover, entrapped biomolecules may display a weakened bioactivity due to the randomness of their immobilization and may undergo uncontrolled release due to their weak physical interactions with the conducting polymer.

A more versatile strategy to immobilize biomolecules at the surface of conducting polymer films is chemical functionalization followed by the covalent attachment of biomolecules (*e.g.* proteins). Chemical functionalization can be achieved by electropolymerization in presence of monomers or dopants holding a reactive functional group.[13-17] However, the chemical modification of monomers to introduce functional groups yields films with low electrical conductivity.[18, 19] The insertion, into conducting polymers films, of species bearing functional groups (*e.g.* chondroitin sulphate, heparan sulfate, COOH-terminated PEG) for subsequent attachment of biomolecules has also been achieved with electropolymerization and vapor phase polymerization.[18, 20, 21] More recently, covalent attachment of biomolecules at surface of solution-processable conducting polymer films has been achieved by adding polyvinyl alcohol to a commercially available dispersion of PEDOT:PSS prior to spin coating, followed by silanization and condensation reaction with a biomolecule.[22]

In this work, we propose a novel *single-step* approach to immobilize proteins on PEDOT:PSS via amide bonds, based on the mixing of the functional biopolymer carboxymethylated dextran (CMD) with a PEDOT:PSS suspension prior to film spin coating. Our approach permits to immobilize proteins directly on the PEDOT:PSS, without any additional step (e.g. silanization). Dextran is a well-defined polysaccharide in terms of molecular weight and polydispersity and it is widely used for its low-fouling properties, providing a barrier against non-specific adsorption of biological molecules.[23] Carboxymethylation of dextran allows the introduction of a controlled number of reactive –COOH groups, homogeneously distributed along the polymer chain. When films are deposited from mixtures containing CMD and PEDOT:PSS, the –COOH groups of CMD act as reactive sites for covalent grafting of proteins via amide bonds.[24] To assess the ability of the –COOH groups present at the film surface to react with proteins, we used an

enzyme-linked immunosorbent assay (ELISA), which allowed establishing a direct correlation between optical density measurements and the availability of –COOH groups for subsequent protein immobilization. Film thickness and electrical conductivity measurements were used to evaluate the impact of CMD, the conductivity enhancer glycerol and the crosslinker agent glycidoxypropyltrimethoxysilane (GOPS) on film electrical conductivity and long-term stability in aqueous media.

Materials and Methods

Materials and Reagents. Hydrogen peroxide (30% v/v), sulfuric acid (98% purity), isopropyl alcohol (98% purity), sodium hydroxide (98.7% purity) and potassium hydroxide (85+% purity) were purchased from VWR International Inc. MilliQ quality water (18.2 MΩ·cm; total organic compounds = 3 ppb) was generated with a Millipore Reference purification system. The aqueous suspension of PEDOT:PSS (Clevios™ PH1000) was purchased from Heraeus Electronic Materials GmbH (Leverkusen, Germany). Glycerol (99.5+ % purity) was donated by Caledon Laboratories Ltd (Georgetown, ON). Dodecylbenzenesulfonic acid (DBSA, 95+% purity), anhydrous 3-glycidoxypropyltrimethoxysilane (GOPS, 98+% purity), Phosphate Buffered Saline (modified PBS, without calcium chloride and magnesium chloride), Tween 20, N-hydroxysuccinimide (NHS, 98% purity), ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 99+% purity), 2-(N-morpholino)ethanesulfonic acid (MES) hydrate (99.5+% purity), MES sodium salt (99.5+% purity) and monochloroacetic acid (99+% purity) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). Dextran (M_w = 10, 70 and 500 kDa) was obtained from Pharmacosmos A/S (Holbaek, Denmark). Recombinant human epidermal growth factor (hEGF, catalog #236-EG) as well as DuoSet enzyme-linked

immunosorbent assay (ELISA) kit containing mouse anti-human EGF antibody (capture antibody), biotinylated goat anti-human EGF antibody (detection antibody), streptavidin-horseradish peroxidase (streptavidin-HRP), bovine serum albumin (BSA), and substrate solution (hydrogen peroxide/tetramethylbenzidine) were obtained from R&D Systems (Minneapolis, MN).

Surface Treatment. Prior to PEDOT:PSS film deposition, the glass slides used as substrates (2.5 cm × 2.5 cm) were immersed in a 3:1 mixture of sulfuric acid and hydrogen peroxide (Piranha solution) for 20 min at 80°C. After intensive rinsing with Milli-Q water, the surfaces were dried using a pressured steam of nitrogen. Subsequently part of the glass were silanized with GOPS using a previously reported procedure.[25] Briefly, the slides were immersed in a dry Schlenk containing 1% v/v GOPS in anhydrous toluene for 30 min under anhydrous argon atmosphere, subsequently rinsed in toluene, dried under a gentle stream of nitrogen and dried in an oven at 120 °C for 30 min.

Deposition and Characterization of PEDOT:PSS films. The PEDOT:PSS aqueous suspension (Clevios™ PH1000) was first sonicated for 15 min at room temperature (RT) to ensure an uniform distribution of the PEDOT:PSS particles. Spin coating was carried out from mixtures containing all or part of the following components (see Results and Discussions for details): Clevios™ PH1000, the surfactant dodecylbezenesulfonic acid (DBSA, constant concentration *of* 0.25% v/v for all films), used to facilitate film processing, the conductivity enhancer glycerol (0.5% to 5% v/v) and the biopolymers dextran or CMD (0.05 to 0.5% w/v). The crosslinking agent GOPS (1% v/v) was added just prior to spin coating, after filtering the mixture through a 5-µm polyvinyldiene fluoride filter. Spin coating was carried out at 500 rpm for 9 s followed by 1500 rpm for 45 s. Finally, the films were dried in an oven (120°C for 120

min). Film thickness was measured using a Stylus Profilometer (Dektak 150). Electrical conductivity measurements were performed using a four-point probe station (Jandel).

