A Novel Electrochemically Switchable Conductive Polymer Interface for Controlled Capture and Release of Chemical and Biological Entities

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1. Introduction

Materials and methods that enable the selective and controllable capture and release of chemical and biological entities are in great demand. A significant example of such need is in realizing the promise of liquid biopsies for precise and personal diagnostics.[1] Research efforts have been directed towards the capture and release of a wide range of clinically relevant targets, including small drug molecules,[2] proteins,[3] oligonucleotides,[4] extracellular vesicles,[5] and various types of cells, including circulating epithelial cells (CECs) in preneoplastic or benign disease[6] and circulating tumor cells (CTCs) in cancers.[7]

Controlled release of small molecules offers opportunities for a number of applications, including in drug delivery[2,8] and for cell stimulation.[9] Sulfur containing molecules, such as reactive sulfur species (RSS)
and biothiols, are biologically important compounds involved in many physiological processes. Biothiols, for example, undergo facile and reversible oxidation and reduction reactions, providing mechanisms for the redox control of cellular metabolism. However, performing research on RSS and biothiols is challenging due to their highly reactive and unstable nature. As a result, materials and interfaces that can capture, stabilize and release them when required are needed. Antibodies are another class of biologically important molecules that have been of great interest in controlled delivery systems with applications in antibody purification and antibody-based therapeutic platforms. Local and controlled delivery systems can improve the therapeutic effects of antibodies by providing a highly regulated antibody concentration at the targeted body site over time, thus improving their bioavailability and reducing the possible side effects.

The isolation of CTCs is of great importance due to their potential use in cancer diagnostics and improved clinical management of cancers. Given the extremely rare abundance of CTCs in the blood, highly efficient, specific and rapid technologies are required for their isolation. The complete and non-destructive release of captured CTCs is also crucial for their downstream biological analysis, particularly when assessing their functions as viable cells. It has been shown that highly efficient capture of CTCs can be achieved by nanostructured materials that resemble the intrinsic nanoscale biological interactions. Electrospun nanofibers of biocompatible biomaterials provide a unique class of materials that mimic the extracellular matrix and have a great potential for CTC capture. For example, Zhao et al. developed two types of metal oxide-based electrospun membranes that can efficiently capture CTCs. However, these substrates suffer either from a lack of release functionality or require the use of harsh release conditions that destroy the capture interface. Antibody-functionalized devices have been designed for efficient CTC capture, utilizing both the intrinsic mechanical properties of CTCs (i.e., size and rigidity) and their surface receptors expression. Despite promising CTC capture results, these devices do not offer a simple means of live cell CTC release to allow downstream functional analyses. A number of stimuli-responsive strategies have been developed to address this issue, utilizing heat, light, enzymatic treatment, pH, mechanical stress, chemical exchange and DNA hybridization as a driving force for release. Recently, Tian et al. reported a sugar-responsive dynamic biointerface, which enabled specific and efficient capture (83.6%), rapid and sensitive electrochemical detection and highly efficient release (98.5%) of targeted tumor cells upon treatment with fructose.

