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Porous bioelectronic substrates for simple electrochemical conjugation and subsequent, controlled electrochemical release of antisense oligonucleotide drug†

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Materials and methods that enable selective and controllable conjugation and release of chemical and biological substances are in demand for various applications, such as drug delivery, cell stimulation, and cancer therapy. Stimuli-responsive systems for controlled release and enhanced drug efficacy are of particular interest. We have here developed an electrochemically addressable system for the specific conjugation and release of connexin43 antisense as a wound-healing agent. The system is based on a conducting terpolymer, P(EDOT-co-EDOTSAC-co-EDOTEG), which was coated onto both carbon cloth and highly porous electrospun substrates. We optimized the conducting terpolymer composition and characterized and compared the terpolymer-coated substrates. The conductive terpolymer contains EDOTSAC, which has an acetylthiomethyl group that can be transformed into a “free” thiol. This thiol can undergo reversible oxidation/reduction cycles at moderate potentials (+1.0 and −0.8 V, respectively) to enable electrochemical conjugation and controlled release. The system was able to quickly conjugate (1 min) and release (5 min) a model thiol molecule in a highly controllable manner, which was demonstrated with, and compared between, carbon cloth and electrospun fiber substrates. Furthermore, successful conjugation and release of connexin43 antisense were demonstrated. Overall, this electrochemical system offers a porous and flexible platform with electrochemically stimulated conjugation and release capabilities that holds significant potential for wound dressing applications.

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Introduction

There is a significant demand for materials and methods that allow for the selective and controllable capture and release of chemical and biological substances.^{1–3} Research efforts have been directed toward the capture and release of a wide range of clinically relevant targets, such as proteins,^{4,5} small drug molecules,^{6,7} extracellular vesicles (EVs),^{8,9} cells^{1,10–12} and DNA^{5,13} for a number of applications, including drug delivery,¹⁴ cell stimulation¹⁵ and cancer therapy.^{16,17} Stimuli-responsive systems for gene and

drug delivery have gained widespread interest as they offer controlled and sustained release, enhanced drug efficacy and reduced potential side effects.^{1,5,18,19} Different methods have been developed to capture and release oligonucleotides by using stimuli, such as heat,²⁰ light,^{5,21,22} aminosilane-modified bacterial magnetic particles,²³ modified Fe₃O₄/Au nanoparticles,²⁴ host–guest binding and electrostatic interaction^{5,25} and surfactants.²⁶

The electrochemically-induced release is a promising method for delivering chemical and biological entities rapidly and easily, using electrical potential or current to control cargo release without additives.^{1,8,27} This approach requires electrically conductive materials that undergo oxidative/reductive cycles at mild potentials to efficiently capture and release the desired cargo.^{28,29} For instance, Gao *et al.*³⁰ developed an electrochemical system for controllable capture and release of cancer cells using β -cyclodextrin (β -CD) and ferrocene (Fc) through a host–guest interaction. Cancer cells were modified with a polyethylenimine (PEI) polymer scaffold conjugated with Fc and co-linked with folic acid (FA). Reduction potential facilitated binding of uncharged Fc to immobilized β -CD on

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the electrode surface, while oxidation potential dissociated the Fc- β -CD complex, thereby releasing the captured cells.

Abidian *et al.*²⁹ demonstrated a noteworthy approach to achieve controlled release from electrospun nanofibers composed of biodegradable poly(L-lactide) infused with dexamethasone. Their study involved the electrochemical deposition of a PEDOT conducting-polymer layer around the drug-loaded nanofibers. By applying an external electrical stimulation of +1 V to the PEDOT nanotubes, they effectively controlled the release of dexamethasone from the nanofibers.

Yuan *et al.*³¹ synthesized non-covalently grafted polymers that are capable of self-assembling into micellar structures using β -cyclodextrin modified dextran (Dex-CD) and Fc terminated poly(ϵ -caprolactone) (PCL-Fc). The presence of an Fc group on the polymer allowed for potential-controlled modulation of hydrophilicity/hydrophobicity, enabling self-assembly and disassembly of the micelles. These micellar structures were explored as drug carriers for controlled-release applications.³¹ Jiao *et al.*³² integrated poly(*N*-isopropylacrylamide) hydrogel with a redox-responsive cross-linkers (*N,N'*-bis(acryloyl)cystamine, BAC) into a microfluidic device using photopolymerization. The hydrogel “dots” captured and released modified protein bovine serum albumin (BSA) by reforming redox-responsive disulfide bonds, thereby altering the hydrogen dot densities. While several methodologies have been developed recently for electrochemical release and delivery, they have limitations, such as the need for high release potentials and the complexity of fabrication.¹

On-skin electronics have attracted significant attention and have been successfully utilized in a wide range of applications in human healthcare, human-machine interfaces, soft robotics, biological studies and augmented reality.^{33–35} Despite significant recent developments, current on-skin devices are not sophisticated enough to measure complex physiological conditions. Chronic non-healing wounds exemplify a challenge for existing skin electronics, as they fail to provide an efficient solution.³⁶ This can be attributed to the intricate and dynamic nature of the wound-healing process, which is influenced by various factors. These factors encompass patient co-morbidities like diabetes and renal failure, as well as local wound characteristics such as growth factors, inflammatory mediators, and the presence of microbial burden.^{37–39} Modulation of the wound environment by delivery of therapeutics in a controlled and personalised manner offers a different approach to treating chronic wounds.^{40–42} Electrochemical techniques offer new opportunities for the retention of wound healing agents and bioactives and their controlled release to the wound surface.^{27,43}