Synthesis and Characterization of Carboxymethylated Dextran (CMD). As previously reported,[24] 400 mg of dextran were dissolved in 10 mL of 3 M NaOH containing 1 M monochloroacetic acid. The solution was stirred for 2 h at RT. The carboxymethylation reaction was stopped by adjusting the pH to neutral with 18 M H_2SO_4. The solution was then filtered through a 0.2-μm PTFE filter, dialyzed five times against Milli-Q water for 1 h in order to remove excess reagents and salts, and finally lyophilized. As assessed by ^{1}H NMR spectroscopy,[24] the carboxymethylation degree of our 500-kDa dextran was 55%.

Capture Antibody Grafting and Assay Procedure. Capture antibodies were covalently grafted on PEDOT:PSS films containing carboxymethylated dextran (CMD) using carbodiimide chemistry in aqueous solution.[26] The films were immersed into a solution containing 200 mM of EDC and 50 mM of NHS in Milli-Q water for 5 min and successively rinsed with MilliQ water. The activated films were incubated with 4.0 μg/mL of capture antibody in 100 mM MES (pH 5.5) containing 2 mg/mL of 500-kDa pristine dextran for 15 min (see Results and Discussions for details). The films were then washed with a buffer consisting of 10 mM PBS and 0.05 % v/v Tween 20 (PBS-T). Remaining reactive groups were deactivated by immersion in ethanolamine (1 M, pH 8.5) for 20 min, followed by rinsing with PBS-T. The films on which antibodies had been grafted were incubated with 34 nM hEGF in 10 mM PBS (pH 7.4) containing 1% bovine serum albumin (PBS-BSA) for 30 min. After rinsing with PBS-T, the surface was incubated with 50 ng/mL of biotinylated detection antibody in PBS-BSA for 30 min. After a PBS-T wash, the surface was exposed to streptavidin-HRP (diluted 200 times with PBS-BSA) for 20 min. Following a final PBS-T wash, the HRP-catalyzed reaction was revealed with a substrate

solution (1:1 mixture of hydrogen peroxide/tetramethylbenzidine). After a 15-min incubation in the dark, the colorimetric reaction was stopped by adding 1 M H_2SO_4 (one third the volume of substrate solution). 100 μL of the colored solution was then transferred into a 96-well polystyrene plate. The optical density (O.D.) at 450 nm and 540 nm (for correction) was measured using an ELISA plate reader (Victor ^{3}V Multilabel Counter, Perkin Elmer).

Statistical Analysis. The results are expressed as mean ± standard deviation (*n,* number of independent results, $n \geq 3$). The mean values were obtained on at least three independent tests on at least three different samples. Statistical analysis was carried out using independent two-sample *t*-test with equal variances. A *p*-value lower than 0.05 was considered significant for all tests.

Results and Discussion

PEDOT:PSS films containing reactive –COOH groups were generated by mixing CMD with Clevios™ PH1000. To achieve a good balance between film electrical conductivity, chemical reactivity (i.e. availability of –COOH groups), and long term stability in aqueous environment, the conductivity enhancer glycerol and the crosslinking agent GOPS were added to the film processing mixture. In what follows, we discuss the effect of the various components present in the film processing mixture on film conductivity, availability of surface-exposed –COOH groups, and stability in aqueous environment.

Influence of Dextran and CMD on PEDOT:PSS Film Conductivity. The addition of CMD to PEDOT:PSS may have an impact on film conductivity, due to the presence of long polymeric chains and of –COOH groups. To disentangle and, thus, better understand the effects of the addition of dextran chains and –COOH groups, we first performed experiments with pristine dextran and successively with CMD.

Dextran chains of different molecular weights (*Mw* = 10, 70 and 500 kDa) were dissolved in Clevios™ PH1000 at concentrations ranging from 0.05 to 0.5% w/v. A slight increase in film conductivity (about two orders of magnitude, Fig. 1A condition B) was observed for dextran concentrations ranging between 0.05 and 0.1% w/v, with respect to pristine PEDOT:PSS films (*ca.* 0.05 S/cm, Fig. 1A condition A). The beneficial effect of dextran on conductivity started to decrease for concentrations above 0.2% w/v and totally vanished at 0.5% w/v. We found a similar trend for all the investigated dextran M_w, *i.e.* 10, 70 and 500 kDa (Fig 1B). Interestingly, when CMD was used instead of pristine dextran, we did not observe significant changes in film conductivity (Fig. 1A, conditions B and C, $p > 0.05$). This means that the carboxymethylation does not impact the film electrical properties. Based on the above results, for subsequent experiments requiring the presence of –COOH groups, we fixed the CMD concentration at 0.1% w/v and used the highest *Mw* (500 kDa).

Spin coated PEDOT:PSS films with electrical conductivities above 500 S/cm are nowadays routinely obtained from commercially available aqueous suspensions mixed with a conductivity enhancer (e.g. glycerol, ethylene glycol, dimethyl sulfoxide or sorbitol).[27] Despite the moderate increase in conductivity resulting by mixing Clevios™ with CMD, the addition of a conductivity enhancer to the processing mixture is still required to reach values of the order of a few hundreds S/cm. As reported elsewhere, the conductivity increase upon addition of conductivity enhancer is likely due to the fact that they alter the film morphology during drying, leading to lower energy barrier for charge carrier transport between individual PEDOT:PSS clusters.[27-29] Among the several available conductivity enhancers, we believe glycerol to be the most suitable for biological applications because of its low toxicity, ready availability at low cost and ease of process. When 5% v/v of glycerol was added to a film processing mixture containing 0.1% w/v

CMD or dextran, the films showed conductivities as high as *ca.* 500 S/cm (Fig. 1A, conditions E and F, $p > 0.05$), i.e. similar to those of PEDOT:PSS film processed in absence of CMD (Fig. 1A, conditions D, $p > 0.05$). These results indicate that low amounts of CMD (or dextran) in a film processing mixture containing a conductivity enhancer still yield PEDOT:PSS films with excellent electrical conductivity.