Electrochemically induced release is a promising, clean, and robust methodology that combines several advantages of being rapid, highly tunable, and easy to operate by simply controlling electrical potential or current, without any additives. Electrochemically switchable interfaces require materials that conduct electricity, are safe to use, and undergo oxidative/reductive cycles at mild potentials, resulting in the fast and efficient capture and release of desired cargo. Several methodologies have been developed recently for the electrochemical release and delivery of chemical and biological entities. However, these methodologies have limitations related to the high release potentials used and complexity of fabrication. For example, organic electronic ion pumps (OEIP) use electrical voltages to transport and deliver a wide range of chemical and biological entities from small ions to large-sized biomolecules. However, OEIPs have limitations related to high operating voltages, complicated patterning procedures, and low transportation efficiencies. Furthermore, the majority of the existing electrochemical release techniques utilize electrochemistry for the release step only, without providing opportunities for the electrochemically induced capture of the cargo, or for the device re-use. Parker et al., for example, reported a photoelectrochemical platform based on an electrochemically cleavable semiconducting silicon surface for the capture and release of rare single cells. The release is based on a quinone species that can be reduced at –1.2 V versus Ag/AgCl to a cyclized hydroquinone via a so-called “trimethyl lock” lactonization system, which then results in the release of the attached species. However, the preparation of this substrate is complicated, with multiple functionalization steps, and the release requires a relatively high electrical potential. Moreover, this platform is not fully electrochemically controllable, as the oxidation of hydroquinone to quinone species is performed using a chemical oxidative reagent, i.e., N-bromosuccinimide. We have recently developed a gold particle-functionalized conductive polymer interface that can selectively capture and electrochemically release intact extracellular vesicles (EVs) by cathodic cleavage of gold-thiol bonds. Although the capture and release was found to be highly efficient, fast, selective and the interface easy to fabricate, the release required a relatively high negative potential of –1.2 V (Ag/AgCl). Moreover, the porous interface is ideal for small sized entities, such as EVs (<500 nm), but not for large biological entities such as cells (>10–20 µm). Herein, we describe a new electrochemical capture and release substrate based on a novel electrochemically switchable, functionalized conductive terpolymer, coated onto a highly porous and biocompatible electrospun membrane. The functionalized terpolymer can electrochemically bind thiolated molecules through electro-oxidative formation of a disulfide covalent bond (at +1.0 V vs Ag/AgCl). Using a suitable thiolated linker molecule, we demonstrate the simple conjugation of an antibody to the substrates via standard protein attachment chemistry. Subsequent reduction of the disulfide bond (at –0.8 V) leads to the non-destructive release of the antibody cargo for further analysis, and consequent regeneration of the free thiol groups on the substrate allow its re-use in subsequent capture/release cycles. Furthermore, we utilize this methodology to demonstrate capture/release of MCF7 breast cancer cells. For this purpose, we patterned an array of 30 µm diameter micropores on the substrate using femtosecond laser machining. This microporous array enabled vertical flow filtration of a suspension of MCF7 breast cancer cells, allowing the highly efficient and selective capture and electrochemical release of the cells intact and alive.

2. Results and Discussion

2.1. Design, Fabrication, and Characterization of the Electrochemically Switchable Electrospun Substrate

Our capture/release substrate was designed to be porous and have the following functionalities: electrical conductivity, excellent wettability and hydrophilicity, and antifouling
properties while providing redox active moieties for electrical potential-controlled capture and release. Figure 1a shows the steps involved in the substrate fabrication process. The first step was electrospinning a polycaprolactone (PCL) nanofiber membrane. PCL is a biocompatible, nontoxic and easy to spin polymer. A 20 nm thick gold layer was then sputter-coated onto both sides of the membrane to make the porous, electrically conductive substrate. Scanning electron microscopy (SEM) images of the substrate at this stage of fabrication (Figure 2a) show a highly porous, fibrous structure with a mode fiber diameter of 410 nm. To enable the capture and release functionality, a 3,4-ethylenedioxythiophene (EDOT) monomer with an acetylthiomethyl substituent (S-((2,3-dihydrothieno[3,4-b][1,4]dioxin-2-yl)methyl) ethanethioate, (denoted "EDOT-CH2S-Ac") was synthesized (Figure 1a and Figure S1, Supporting Information). Another EDOT derivative, 2-(2,5,8,11-tetraoxadodecyl)-2,3-dihydrothieno[3,4-b][1,4]dioxine, bearing three ethylene glycol units (denoted “EDOT-EG”) was synthesized to act as a hydrophilic spacer, with the EG substituents also expected to provide antifouling properties to the substrate (Figure 1a and Figure S2, Supporting Information). The third co-monomer used was unmodified EDOT, serving as a spacer for the other two functionalized EDOTs. A conductive terpolymer of EDOT-CH2S-Ac, EDOT-EG and EDOT (named “PEDOT-EG-SA c”) was then electrodeposited onto 0.5 × 0.5 cm pieces of the electrospun, gold-coated PCL membrane using chronoamperometry (Figure S3, Supporting Information). The SEM images of the resulting terpolymer-functionalized electrospun substrate showed a clear increase in the fiber diameter with the mode fiber diameter 890 nm (Figure 2b,c), compared to the gold-coated electrospun membrane (410 nm, Figure 2a).