We have recently reported the development of a sensor for detecting biothiols based on a conductive polymer called poly(EDOT-thioacetate-*co*-EDOT).²⁸ This sensor utilizes the thioacetate moieties on the conducting polymer, which can be electrochemically deprotected to the corresponding thiols that can then form disulfide bonds upon electrochemical oxidation, allowing for the reversible capture and release of biothiols.²⁸ We have further developed this technology by using similar

conductive polymer-coated substrates, to create an electrochemically switchable interface for the controlled capture and release of biological targets, such as human cancer cells, antibodies and extracellular vesicles (EVs) from breast cancer cells and placenta.^{1,8,9,44,45}

Building on these previous works, we present a system with the conducting-polymer deposited onto carbon cloth (CC) and electrospun polycaprolactone (PCL) fibers (EF) mats. The benefit of CC and electrospun fibers are that they have high surface porosity, conductivity and biocompatibility^{8,9} which render them suitable surface-conforming materials for wound healing applications, where these properties are key attributes. Through optimization of the terpolymer composition and its redox chemistry, the terpolymer [P(EDOT-*co*-EDOTSac-*co*-EDOTEG)] was electropolymerized on the CC and EF mats substrates. These were investigated as novel platforms to actively electrochemically control the delivery of wound healing agents using simple redox chemistry.

Anti-sense oligodeoxynucleotides (ODNs) are synthetic DNA molecules that allow for *in situ*, site-specific downregulation of connexin protein expression.⁴⁶ The downregulation of connexin expression in wound healing has multiple therapeutic potentials, including wound healing, reduced inflammation, decreased scar formation, and skin rejuvenation and thickening.⁴⁶ Our platform enables the controlled and customized direct delivery of connexin43 antisense for various wound conditions, especially chronic wounds where connexin43 expression is dysregulated.^{47,48} Matching the release rate of the connexin43 antisense molecule to the specific healing stages and conditions of the patient's wounds has the potential to enhance the effectiveness of personalized wound treatments.

Here, we successfully demonstrate that the conductive terpolymer-coated substrates enable electrochemical conjugation and on-demand release of connexin43 oligodeoxynucleotides *via* reversible oxidation/reduction cycles at +1.0 V and −0.8 V (vs. Ag/AgCl), respectively. These findings open a door to future studies towards the delivery of connexin43 and other therapeutics to wounds, including chronic wounds.

Experimental section

Materials

Epichlorohydrin, triethylene glycol monomethyl ether, potassium *tert*-butoxide (*t*BuOK), polycaprolactone (PCL, MW 80 000), 2-mercaptoethanol, (2ME), 6-mercaptohexanoic acid (MHA), *n*-hydroxysuccinimide (NHS), DL-alanine, fetal bovine serum (FBS), glutaraldehyde, paraformaldehyde (PFA), lithium perchlorate, sodium chloride, and calcein-AM were purchased from Sigma-Aldrich. Thioacetic acid *s*-potassium salt, dimethoxythiophene, 3,4-ethylenedioxythiophene, *p*-toluenesulfonyl chloride, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were purchased from AK Scientific. *o*-Phthalaldehyde was purchased from Acros Organics. Thiolated-ODN of connexin43 antisense (Nexagon) (5'-GTAATTGCGGCAAGAAGAATTGTTTCTGTC-3') was supplied



The conductivity of samples was measured using a four-point probe conductivity meter (Janel RM2), taking an average of 5 points per electrode.

Statistical analysis

The unpaired *t*-test, one-way ANOVA ($\alpha = 0.05$) and Tukey's HSD multicomparison tests were used to test for statistical significance between the groups. Results are reported as an average $\pm$ standard deviation (SD). The graphs are reported with error bars representing the SD. All statistical analyses were performed using GraphPad Prism 8.

Results and discussion

Design, fabrication and characterization of conjugation/release system using terpolymer-coated conductive substrates

Two distinct conducting substrates were fabricated to construct materials capable of electrochemically-controlled conju-

gation and release. Fig. 1 shows the steps involved in each of the substrate's fabrication processes. Gold-coated electrospun PCL fiber mat (EF) and carbon cloth (CC) were used as the starting substrates. PCL is a nontoxic and biocompatible polymer that could be easily electrospun into a porous fiber mat membrane.^{1,49} The EF was made electrically conductive by sputter-coating both sides of the mat with a 20 nm-thick layer of gold. CC electrodes were washed to remove any impurities before the electropolymerization step. Electropolymerization of three monomers: 3,4-ethylenedioxythiophene (EDOT), an acetylthiomethyl-substituted EDOT *S*-[(2,3-dihydrothieno[3,4-*b*][1,4]dioxin-2-yl)methyl] ethanethioate, denoted as "EDOTSac", and EDOT substituted with three ethylene glycol units 2-(2,5,8,11-tetraoxadodecyl)-2,3-dihydrothieno[3,4-*b*][1,4]dioxine, denoted as "EDOTEG", was performed onto 0.5 ×