Availability of –COOH groups for covalent coupling of biomolecules on PEDOT:PSS/CMD films. The density of carboxyl group at a surface can be determined by several methods, mostly based on colorimetry and spectroscopy (*e.g.* infrared, X-ray photoelectron, fluorescence, time-of-flight secondary ion mass spectroscopy).[30] On the one hand, some colorimetric assays have been shown to be quite specific to surface-exposed functional groups, although they are generally more sensitive to charge.[31] On the other hand, most spectroscopic methods provide a bulk rather than a top surface characterization.[30-32] As our goal is to evaluate the presence of surface-exposed –COOH groups available for covalent coupling with biomolecules (we will hereafter designate them as 'reactive groups' for simplicity sake), we developed a method adapted from an enzyme-linked immunosorbent assay (ELISA). More specifically, the –COOH groups made available by the presence of CMD chains in PEDOT:PSS films were reacted with the free amino groups of a capture antibody, to create a covalent amide bond (Fig. 2A).[26] A standard 'sandwich' ELISA (Fig. 2B) was then used to evaluate the surface density of capture antibody. As a result, a direct relationship between the availability of the –COOH groups at the surface and the optical density (O.D.) value extracted from the ELISA could be established. The appropriateness of our ELISA-based strategy is assessed in Fig. 3, which shows a schematic illustration of the reactivity of the carboxyl groups at the surface of PEDOT:PSS films containing 0.1% w/v CMD.

As depicted in Fig. 2A, the activation of surface –COOH groups via standard NHS/EDC chemistry is followed by covalent coupling of the capture antibody. The NHS-activated –COOH groups remaining at the surface were deactivated by ethanolamine to prevent any further coupling of amine-containing molecules (Fig. 2).[26] Significant differences in the values of O.D. were observed when the deactivation step was performed before (mock surface) rather than after the incubation of the capture antibody, *i.e.* 0.28 and 0.12 for conditions C and A, respectively ($p < 0.05$, Fig. 3). When either the capture antibody or the human EGF was not added in the ELISA (Fig. 3, conditions B and D, respectively), the O.D. value remained low (ca. 0.08). Overall, these observations indicate that i) the O.D. value is related to the surface density of available –COOH groups and ii) the non-specific adsorption of hEGF (O.D. ca. 0.08) as well as that of the capture antibody (0.12 - 0.08 = 0.04), on PEDOT:PSS films are low. Remarkably, the covalent grafting of the capture antibody on PEDOT:PSS films containing CMD was further increased by modifying the buffer used for grafting. When pristine dextran (0.2% w/v) was added in the reaction buffer (100 mM MES, pH 5.5), a significant increase in O.D. from 0.28 ± 0.01 to 0.44 ± 0.03 was observed (compare condition A to E in Fig. 3). We believe that the dextran added to the buffer acted as a blocking agent towards the non-specific adsorption of the capture antibody (which likely lowers its concentration) on the labware used for ELISA.[33, 34]

As mentioned above, PEDOT:PSS films require a conductivity enhancer (in our case glycerol), to achieve reasonably high electrical conductivities. As the presence of glycerol may alter the surface composition of PEDOT:PSS films containing CMD, we evaluated its impact on the availability of –COOH reactive groups at the film surface (Fig. 4). A PEDOT:PSS film containing CMD only was used as the positive control (Fig. 4, condition B, O.D. = 0.41 ± 0.05), while a PEDOT:PSS film containing pristine dextran only was used as the negative control (no

antibody grafting, Fig. 4, condition A, O.D. = 0.14 ± 0.05). No significant influence on the density of covalently grafted antibody was observed upon addition of 0.5% v/v glycerol (Fig. 4, condition C, O.D. = 0.38 ± 0.02), whereas further addition of glycerol led to a significant decrease in grafted antibody (O.D. = 0.20 ± 0.04, Fig. 4, conditions D-F). This decrease may be due to an undesired interaction between glycerol and NHS-activated –COOH groups preventing antibody coupling, as previously observed at high glycerol concentrations.[35]

Stability of PEDOT:PSS/CMD films in physiological buffers. As all organic bioelectronic devices work in aqueous environment (most often physiological buffers), we investigated the stability of our PEDOT:PSS films by measuring changes in thickness and electrical conductivity after immersion for 24 hours in PBS, an electrolyte widely used for biochemical and bioelectronic applications (Fig. 5). For films containing Clevios™ PH1000, DBSA, CMD and glycerol, immersion in PBS led to complete film removal (Fig. 5, condition A). This observation is in agreement with recent findings, reporting that the thickness of films deposited from similar mixtures decreased of about 40% after only 10 minutes of immersion in pure water.[36] To improve the film stability we exploited the crosslinking properties of GOPS.[36-38] GOPS possesses three methoxysilane groups, known to react with silanols present on a glass surface via a silanization reaction, and an epoxy ring, which can react with nucleophilic groups, such as the –OH groups of CMD and glycerol.[39, 40] It has been already shown that the addition of GOPS to PEDOT:PSS film processing mixtures prevents film dissolution or delamination upon immersion into aqueous electrolytes. Here, we used two different approaches to improve films stability: GOPS was either covalently grafted via a silanization reaction on glass prior to film deposition (Fig. 5, condition B) or added (concentration 1% v/v) to the film processing mixture (Fig. 5, condition C). PEDOT:PSS films deposited on glass slides silanized with GOPS showed a

significantly improved stability and experienced a thickness decrease of about 50% (with respect to the initial film thickness) after immersion in PBS for 24 hours (condition B). The GOPS treatment provides a high density of epoxy groups, which can form multiple covalent bonds with the –OH groups of CMD. This multiplicity of bonds may thus favor the entanglement between PEDOT and CMD chains, which may significantly contribute to enhance film stability.