Raman spectroscopy was used to further confirm the successful deposition of the terpolymer (Figure 2d). The presence of PEDOT characteristic peaks at 439 cm\(^{-1}\), 571 cm\(^{-1}\) and 992 cm\(^{-1}\) (oxyethylene ring deformation), 695 cm\(^{-1}\) (symmetric \(\text{C} = \text{S} - \text{C}\) stretching), 1104 cm\(^{-1}\) (C–O–C stretching), 1268 cm\(^{-1}\) (\(\text{C}\alpha\text{C}=\text{C}\beta\) stretching), 1366 cm\(^{-1}\) (C\(\beta\) stretching) and 1510 cm\(^{-1}\) (asymmetric \(\text{C} = \text{S} - \text{C}\) stretching) \(^{34}\) confirmed the successful coating of PEDOT-EG-SA c onto the fibers. Furthermore, the FTIR spectrum of
the terpolymer (Figure 2e) showed all the characteristic peaks of PEDOT at 1493 cm\(^{-1}\) (C=C stretching), 1378 cm\(^{-1}\) (inter-ring stretching mode of C–C), 1170, 1137, 1086, 1046 cm\(^{-1}\) (C–O–C vibration) and 962, 910, 832, and 676 cm\(^{-1}\) (C–S–C).[13]

Water contact angle measurements showed a significant change in contact angle from 82.1 ± 2° to 0.0° upon the electrodeposition of the PEDOT-EG-SAc, which could be attributed to the presence of the hydrophilic ethylene glycol substituents on the polymer (Figure 2f). A hydrophilic surface is expected to be beneficial in the practical biological applications of the substrate, owing to its improved wettability and biofouling resistance.[36] The electrical conductivity of the gold-coated and PEDOT-EG-SAc-coated electrospun substrates were measured using a four-probe conductivity meter (Figure 2g). The gold coated substrate showed a high conductivity of 1448 ± 140 S cm\(^{-1}\). Although the conductivity decreased to 117 ± 29 S cm\(^{-1}\) after the PEDOT-EG-SAc electrodeposition, the substrate still possessed a high conductivity, sufficient for the subsequent electrochemical experiments.

2.2. Capture and Release of a Model Small Thiol-Containing Molecule and a Model Antibody

The electrospun terpolymer substrate was first activated by electrochemical reduction of the acetylthiomethyl moiety on the PEDOT-EG-SAc at −0.8 V (vs Ag/AgCl) for 30 s in PBS to unmask the thiol functionality (Figure 1a, the polymer termed “PEDOT-EG-SH”). The successful unmasking of the thiol functionality is evident from the significant changes in the FTIR spectra of the terpolymer before and after reduction, in particular the disappearance of the peak shoulder at 1733 cm\(^{-1}\) attributed to the C=O stretching of thioacetate group upon reduction (Figure 2e). The capture ability of the activated substrate is based on the oxidative formation of a disulfide bond between the thiol moiety of PEDOT-EG-SH on the substrate and the thiolated target molecules, while release is achieved by the reductive cleavage of the disulfide bond (Figure 1b).

To demonstrate the capture and release ability of our electrospun PEDOT-EG-SH coated substrate, we used 2-mercaptooctanol (2ME) as a model small molecule (Figure S4, Supporting Information). To capture 2ME, a potential of +1.0 V (vs Ag/AgCl) was applied to the substrate in an aqueous solution of 2ME (5 × 10\(^{-3}\) m) containing LiClO\(_4\) (0.1 m) electrolyte for 1 min. The chronoaamperometry trace of the 2ME attachment to the PEDOT-OEG-SH substrate is shown in Figure S5 (Supporting Information). The release of 2ME from the substrate was then studied under zero potential and under −0.8 V potential (vs Ag/AgCl) applied for 2 min, followed by fluorescence detection of 2ME in the release solution through the formation of a fluorescent adduct of 2ME with o-phthalaldehyde and alanine[37] (see the Experimental Section for details). The successful electrochemical release of 2ME from the substrate at −0.8 V was confirmed by the characteristic emission peak of the fluorescent adduct, with the maximum at 441 nm (Figure 3a,b). When compared to the blank signal from PBS, 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 first 2 min release cycle resulted in a strong fluorescence signal, while further second and third 2 min polarization of the substrate at −0.8 V resulted in very low fluorescence signals, implying the efficient and fast electrochemical release of 2ME over the first 2 min of polarization at −0.8 V (Figure 3a, b). These results confirmed the success of the proposed capture/release methodology.
The practical applications of the capture and release methodology rely on the conjugation of the targeted biological entities, such as antibodies, aptamers and cells on the substrates. Therefore, as the next step, we investigated the selective capture and release of a fluorescent Alexa Fluor 488-conjugated antibody (Figure S6, Supporting Information). To conjugate the antibody to the substrate, a mercaptohexanoic acid (MHA) linker molecule was electrochemically attached to the PEDOT-EG-SH substrate through oxidative coupling and disulfide bond formation at +1.0 V in an ethanolic solution of MHA (5 × 10^{-3} M) containing LiClO4 (0.1 M) electrolyte. The antibody was then conjugated to the MHA linker-functionalized substrate using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC):NHS chemistry.