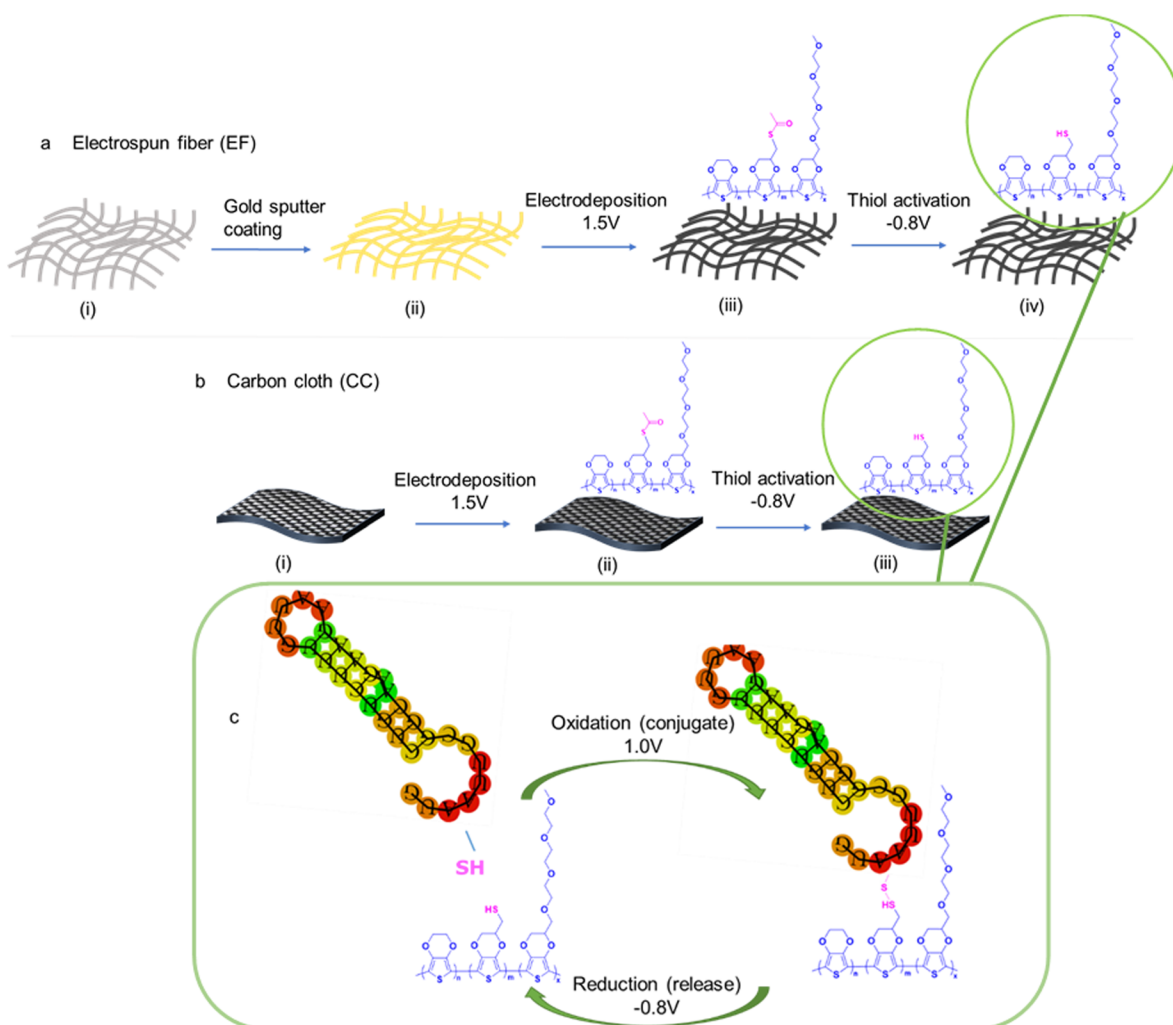


Fig. 1 Schematics showing: (a) The fabrication procedure of the electrospun fiber (EF) mat coated with the terpolymer: (i) PCL membrane electrospinning, (ii) sputter coating of gold on PCL membrane, (iii) P(EDOT-co-EDOTSac-co-EDOTEG) terpolymer electropolymerization, (iv) thiol activation by electrochemical reduction of P(EDOTSac) to P(EDOTSH). (b) The fabrication procedure of the carbon cloth substrate with electropolymerized P(EDOT-co-EDOTSac-co-EDOTEG) terpolymer (ii), and after thiol activation by electrochemical reduction of P(EDOTSac) to P(EDOTSH) (iii). (c) Electrochemical coupling of thiol-functionalised connexin43 through the oxidative formation of disulphide and cleavage by reduction of the disulfide, on both the P(EDOTSH) EF and P(EDOTSH) CC substrates. (The figure is a schematic representation and may not be drawn to an exact scale.)