PEDOT:PSS films with excellent chemical and mechanical stability, showing no thickness loss after a 24 h immersion in PBS, were obtained when GOPS (1% v/v) was added to the film processing mixture prior to spin coating on untreated glass surfaces (Fig. 5, condition C). The addition of GOPS also led to a significant increase of film thickness (*i.e. ca.* 150 nm vs *ca.* 100 nm measured without addition of GOPS in the mixture). The enhanced stability and the higher thickness of films containing GOPS are likely related to a crosslinking between GOPS and other components of the mixture. The details of the crosslinking reaction are still unclear since several chemical reactions can take place between GOPS, the components of the mixture and the glass surface. Possible reaction paths leading to a network structure are: epoxy-hydroxy (etherification) reaction between GOPS and CMD or glycerol, condensation of the GOPS methoxysilane groups with the –OH groups of CMD, glycerol or the glass slide.[39-41] All these reactions are favored by the presence of water and by high temperatures.[40]

Electrical conductivity and reactivity of GOPS-stabilized PEDOT:PSS/CMD films.

The electrical conductivity of PEDOT:PSS/CMD films containing 1% v/v of GOPS increased upon increasing glycerol content, reaching about 200 S/cm for 2% v/v of glycerol (Fig. 6, condition D). No significant changes were observed when the glycerol concentration was increased from 2% to 5% v/v (Fig. 6, condition E). The film conductivity remained unchanged after a prolonged immersion in PBS, thus confirming the stability improvement upon GOPS

addition. As an example, a film containing 2% v/v glycerol (Fig. 6, condition D) showed the same electrical conductivity of about 200 S/cm before and after immersion in PBS for 24 h. Nevertheless, it is known that the presence of GOPS in PEDOT:PSS films, besides increasing mechanical and chemical stability, leads to a decrease of electrical conductivity.[36] For instance, upon addition of 1% v/v of GOPS, the conductivity of PEDOT:PSS/CMD films containing 5% v/v of glycerol decreased from 500 S/cm (Fig. 1A, condition E) to 200 S/cm (Fig. 6, condition E). As recently shown by X-ray photoelectron spectroscopy (XPS), the addition of GOPS to the processing mixture and subsequent crosslinking leads to a large content of non-evaporating and non-conducting species in the PEDOT:PSS films, which may explain both the decrease of electrical conductivity and the increase of thickness.[36] However, conductivity values close to 200 S/cm appear acceptable for bioelectronic applications.

The influence of glycerol addition on the ability of GOPS-stabilized films to covalently graft antibody, was studied using our ELISA essay (Fig. 7). In this experiment, glycerol-free films containing pristine dextran (no –COOH groups, conditions A) and CMD (high density of –COOH groups, condition B) were respectively used as the negative and positive control. As previously shown (Fig. 4), an increasing glycerol content resulted in a decrease of availability of surface-exposed –COOH groups. However, the presence of GOPS limited the detrimental effect of glycerol on the availability of surface-exposed –COOH groups. Indeed, in the absence of GOPS, negligible antibody grafting was observed for glycerol concentrations higher than 0.5% v/v (Fig. 4) whereas the addition of 1% v/v GOPS displaced this limit to 2% v/v (Fig. 7). As an example, for a glycerol concentration of 2% v/v, the loss in chemical reactivity was of *ca.* 35% in the presence of GOPS (with respect to control B in Fig. 7) and 90% in the absence of GOPS (with respect to control B in Fig. 4).

Overall, the presence of GOPS in the film processing mixture enabled to obtain PEDOT:PSS/CMD films displaying a satisfactory balance between protein grafting efficiency and electrical conductivity. In our conditions, *i.e.* 0.1% w/v of 500 kDa CMD and 1% v/v of GOPS in Clevios™ PH1000 suspension, these achievements were reached by adding 2% v/v of glycerol.

Conclusion

We demonstrated a simple and original approach for covalent protein grafting on PEDOT:PSS films. Our approach is based on the addition of the biopolymer CMD, a modified polysaccharide that *per se* reduces the non-specific adsorption of biomolecules, to the suspension used for the processing of the conducting polymer film. The addition of CMD to PEDOT:PSS films results in the presence of –COOH groups at the film surface that enable covalent grafting of proteins via amide bonds without any further functionalization step. By fine-tuning the concentration of CMD, the film crosslinking agent GOPS and the conductivity enhancer glycerol in the conducting polymer processing mixture, we were able to obtain –COOH functionalized PEDOT:PSS films with excellent electrical conductivity and long term stability in aqueous environment. We believe there are no chemical reactions between PEDOT:PSS and CMD for the reasons detailed below. The fact that we are able to graft proteins on PEDOT:PSS films via amide bonds reveals that the carboxylic groups of CMD remain available after mixing with PEDOT:PSS. Hydroxyl groups are present on PEDOT:PSS conductivity enhancers such as glycerol, sorbitol and ethylene glycol. It is well established that these compounds do not change the electronic structure of PEDOT. The addition of dextrane and CMD to mixtures containing PEDOT:PSS and the conductivity enhancer glycerol has no significant effects on electrical

conductivity, which indicates that the presence of the carboxyl group does not impact the electronic structure of PEDOT.

Work is in progress to incorporate our biofunctionalized PEDOT:PSS films into bioelectronic devices such as biosensors based on organic electrochemical transistors, implantable electrodes for *in vivo* stimulation and recording, and smart devices for cell culture and stimulation.