Electrochemical release of the captured antibody was performed into a PBS buffer by applying −0.8 V onto the substrate, and the fluorescent Alexa Fluor 488-conjugated antibody was detected in the PBS release solution. As shown in Figure 3c, electrochemical polarization at −0.8 V for 2 min results in a strong, characteristic fluorescence signal of the Alexa Fluor 488, confirming both successful capture and electrochemical release of the antibody. The second and third electrochemical polarizations at −0.8 V for 2 min did not result in a considerable fluorescence signal, suggesting that the antibody release is fast and effective, only requiring a single polarization (Figure 3c,d). When no potential was applied to the substrate, a negligible fluorescence signal was detected, indicating very low nonspecific adsorption of the antibody to the substrate. To further study the nonspecific binding of the Alexa Fluor 488 antibody to the substrate, the activated PEDOT-EG-SH substrate (without the MHA linker) was incubated with the antibody solution and subjected to the electrochemical release cycles. As shown in Figure 3e, a minimal fluorescence signal was detected, indicating a good resistance of our capture/release substrate to nonspecific adsorption of antibodies.

Release potential is expected to be a key parameter in affecting the release efficiency, release time, and notably the intactness of the released cargo. Therefore, it was of importance to find the smallest negative potential that could efficiently release the cargo. As seen in Figure 3f, the Alexa Fluor 488 antibody was not released at potentials more positive than −0.4 V (vs Ag/AgCl). The electrochemical release process started at −0.6 V and increased markedly at −0.8 V. A further increase of the potential to −1.0 V did not significantly affect the release; thus −0.8 V was chosen as the optimum release potential.

To evaluate the reusability, substrates that were already run through a first capture and release step were subjected to a second round of functionalization with MHA linker, EDC/NHS antibody coupling and electrochemical release. Figure S7 (Supporting Information) shows the fluorescence intensities of the first- and second-release solutions of Alexa Fluor 488 antibody

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Figure 3. a) The fluorescence emission spectra confirming the electrochemical release of 2ME using the electrospun, PEDOT-EG-SH-coated, substrate (λ<sub>e</sub> = 330 nm). 2ME is captured by electrochemical oxidative addition at +1.0 V (Ag/AgCl) and then released from the substrate into PBS at −0.8 V in 3 × 2 min cycles: 1st cycle (green line), 2nd cycle (blue line), 3rd cycle (orange line); PBS solution incubated with 2ME-captured substrate for 5 min without electrical polarization (red line); PBS blank solution containing 1 × 10^{-3} M o-phthalaldehyde and alanine (black line). b) The fluorescence intensities at 441 nm from the spectra in graph (a) (n = 3). c) The fluorescence emission spectra confirming the electrochemical release of Alexa Fluor 488 antibody (λ<sub>e</sub> = 493 nm). The antibody was captured to the substrate by electro-oxidative coupling of MHA and EDC/NHS antibody conjugation, followed by the release into PBS at −0.8 V in 3 × 2 min cycles: 1st cycle (green line), 2nd cycle (blue line), 3rd cycle (orange line); PBS solution after incubation of the antibody-captured substrate for 5 min without electrical polarization (red line); PBS blank solution (black line). d) The fluorescence intensities at 518 nm from the spectra in graph (c) (n = 3). e) Comparison of the fluorescence signal of the Alexa Fluor 488 antibody released from the substrates with and without an MHA linker (n = 3). f) Effect of the potential on the release of Alexa Fluor 488 antibody (n = 3). ****p < 0.0001, ***p < 0.001, **p < 0.01 (based on one-way ANOVA (α = 0.05), and Tukey’s HSD multicomparison tests).
at 518 nm, which indicated the substrate preserves 81% of its activity when reused.