0.5 cm pieces of gold-coated EF and 0.5×0.5 cm pieces of CC using chronoamperometry. The incorporation of the EDOTSac enables the conjugation and subsequent release functionality of these substrates through electrochemically forming and breaking disulfide bonds. The EDOTEG was used to increase the substrate's hydrophilicity and antifouling properties, and the unmodified EDOT was used as a spacer for the other two functionalized EDOTs.²⁸

The electropolymerization of the terpolymer P(EDOT-co-EDOTSac-co-EDOTEG) on CC and PCL EF mats was conducted in 0.1 M LiClO₄ water:acetonitrile (1:1 vol:vol) containing 5.3 mM EDOTSac (5%), 40 mM EDOT (80%) and 8 mM EDOTEG (15%). The potentiodynamic polymerizations of the terpolymers were performed in a potential range from -0.2 V to $+1.25$ V (vs. Ag/AgCl) at a 100 mV s^{-1} scan rate (Fig. 2a) or using a constant potential at $+1.5$ V for 2 min (Fig. 2b). The electropolymerization voltammograms of the terpolymer onto

CC showed increased currents on the subsequent potential sweep cycles, suggesting the successful coating of the terpolymer onto the CC (Fig. 2a). The onset potential of electropolymerization for the terpolymer was $+1.1$ V.

It was shown earlier by us that a potential of -0.8 V (vs. Ag/AgCl) reduces the acetylthiomethyl moiety on the EDOTSac to a thiol (denoted as EDOTSH);²⁸ here -0.8 V was applied for an optimized time of 30 s for the activation of the thiol (Fig. 2c). Thiolated-ODN were then conjugated to the P(EDOT-co-EDOTSH-co-EDOTEG) through the electro-oxidative formation of a disulfide covalent bond between the two thiol groups by applying a potential of $+1.0$ V for 60 s (Fig. 2d). The conjugated ODN was shown to be able to be released by applying a potential of -0.8 V (vs. Ag/AgCl) for 300 s (Fig. 2e).

Water contact angle measurements showed a significant decrease in contact angle from $112.45 \pm 2^\circ$ for CC and $68.22 \pm 4^\circ$ for gold-coated EF to 0.0° upon the electrodeposition of the