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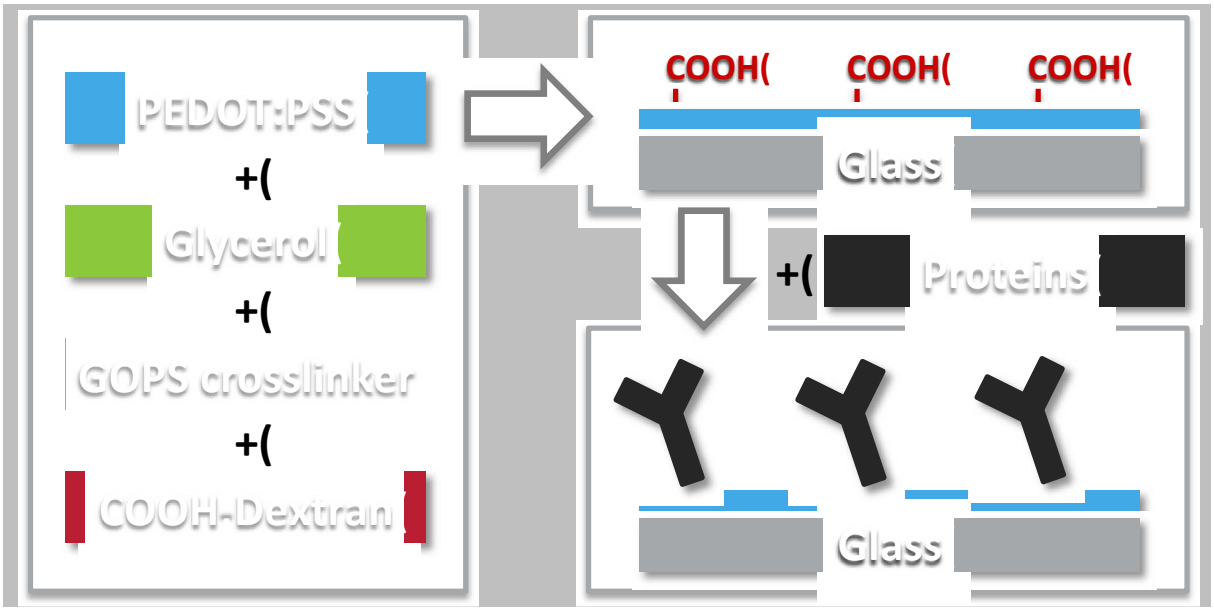
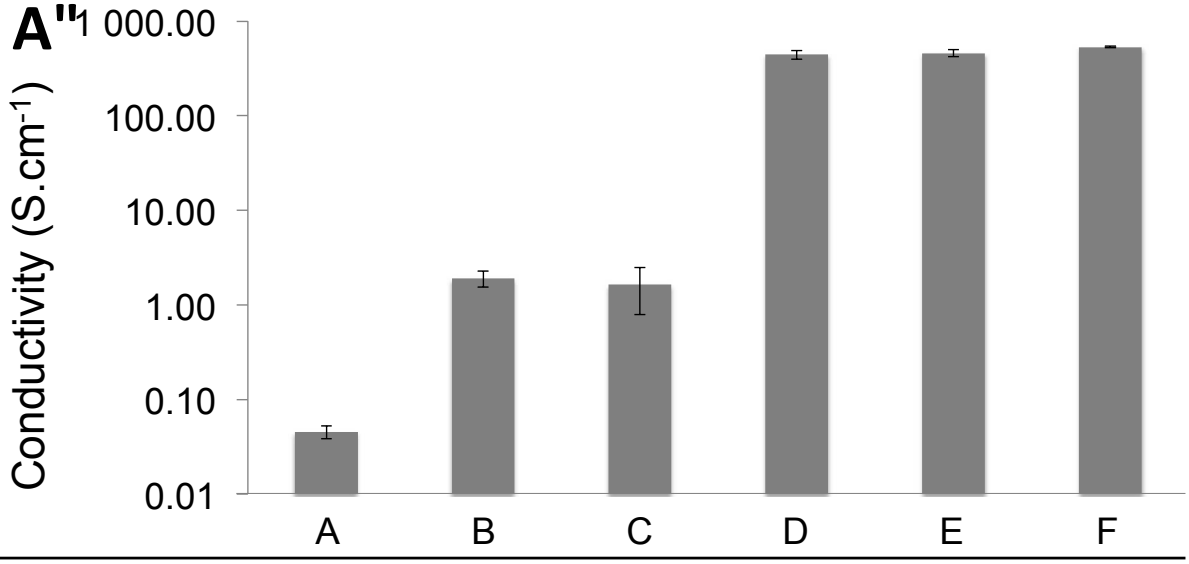


Figure 1. (A) Electrical conductivity of PEDOT:PSS films deposited by spin coating from a mixture containing all or part of the following components (the + signs indicate the presence of the component): Clevios™ PH1000, 0.1% w/v of unmodified 500-kDa dextran, 0.1% w/v of 500-kDa carboxymethylated dextran (CMD, degree of carboxymethylation = 55%) and 5% v/v of glycerol. (B) Impact of dextran and carboxymethylated dextran (CMD) on the conductivity of PEDOT:PSS films. Dextran (10 kDa, 70 kDa, 500 kDa) and CMD 500 kDa was added to the PEDOT:PSS suspensions (Clevios™ PH1000) prior to spin coating.



	A	B	C	D	E	F
PEDOT:PSS	+	+	+	+	+	+
Dextran, 0.1%		+		+		
CMD, 0.1%			+		+	
Glycerol, 5%				+	+	+