2.3. Capture and Release of Breast Cancer Cells

The high surface area, excellent wettability in aqueous solutions, antifouling nature and ability to be functionalized with capture molecules, such as antibodies, suggest that these substrates could be useful interfaces for selective and efficient isolation of cells, including cancer cells, with a potential future application in the capture/release of CTCs.

To demonstrate the application of our substrate for capture/release of cancer cells, we modified the electrospun substrate in several ways to enable maximum cell capture and release. A schematic illustration of the cell capture and release system is presented in Figure 1c. Femtosecond laser machining was used to fabricate an array of 30 μm diameter micropores (actual diameter 26.97 ± 2.699 μm) on the electrospun substrate (0.6 × 0.6 cm, Figure 4a–c) to provide a membrane suitable for the vertical flow filtration of a sample of suspended cells in a test system; in this case a suspension of MCF7 human breast cancer cells.[21c] The MHA-functionalized PEDOT-EG-SH substrate was conjugated with EpCAM antibody, in the same way as described above for the fluorescent antibody. This antibody targets an EpCAM protein epitope on the outer external surface of epithelial cells, including MCF7 cells. Additionally, the substrate was treated for 1 h with 3% bovine serum albumin (BSA), a known blocking reagent,[39] to minimize nonspecific cell attachment.

Cell capture was then undertaken in a custom built, easy to assemble filtration cartridge, equipped with a platinum counter electrode and Ag/AgCl wire reference electrode. MCF7 cells were spiked into Dulbecco’s Modified Eagle Medium (DMEM) containing 1% BSA at a concentration of 10^5 cells mL^-1 and passed through the antibody-functionalized substrate at a flow rate of 20 μL min^-1 for 20 min, using a syringe pump. The filtration substrate was then washed at 20 μL min^-1 with PBS for 10 min to remove any unbound cells. Cell capture efficiency was evaluated by direct visualization using SEM imaging, as well as by counting cells in the filtrate and in the washing solutions according to the Equation (1)

\[
\text{Cell capture efficiency} = \frac{C_i - C_f - C_w}{C_i} \times 100 \tag{1}
\]

where, \(C_i\), \(C_f\), and \(C_w\) are the concentrations of cells in initial, filtrate, and washing solutions, respectively.

As depicted by the SEM images in Figure 4d–f, MCF7 cells were successfully captured on the substrate. Analysis of the cell concentrations in solution, showed a 92 ± 8% capture efficiency. The excellent capture efficiency could be attributed to specific EpCAM-functionalization of the substrate and the nanofibrillar, porous structure of the substrate that mimics the native extracellular matrix.[19,40] After PBS washing, −0.8 V was applied to the substrate under a PBS flow rate of 20 μL min^-1 to release 33 ± 6% of the captured cells (Figure S8, Supporting Information), calculated according to the following equation:

\[
\text{Cell release efficiency} = \frac{C_i}{C_i - C_f - C_w} \times 100 \tag{2}
\]

where, \(C_i\) is the concentration of cells in the release solution.

We hypothesize that the release efficiency was diminished because, following an initial capture via an antibody and upon prolonged time, live cells can extend protrusions to attach to a surface preventing their release (see Figure S9, Supporting Information, for SEM images).

Additionally, we tested the effect of the oxidation state of the terpolymer prior to the cell capture, as that is an important parameter that can affect the surface charge of the substrate, thus changing the cell-substrate interactions.[41] Since a +1.0 V potential was used for MHA linker conjugation to the terpolymer on the substrate in all of the previous experiments, the substrate was in an oxidized form, providing a positively charged surface.[42] Cyclic voltammetry was utilized to find the
significant difference in EpCAM expression levels for MCF7 and HDFa cells. As expected, the results revealed a prior to the cell capture to verify the EpCAM expression level substrate with the cells. Fluorescence microscopy blasts) cells, were used to study the nonspecific interactions EpCAM antibody is present on the substrate, live EpCAM +

Figure 5. Fluorescence microscopy images showing EpCAM protein expression level of adherent a) HDFa and b) MCF7 cells. c) Western blot analysis indicating the relative abundance of EpCAM protein in MCF7 and HDFa cells. d) SEM image of the EpCAM antibody-functionalized PEDOT-EG-SH substrate after passage of HDFa cells. e) SEM image of the control PEDOT-EG-SH electrospun substrate with no antibody, after passage of MCF7 cells. f) Trypan blue cell viability assay of control and released MCF7 cells (n = 3, statistical analysis based on unpaired t-test). g) Bright-field and h) fluorescence microscopy images of the electrochemically released MCF7 cells (green-live, calcein-AM and red-dead, propidium iodide).