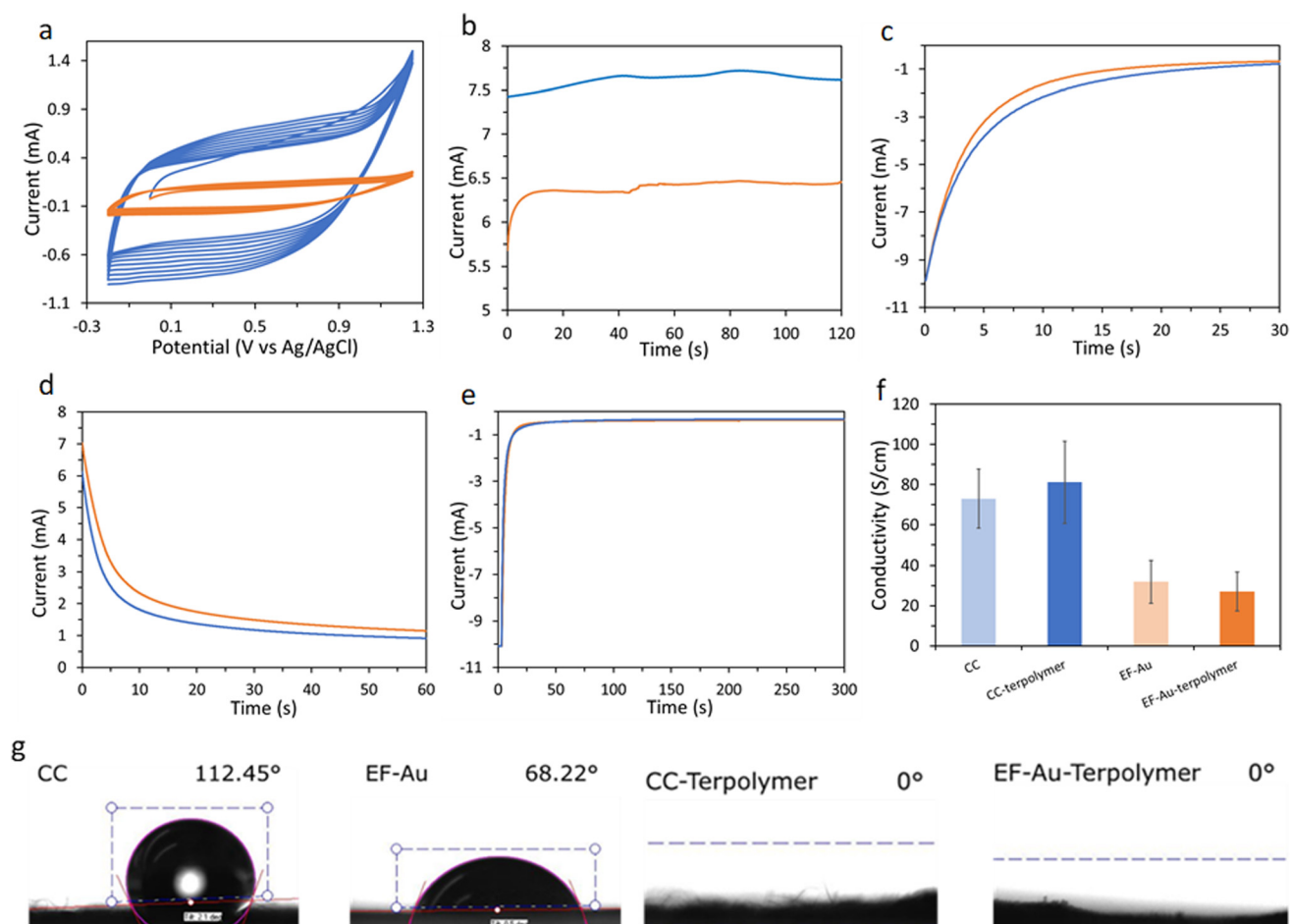


Fig. 2 (a) Potentiodynamic electropolymerization of P(EDOT-co-EDOTSac-co-EDOTEG) on carbon cloth (CC) (blue line) and PCL electrospun fibers (EF) (orange line) in 0.1 M LiClO₄ in water:acetonitrile (1:1 vol:vol) at 100 mV s^{-1} , in the potential range from -0.2 to $+1.25$ V. (b–e) Chronoamperograms of carbon cloth (CC) (blue line) and PCL electrospun fibers (EF) (orange line): (b) the electropolymerization of the terpolymer P(EDOT-co-EDOTSac-co-EDOTEG) at $+1.5$ V (vs. Ag/AgCl) for 2 min. (c) electrochemical reduction of P(EDOT-co-EDOTSac-co-EDOTEG) at a potential of -0.8 V (vs. Ag/AgCl) for 30 seconds, (d) Conjugations of thiolated-ODN to the P(EDOT-co-EDOTSH-co-EDOTEG) at the potential of $+1.0$ V (vs. Ag/AgCl) for 60 s. (e) chronoamperograms of the electrochemical release of ODN at -0.8 V (vs. Ag/AgCl) for 5 min. (f) Electrical conductivity of the CC and the gold-coated EF mats before and after electrodeposition of P(EDOT-co-EDOTSac-co-EDOTEG). (g) Water contact angle measurements on CC and gold-coated EF before and after electrodeposition of P(EDOT-co-EDOTSac-co-EDOTEG).



P(EDOT-co-EDOTSac-co-EDOTEG) (Fig. 2g). The increase in the hydrophilicity of the terpolymer-coated samples could be attributed to the presence of the hydrophilic ethylene glycol substituents on the polymer. The hydrophilic nature of the surface is expected to be advantageous in its practical biological applications, as it leads to increased wettability and decreased biofouling. The electrical conductivity of CC and gold-coated EF was measured before and after the electrodeposition of P(EDOT-co-EDOTSac-co-EDOTEG) layer using a four-probe conductivity meter (Fig. 2f). The CC substrate

showed a conductivity of $73.04 \pm 14 \text{ S cm}^{-1}$ which was higher compared to the gold-coated EF substrate ($31.89 \pm 10 \text{ S cm}^{-1}$). The conductivity of CC and EF substrates did not change significantly after the P(EDOT-co-EDOTSac-co-EDOTEG) electrodeposition.

Scanning electron microscopy (SEM) analysis revealed the morphology of the EF and the CC before and after the electrodeposition of the terpolymer (Fig. 3). The SEM images of the bare CC (Fig. 3a–c) and EF (Fig. 3g–i) showed fibers with smooth surface morphologies.