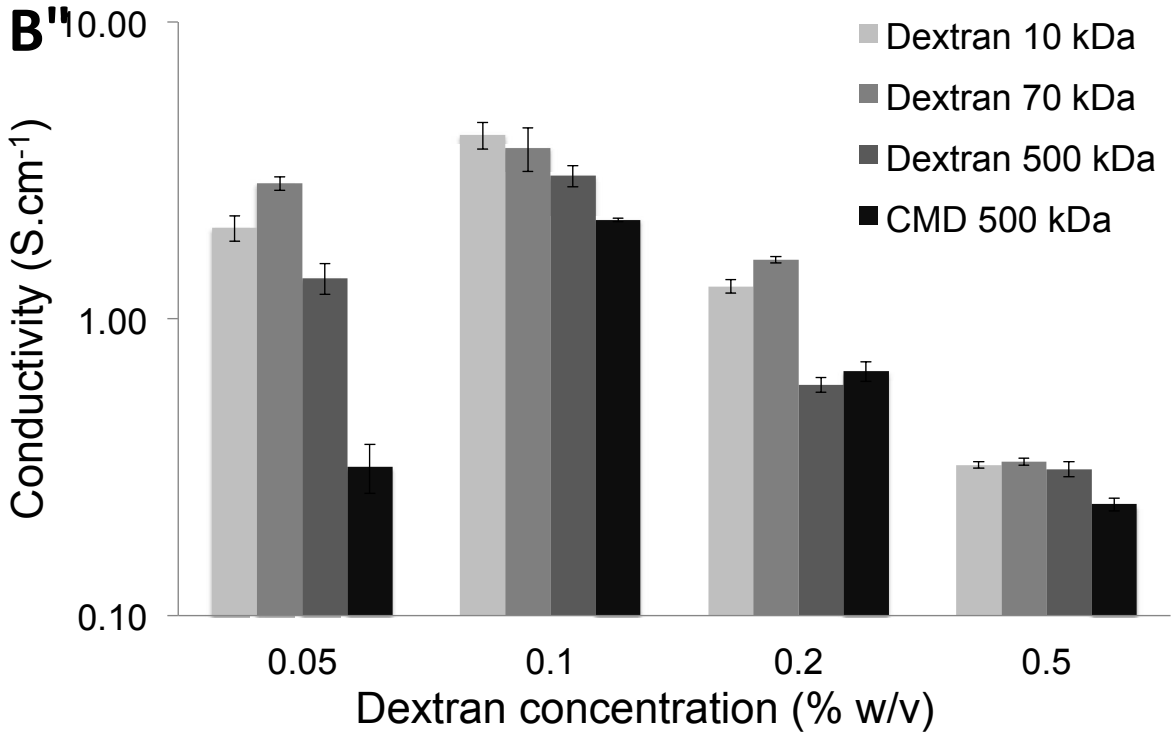


Figure 2. Schematic illustration of (A) the capture anti-human EGF antibody covalent grafting and (B) the ELISA procedure that was used to evaluate the amount of –COOH groups that are available to graft covalently anti-human EGF antibody on PEDOT:PSS/CMD films. Capture antibody (Ab) was covalently grafted onto the COOH-functionalized surface of the PEDOT:PSS film via NHS/EDC chemistry. Following the successive incubation of human EGF (hEGF), biotinylated detection Ab and streptavidin-horseradish peroxidase (streptavidin-HRP), a substrate solution was added. The uncolored substrate solution turns to colored after being oxidized by the HRP enzyme.