The above results suggest the following: i) when the specific EpCAM antibody is present on the substrate, live EpCAM** MCF7 cells attach to the substrate via antibody-antigen (EpCAM) interactions, whereas EpCAM** HDFa cells do not; ii) neither cell type attaches to the substrate in the absence of EpCAM antibody on the substrate. In other words, our novel electrospun, PEDOT-EG-S-antibody terpolymer substrate displays low levels of nonspecific cell attachment and is capable of the specific capture/release of targeted cells.

The ability to release intact cancer cells obtained from liquid biopsies after capture is important for post expansion and downstream studies of malignant diseases. It has been shown that electrochemical treatment of cells at high potentials can damage the cell membrane through electroporation. The released cells from our substrate showed 91.8 ± 2.1% of viable cells as compared to 92.7 ± 0.9% for the control MCF7 cells, indicating the capture and low-potential electrochemical release process has no effect on the cell survival (Figure 5f). To further study the possible effect of the electrochemical release on cell viability and proliferation, the subpopulation of released MCF7 cells from the substrate was transferred into culture dishes and subjected to standard incubation conditions. Upon culture, the cells attached and proliferated (Figure S12, Supporting Information). The slightly lower proliferation of the released cells compared to the control cells could be due to the stress experienced by the cells during the capture/release process at room temperature. In addition, bright-field and fluorescent live/dead staining images of the cells on Day 4 of cell culture indicated a regular cell morphology and a very few dead cells (Figure 5g,h).

We conclude, in line with previous reports, that the capture and electrochemical release of MCF7 cells at a mild −0.8 V potential does not affect cell viability.

Table S1 (Supporting Information) compares the performance of recently published methodologies for capture and release of cancer cells. The capture efficiency of our electrospun substrate is comparable or better than most of these other methods. This could be attributed to the unique design, specific antibody functionalization, nanofibrous morphology and
All reagents and materials were used without further purification.

**Synthesis of** [(2,3-dihydrothieno[3,4-b][1,4]dioxin-2-yl)methyl] Ethanethioate, EDOT-CH$_2$-S-Ac. EDOT-CH$_2$-S-Ac was synthesized in three steps from epichlorohydrin (Figure S1, Supporting Information). First, the epoxide in epichlorohydrin underwent a ring-opening to provide the corresponding diol which was then reacted with dimethoxyphosphine, forming EDOT-CH$_2$-Cl. In order to install the thiol functionality, EDOT-CH$_2$-Cl was reacted with potassium thioacetate, forming EDOT-CH$_2$-S-Ac.

**Dioxine, EDOT-EG**. EDOT-EG was synthesized in two steps (Figure S2, Supporting Information). First, ethylene glycol was reacted with tosyl chloride to form a tosylate which was then reacted with hydrazinyl EDOT in the presence of BuOK to provide EDOT-EG.

**Fabrication of the Electrospun PCL Interface**: 12% of PCL was added to a mixture of dimethylformamide:tetrahydrofuran (1:1) solution and stirred until completely dissolved at room temperature. PCL solution was drawn into a 5 mL glass syringe with a 21G metallic needle and placed in the syringe pump component of an electrosprining setup (Adelab Scientific). The electrosprining was carried out at 15 kV, a flow rate of 2 mL h$^{-1}$ and a distance of 15 cm. The fibers were homogeneously collected on a paper-covered rotating drum at a speed of 200 rpm. 8 mL of PCL solution was electrosprined in total. The PCL electrospun membrane was then sputter-coated on both sides with a 20 nm gold layer at a speed of 4 nm min$^{-1}$ using a Q150R sputter coater (Quorum).

**PEDOT-EG-SAc Terpolymer Electrodeposition**: All solutions used for electrochemistry were degassed with nitrogen prior to the experiments. A three-electrode electrochemical setup was used to deposit PEDOT-EG-SAc onto a 5 × 5 mm area of the gold-coated electrospun substrate using a BioLogic potentiostat, where the electrospun substrate was used as the working electrode, a platinum mesh as the counter electrode and Ag/AgCl as the reference electrode (MF-2052, BasI). The polymerization was performed at a potential of 1.5 V (vs Ag/AgCl, +0.230 V vs SHE) for 1 min in 0.1 M LiClO$_4$ water:acetonitrile (1:1) solution containing 5.3 × 10$^{-3}$ M EDOT-CH$_2$-S-Ac (10%), 40 × 10$^{-3}$ M EDOT (75%) and 8 × 10$^{-3}$ M EDOT-EG (15%). The PEDOT-Sac-coated substrate was then washed with water:acetonitrile (1:1) solution three times to remove the electrolyte and unreacted monomers.