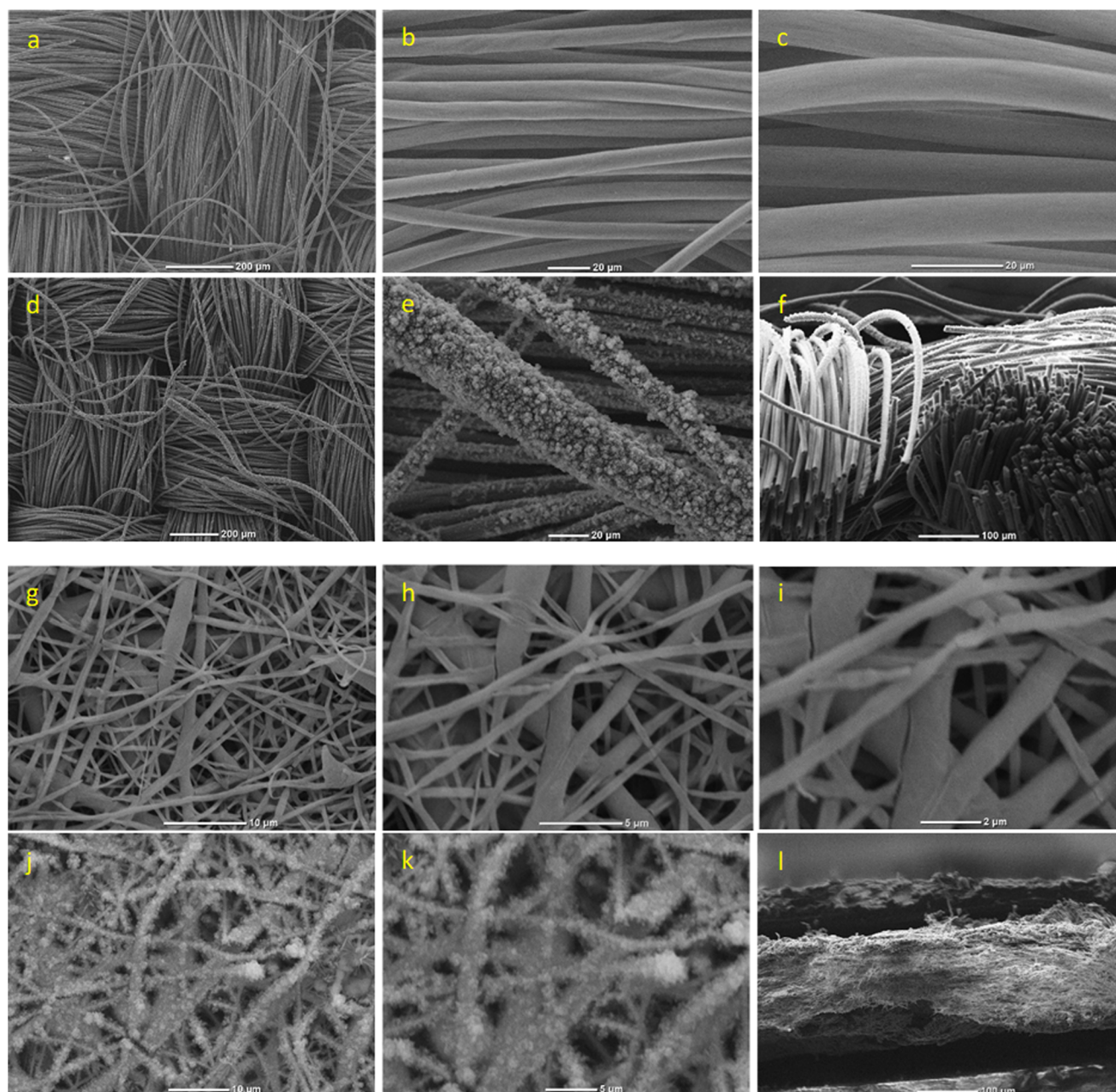


Fig. 3 SEM images of: (a–c) the uncoated carbon cloth (CC), (d and e) EDOT-co-EDOTSac-co-EDOTEG coated CC, (f) Cross section of EDOT-co-EDOTSac-co-EDOTEG coated CC. (g–i) uncoated electrospun fibers (EF). (j and k) EDOT-co-EDOTSac-co-EDOTEG coated EF, (l) cross-section of EDOT-co-EDOTSac-co-EDOTEG coated EF.



In contrast, the cross-section of terpolymer-coated electrospun fibers exhibited a prominently thick, dense, and uniform PEDOT layer on the surface of the gold-coated fibers (Fig. 3i). A

The observed increase in the CV currents (Fig. 4a) upon electrodeposition of terpolymer onto the CC and gold-coated