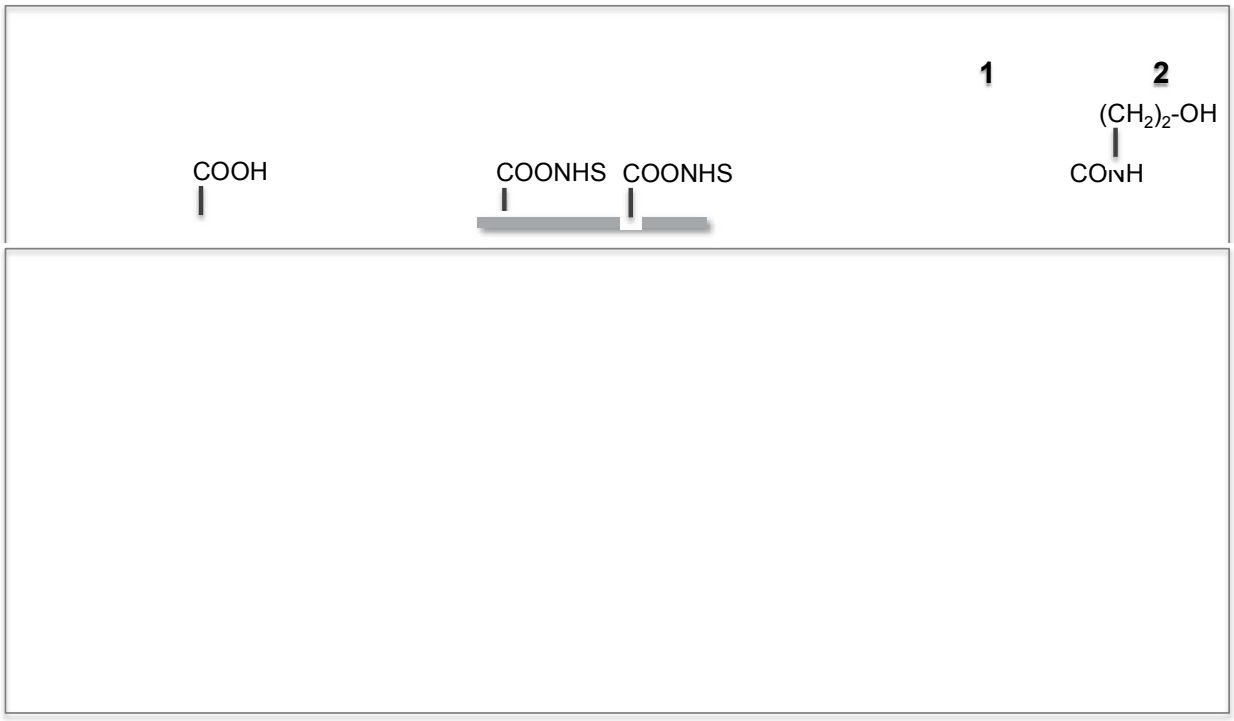
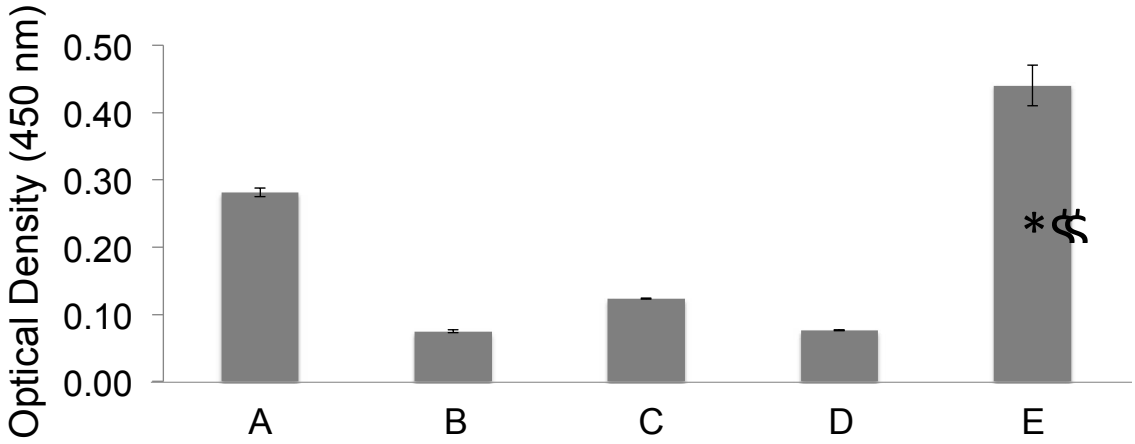
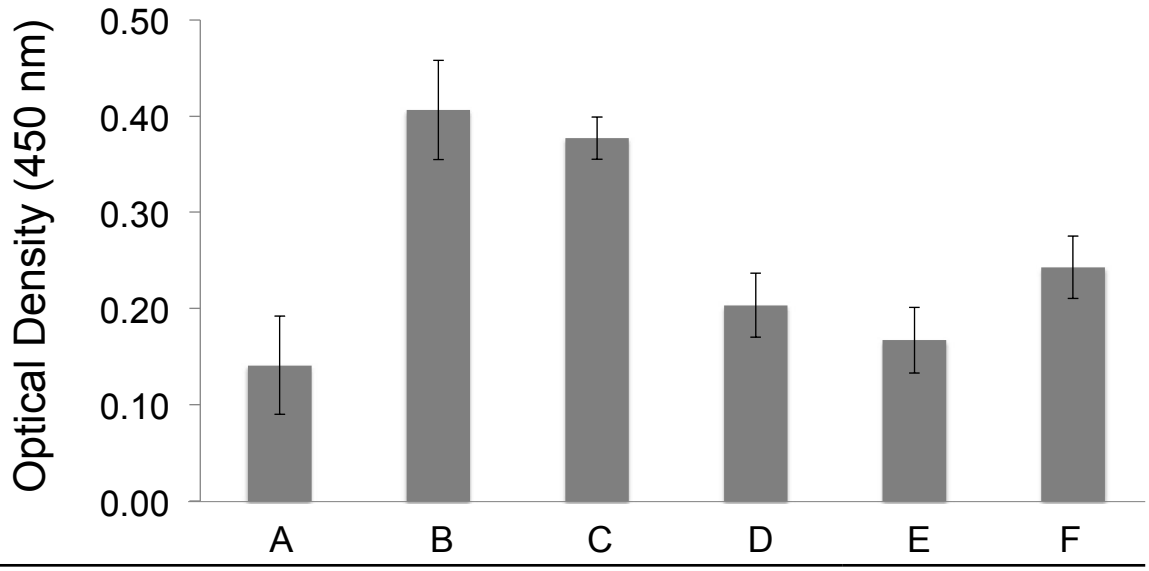


Figure 3. Reactivity of the carboxyl groups of PEDOT:PSS/CMD films: specificity of ELISA reagents. The tests were performed on PEDOT:PSS films containing 0.1% w/v CMD. Capture antibodies were grafted in 100 mM MES buffer (pH 5.5) The star (*) denotes that 0.2% w/v of unmodified dextran was added to the buffer. ELISA steps related to detection antibody (Ab), HRP and substrate were identical for all conditions.



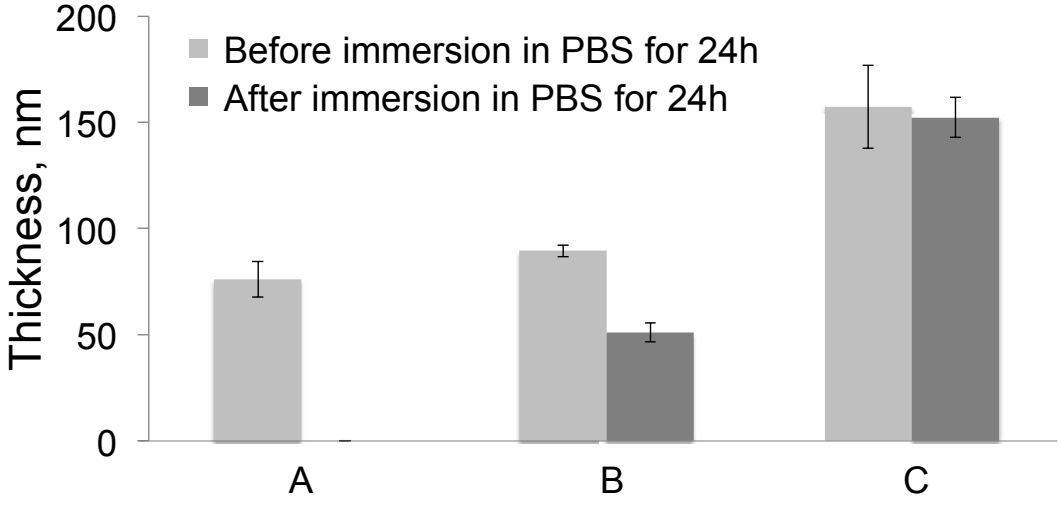
	A	B	C	D	E
NHS/EDC activation	+	+	+	+	+
NHS deactivation with ethanolamine			+		
Capture Ab	+		+	+	+
NHS deactivation with ethanolamine	+	+		+	+
hEGF	+	+	+		+

Figure 4. Influence of glycerol, on the reactivity of the carboxyl of PEDOT:PSS/CMD films. PEDOT:PSS suspensions containing 0.1% w/v unmodified dextran or CMD (concentration ranging from 0 to 5% v/v) were mixed with glycerol.