**Capture and Release of 2ME**: 2ME was electrochemically attached to the thiol-activated electrospun substrate (PEDOT-EG-SH) by an oxidative disulfide bond formation at 1.0 V for 60 s in 0.1 M LiClO$_4$ and 5 × 10$^{-3}$ M 2ME (aq.). After the 2ME assembly process, the substrate was rinsed with copious amounts of water to remove the unbounded 2ME molecules. The electrochemical release of 2ME was performed in PBS at −0.8 V for 120 s. The released 2ME was detected through the formation of a fluorescent adduct in solution between 2ME, ethanolamine and maleimide (1 × 10$^{-3}$ M) and alane (1 × 10$^{-3}$ M) with the maximum excitation and emission wavelengths of 330 and 450 nm, respectively (Figure S13, Supporting Information).[37] A PerkinElmer spectrometer was used for fluorescence measurements.

**Alexa Fluor 488 Fluorescent Antibody Conjugate Capture and Release**: Prior to the antibody attachment, MHA linker was electrochemically attached to the electrospun PEDOT-EG-SH substrate through the oxidative disulfide bond formation at 1.0 V for 60 s in an ethanolic solution of LiClO$_4$ (0.1 M) and MHA (5 × 10$^{-3}$ M). After washing with ethanol and then PBS, the carboxyl (COOH) groups of the MHA linker-functionalized substrate were activated in a 2:1 mol:mol ratio of EDC:NHS (100 × 10$^{-3}$ M EDC, 50 × 10$^{-3}$ M NHS, pH = 6.3) in 2 mL PBS at 28 °C for 2 h. The activated substrate was rinsed with PBS and incubated in Alexa Fluor 488-AffiniPure Anti-Human IgG solution in PBS (2 μg mL$^{-1}$) for 1 h at room temperature to covalently bond the free amine groups of the antibody to the NHS activated substrate. After washing with PBS, the captured antibody was released into a PBS buffer at −0.8 V for 120 s and the fluorescence signal from the release solution was measured at an excitation wavelength of 493 nm.

**Laser Machining**: Laser machining was used to fabricate an array of micropores on the electrospun PCL substrate to provide a path for the vertical flow filtration of cancer cells. The laser source consisted of a Ti:Sapphire laser system combining an ultrafast oscillator (VITARA)
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Fluorescence Microscopy for Visualizing EpCAM Expression Levels

Live Cell Capture and Release: The process for conjugation of EpCAM antibody was similar to that used for the attachment of the fluorescent antibody with some minor modifications. 100 µL of the EpCAM antibody in PBS (10 µg mL−1) was pipetted onto the PEDOT-OEG-SH substrate pre-functionalized with the NHS-activated MHA linker, and incubated at room temperature for 1 h and then at 4 °C overnight. Cell capture was performed on a custom-built filtration cartridge with ports to assemble the electrospray substrate, a platinum counter and Ag/AgCl wire reference electrodes. MCF7 cells were diluted in DMEM (containing 1% BSA) at a density of 106 cells mL−1 and passed through the antibody-functionalized substrate at a flow rate of 20 µL min−1 using a syringe pump. The filtrate solution was collected for cell concentration analysis. The captured cells were then washed with PBS at a flow rate of 20 µL min−1.

Characterization of Cell Viability and Proliferation: Cell viability was measured using trypan blue and calcine-AM/propidium iodide staining. For the calcine-AM/propidium iodide staining, MCF7 cells were washed with PBS, followed by addition of calcein-AM (1 µM) and propidium iodide (25 µg mL−1) for 30 min in a standard incubator (37 °C and 5% CO2). After the incubation, the staining solution was removed, PBS added, and the cells were imaged using a Nikon Ti-E microscope to assess viability. Cell proliferation was measured using PrestoBlue colorimetric assay. Briefly, 100 µL of cell suspensions in DMEM with a density of 105 cells mL−1 were seeded in 96 well plates and incubated at 5% CO2 at 37 °C. After 24, 48, and 72 h, 10 µL of PrestoBlue reagent was added to each well and incubated for 30 min at 5% CO2 at 37 °C. The absorbance was then measured at 570 nm and normalized to the absorbance at 600 nm.