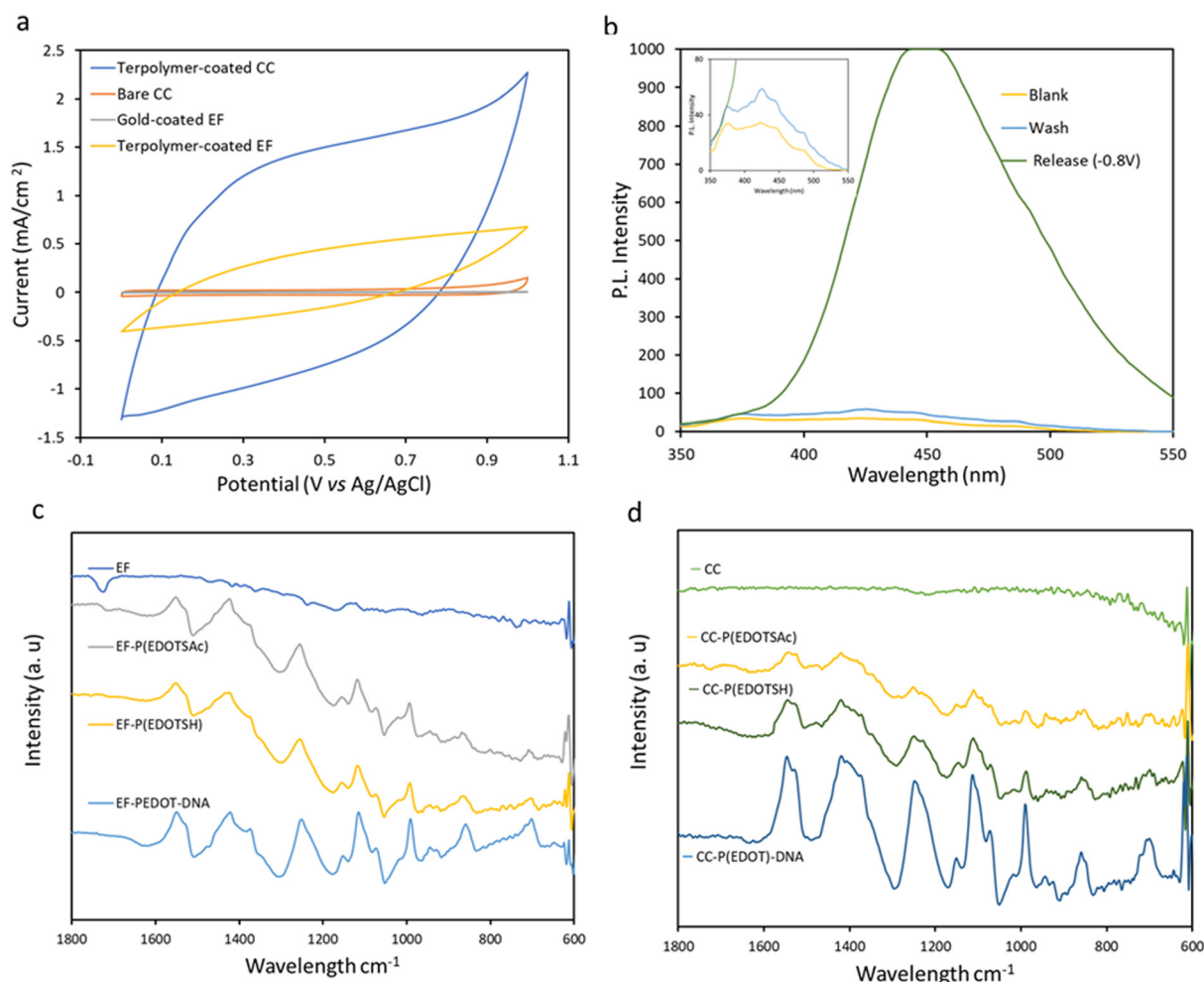


Fig. 4 (a) Cyclic voltammetry profile of the CC and gold-coated EF before and after electrodeposition of the terpolymer. (b) The fluorescence emission spectra confirming the electrochemical release of 2ME from terpolymer-coated CC ($\lambda_{\text{ex}} = 330 \text{ nm}$). 2ME is conjugated by electrochemical oxidative coupling at +1.0 V (Ag/AgCl) on the CC; then, CC is kept in a PBS solution for 30 min before release (wash). 2ME was then released at -0.8 V from the CC substrate into PBS containing $1 \times 10^{-3} \text{ M}$ *o*-phthalaldehyde and alanine. PBS solution of $1 \times 10^{-3} \text{ M}$ *o*-phthalaldehyde and alanine was used as the blank sample. FTIR spectra of terpolymer coated (c) EF and (d) CC before and after electropolymerization, thiol activations and conjugation of ODN.

EF can be attributed to the deposited electroactive terpolymer. The CVs taken in PBS at a scan rate of 100 mV s^{-1} showed pseudo-rectangular CV shapes.

Based on the integrated area of the CV curves in Fig. 4a, the terpolymer-coated CC exhibited higher capacitance ($7.78 \pm 0.64 \text{ mF cm}^{-2}$) than the terpolymer-coated EF ($2.11 \pm 0.31 \text{ mF cm}^{-2}$), indicating higher electrodeposition of PEDOT on CC than on EF. At the scan rate of 100 mV s^{-1} , the capacitance of the samples completely screened the currents from redox processes. The CV profile of the terpolymer-coated samples at a lower scan rate of 5 mV s^{-1} (Fig. ESI 1†) showed a reduction peak of the terpolymer at -0.68 V .

2-Mercaptoethanol (2ME) was used as a small model molecule to demonstrate the terpolymer-coated CC-coated substrate's conjugation and release ability. A potential of $+1.0 \text{ V}$ (vs. Ag/AgCl) was applied to the CC in an aqueous solution of 2ME ($5 \times 10^{-3} \text{ M}$) containing 0.1 M LiClO_4 electrolyte for 1 min. Then the CC substrates were washed and kept in a 2 mL PBS solution before further use. The release of 2ME from the substrate was typically performed the next day at the constant potential of -0.8 V (vs. Ag/AgCl) for 5 min, followed by fluorescence detection of 2ME adduct with *o*-phthaldialdehyde and alanine in the release solution (with the maximum excitation and emission wavelengths of 330 and 450 nm, respectively).¹ The characteristic emission peak of the fluorescent adduct, with a maximum emission at 441 nm, confirmed the successful electrochemical release of 2ME from the substrate at -0.8 V (Fig. 4b). No fluorescence was observed in the absence of the electrochemical polarization, which suggests the absence of nonspecific attachment of the 2ME to the substrate.

The deposition of the terpolymer was also confirmed by FTIR of the terpolymer on EF and CC by the presence of characteristic peaks of PEDOT at 1420 cm^{-1} (C-S stretching), 1350 cm^{-1} (C-C stretching) (Fig. 4c and d) that were not present on the bare substrates.

Compared to P(EDOT) there was an additional signal for the terpolymer and P(EDOTSac) at 1620 cm^{-1} , related to the carbonyl C=O stretching vibrations from the carbonyl present in the thioacetate of the EDOTSac unit. After the thiol activation step, the peak at 1600 cm^{-1} disappeared, indicating the electrochemical reduction of thioacetate to -SH groups. FTIR was also carried out on the CC and EF after the conjugation of ODN. The appearance of a new peak at 1600 cm^{-1} , which could be attributed to the C=O stretching vibrations of the nucleic acid bases, confirms that the ODN has been successfully conjugated to the substrate.