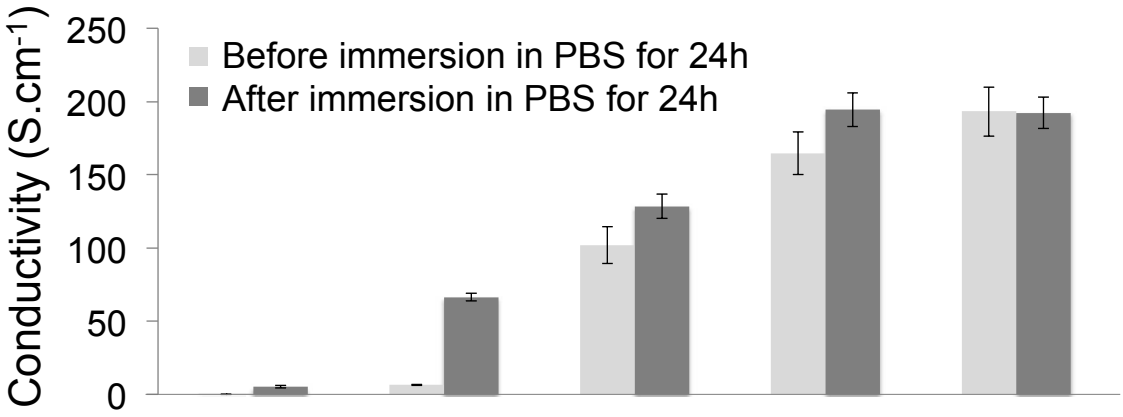
	A	B	C	D	E	F
PEDOT:PSS	+	+	+	+	+	+
Dextran	+					
CMD		+	+	+	+	+
Glycerol (%)			0.5	1	2	5

Figure 5. Stability of PEDOT:PSS films in PBS. PEDOT:PSS suspensions containing CMD (0.1% w/v), glycerol (1% v/v), with or without glycidoxypropyltrimethoxysilane (GOPS, 1% v/v) were spin-coated on pristine (conditions A and C) or GOPS-treated (condition B) glass surfaces. A profilometer was used to measure the thickness before and after immersion in phosphate buffered saline (PBS, 10 mM, pH 7.4).



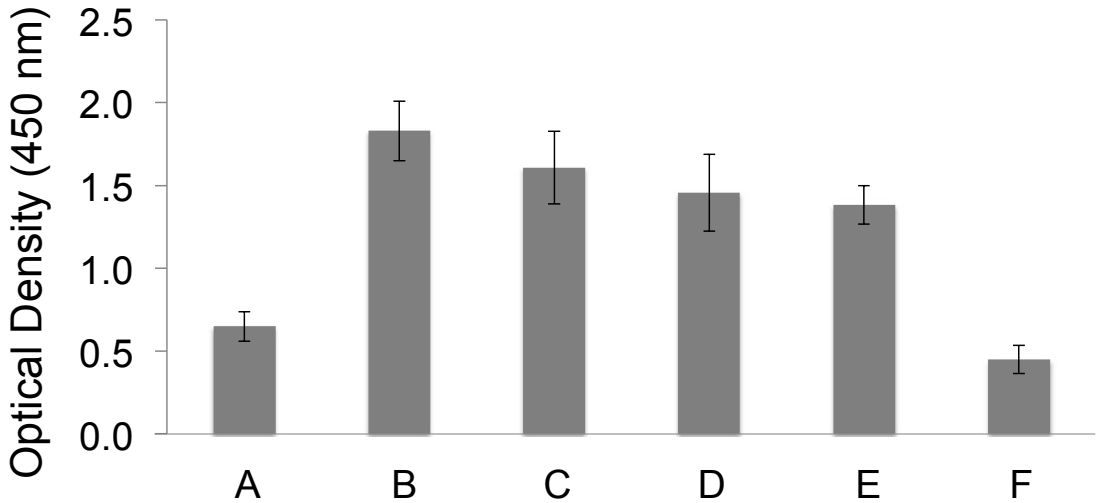
	A	B	C
Glass silanization with GOPS		+	
PEDOT:PSS	+	+	+
CMD	+	+	+
Glycerol (1%)	+	+	+
GOPS			+

Figure 6. Impact of glycerol on the conductivity of GOPS-containing PEDOT:PSS films. Prior to the spin coating and the drying processes, PEDOT:PSS suspensions containing 0.1% w/v CMD and 1% v/v GOPS were mixed with glycerol (concentration ranging from 0.8% v/v to 5% v/v).



	A	B	C	D	E
PEDOT:PSS	+	+	+	+	+
GOPS	+	+	+	+	+
CMD	+	+	+	+	+
Glycerol (%)		0.8	1	2	5

Figure 7. Impact of glycerol on the –COOH groups reactivity in GOPS-containing PEDOT:PSS films. Prior to the spin coating and the drying processes, PEDOT:PSS suspensions containing 0.1% w/v CMD and 1% v/v GOPS were mixed with glycerol (concentration ranging from 0 to 5% v/v).



	A	B	C	D	E	F
PEDOT:PSS	+	+	+	+	+	+
GOPS	+	+	+	+	+	+
Dextran	+					
CMD		+	+	+	+	+
Glycerol (%)			0.8	1	2	5

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By mixing a PEDOT:PSS suspension with the modified biopolymer carboxymethylated dextran (CMD), we obtain conductive films displaying carboxyl (–COOH) groups allowing for covalent grafting of proteins via amide bonds.