SEM Observation of Captured Cancer Cells: Prior to SEM imaging, the captured cells on the electrospray terpolymer substrate were fixed with 2% glutaraldehyde in PBS for 1 h at room temperature and then overnight at 4 °C, followed by a PBS wash. The cells were then dehydrated through a series of ethanol concentrations (35%, 50%, 70%, 90%, and 100%, 20 min each) at room temperature. After complete drying in air and gold sputtering (10 nm), the samples were visualized on a JCM-6000 Versatile Benchtop SEM from JEOL.

Fluorescence Microscopy for Visualizing EpCAM Expression Levels: 200 µL of MCF7 or HDFa cells at a density of ~1×104 mL−1 in complete DMEM were added to each compartment of an 8-chamber LabTekII seedling chamber slide (ThermoFisher). The chambers were incubated in standard conditions for 48 h. Once the cells were immobilized on slides, medium and nonadherent cells were removed from the chamber slides, and the chamber slides were then washed 2x in 200 µL PBS. Cells were fixed with 200 µL of 4% PFA in PBS for 30 min at RT, then washed twice with 200 µL PBS. Following fixation and washing, the chamber slides were then blocked in 3% BSA (Gibco) in PBS, for either 30 min at RT, or overnight at 4 °C, then washed 2x with PBS. Incubation with rabbit anti-EpCAM polyclonal antibody at 1:200 in PBS was either performed overnight at 4 °C, or for 1 h at RT, both in the dark. Slides were then washed 2x with 2 mL PBS before labeling in the dark with secondary goat anti-rabbit IgG DyLight 488 conjugate in PBS at 1:5000 for 1 h at room temperature. Slides were visualized using fluorescence microscopy (Nikon Ti-E microscope) using the excitation and emission wavelengths of 490 and 520 nm, respectively.

Western Blot: PBS-washed MCF7 and HDFa cell pellets were lysed in 1 mL modified RIPA buffer (150 × 10−3 M NaCl, 50 × 10−3 M Tris HCl pH 7.5, 1% NP-40, 0.1% SDS, 1× complete protease inhibitor tablet 50 mL−1), sonicated for 60 s, incubated on ice for 15 min and centrifuged at 13000 × g for 5 min at 4 °C. The protein concentration was determined using the BCA protein determination kit (Pierce) to normalize protein gel loading. 7 µg protein was mixed with 4x SDS-PAGE sample buffer (200 × 10−3 M Tris-HCl pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Blue G-250, 20 × 10−3 M DTT), and incubated at 95 °C for 5 min. The preparation was then centrifuged briefly and run on SDS-PAGE BioRad Stain-free TGX 4–20% gels in a tris-glycine-SDS buffer (250 × 10−3 M Tris, 1.92 µg mL−1, 1% SDS) at 200 V for 30 min. The proteins were then transferred onto a BioRad mini-PVDF membrane using a BioRad TurboBlot at 5 V for 60 min and blocked overnight in 3% BSA in TBST (20 × 10−3 M Tris, 150 × 10−3 M NaCl, 0.1% Tween 20, pH 7.6) at 4 °C. Following 3 × 5 min TBST washes, the membranes were incubated with primary anti-EpCAM antibody (1:200) in 3% BSA in TBST at room temperature for 1 h on a tilting platform shaker. Following 3 × 5 min washes in TBST, the membranes were incubated with a 1:1000 goat anti-rabbit IgG HRP-linked conjugate in TBST at room temperature for 1 h. The membranes were then imaged with a BioRad Clarity ECL kit at a 1:1 reagent ratio on a ChemiDoc analyzer.

Statistical Analysis: The unpaired t-test, one-way ANOVA (α = 0.05), and Tukey’s HSD multocomparison tests were used to test for statistical significance between groups. Results are reported as an average ± standard deviation (SD). The graphs are reported with error bars representing the SD. All statistical analyses were performed using GraphPad Prism 8.

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.
Data Availability Statement
The data that support the findings of this study are available from the corresponding author on reasonable request.

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antibody, circulating tumor cells, conducting polymers, electrochemical release, electrospinning, femtosecond laser machining

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