Conjugation of fluorescent ODNs and their electrochemical release

The thiol groups of the terpolymer-coated CC and EF were exposed to an oxidation potential of $+1.0 \text{ V}$ for 60 s in PBS containing thiolated ODN (at a concentration of $50 \mu\text{g mL}^{-1}$) to form disulfide linkages between the ODN and the thiolated moieties of the substrate (Fig. 1a-c). The release of the fluorescent ODN was then performed the next day by applying a

potential of -0.8 V for 5 min, reducing the disulfide bonds in PBS solution back to thiol (Fig. 1c). The characteristic emission peak of the released fluorescent ODNs in PBS at 518 nm, from both functionalized CC and EF substrates, confirmed the successful conjugation and the electrochemical release of ODNs (Fig. ESI 2†).

The fluorescent intensity of the ODNs in the concentration range of $0.8 \mu\text{g mL}^{-1}$ to $0.008 \mu\text{g mL}^{-1}$ was recorded to establish the calibration graph and to quantify the amount of the released fluorescent ODNs from CC and EF substrates (Fig. 5a).

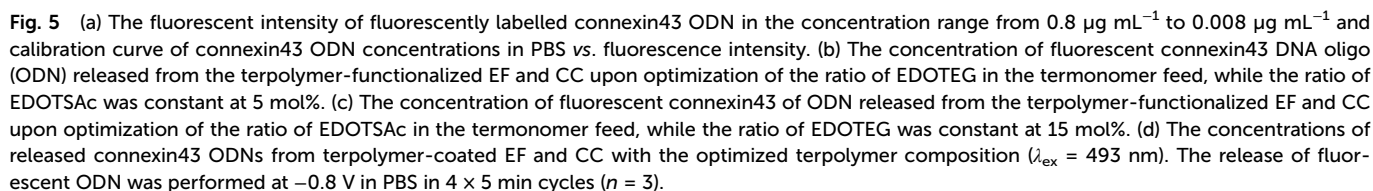
Two series of terpolymers were prepared from different monomer feed compositions on two different substrates (CC and EF) to determine the optimum composition of the terpolymer to achieve maximum conjugation/release efficiency. Fluorescence intensity of the released ODNs from terpolymer-coated EF and CC, polymerized with different EDOTSac : EDOTEG : EDOT ratios of 1 : 0 : 19, 1 : 2 : 17, 1 : 3 : 16, 1 : 4 : 15, 1 : 6 : 13 while keeping the concentration of EDOTSac fixed at 5 mol%, are shown in Fig. 5b. The monomer ratio of 1 : 3 : 16 (15 mol% EDOTEG) exhibited the highest release efficiency, which could be attributed to the presence of highly hydrophilic ethylene glycol units of EDOTEG and its antifouling properties.^{8,44} With a further increase in EDOTEG concentration, the efficiency of the conjugation and release decreased for both EF and CC. Hence the 15 mol% EDOTEG was chosen as the optimized concentration of EDOTEG for the further optimization experiments.

The concentration of EDOTSac was also optimized in another series of fluorescence ODN release experiments on EF and CC with different EDOTSac : EDOTEG : EDOT ratios of (0 : 3 : 17, 1 : 6 : 33, 1 : 3 : 16, 2 : 3 : 16, 4 : 3 : 13) while keeping the concentration of EDOTEG fixed at 15 mol% (Fig. 5c). The fluorescence intensity of released ODN increased up to monomer ratio of 1 : 3 : 16 (5 mol% of EDOTSac), but decreased at higher EDOTSac ratios. This could be attributed to the reduction of spacing between EDOTSac units, leading to steric blocking caused by the high density of ODN on the surface, or self-reactivity of the SH groups at densities beyond 5%.

Accordingly, the optimal spacing between EDOTSac units on the surface is a crucial parameter to achieve a highly efficient conjugation and release from CC and EF. A monomer ratio of 1 : 3 : 16 containing 2.52 mM EDOTSac (5%) : 5.05 mM EDOTEG (15%) : 44.7 mM EDOT (80%) was chosen as the optimal composition for subsequent experiments.

Release potential and release time are the key parameters affecting release efficiency. Fluorescent ODNs were released at -0.8 V in PBS in $4 \times 5 \text{ min}$ cycles. As shown in Fig. 5d, the first release cycle resulted in a strong fluorescence signal, while further second and third 5 min polarization of the substrate at -0.8 V resulted in lower fluorescence signals. A low fluorescence signal was recorded for the EF and CC substrates when no potential was applied to both substrates, indicating very low nonspecific adsorption of the ODN to the substrates, confirming the success of the electrochemically controlled





We have developed an electrochemical system based on a highly porous and flexible carbon cloth and electrospun substrates functionalized with a conducting terpolymer P(EDOT-*co*-EDOTS-*co*-EDOTEG) for the covalent conjugation and release of connexin43 antisense as an model drug and a reported wound healing agent. The conjugation was facilitated

by the electrochemical oxidative coupling of the thiols present on the substrates and on the drug molecule, while reduction of the formed disulfide bond afforded electrochemical controlled of the drug release. The disulfide bond between both of the investigated substrates (EF AND CC) substrate and the conjugated connexin43 antisense cleaved at a mild potential of -0.8 V (vs. Ag/AgCl) applied for a short time (300 s). This novel, flexible, porous and general platform technology holds a great potential in wound dressing and other drug-delivery applications.

Author contributions

The manuscript was written through the contributions of all authors. All authors have given approval for the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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