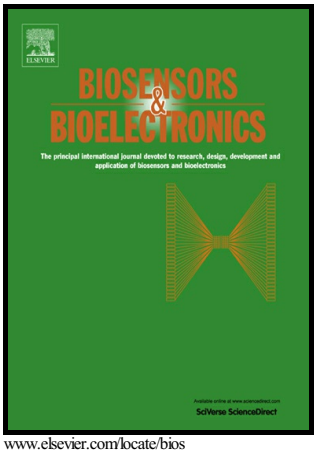

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Conducting polymer-silk biocomposites for flexible and biodegradable electrochemical sensors

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Abstract

Approaches to form flexible biosensors require strategies to tune materials for various biomedical applications. We report a facile approach using photolithography to fabricate poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS) sensors on a fully biodegradable and flexible silk protein fibroin support. A benchtop photolithographic setup is used to fabricate high fidelity and high resolution PEDOT:PSS microstructures over a large (cm) area using only water as the solvent. Using the conductive micropatterns as working electrodes, we demonstrate biosensors with excellent electrochemical activity and stability over a number of days. The fabricated biosensors display excellent nonspecific detection of dopamine and ascorbic acid with high sensitivity. These devices are mechanically flexible, optically transparent, electroactive, cytocompatible and biodegradable. The benign fabrication protocol allows the conducting ink to function as a matrix for enzymes as shown by a highly sensitive detection of glucose. These sensors can retain their properties under repeated mechanical deformations, but are completely degradable under enzymatic action. The reported technique is scalable and can be used to develop sensitive, robust, and inexpensive biosensors with controllable biodegradability, leading to applications in transient or implantable bioelectronics and optoelectronics.

1. Introduction:

Electronics and sensors that can form flexible interfaces with a biological milieu while retaining biocompatibility, stability and desired characteristics are of great interest (Rivnay et al. 2014). Improving both material design and multiscale fabrication platforms towards such organic electronics (OEs) has applications in diverse fields including regenerative medicine, neural stimulation and recording, drug delivery, flexible energy storage, sensors and photonics (Green et al. 2008; Irimia-Vladu 2014; Lin and Yan 2012; O'Neill and Kelly 2011; Oh et al. 2013; Weng et al. 2015). As an alternative to conventional inorganic materials and metals, intrinsically conducting polymers (CPs) provide a promising route for flexible and transient OEs with competitive electronic and ionic transport properties (Berggren and Richter-Dahlfors 2007). CPs such as polypyrroles (PPy), polyanilines and polythiophenes are choices not only due to their structural similarities with biopolymers, but also due to their biocompatibility and conformability, which allows them to be interfaced with intrinsically soft and curvy tissue and biological constructs (Balint et al. 2014; Guimard et al. 2007; Khodagholy et al. 2011). Low temperature processing, oxide free interfaces in contact with aqueous electrolyte, and ion transport at ambient temperatures are few advantages suitable for biosensors and bioelectronics (Park et al. 2015; Rivnay et al. 2014; Weng et al. 2015). They are also environmentally friendly and provide green alternatives to conventional electronic materials in terms of fabrication and degradable behavior.

Poly (3,4-ethylenedioxythiophene): poly (styrene sulfonate) (PEDOT:PSS) and its derivatives stand out (Asplund et al. 2010; Berggren and Richter-Dahlfors 2007), with good electronic and ionic conductivity, favorable biocompatibility (Venkatraman et al. 2011), and optical properties (Xia et al. 2012). Organic light emitting diodes (OLEDs), organic thin film transistors (OTFTs), solar cells, photodetectors and sensors (Khodagholy et al. 2011; Kirchmeyer and Reuter 2005) have been formed using PEDOT:PSS. However, this commercially available polymer is hydrophobic, with reduced processibility and brittleness. Composites with bio-derived polymers such as polylactide, polycaprolactone, chitosan, gelatin, collagen, and heparin can impart properties beneficial to a biological setting (Guo et al. 2013; Li et al. 2006), but have challenges

in terms of performance or high resolution microfabrication. To form bio-integrated devices and sensors using materials such as PEDOT:PSS, it is often necessary to form micro and/or nano-architectures. While inkjet printing and soft lithography are widely used (Xiong and Liu 2012), issues with resolution, lack of uniformity of pattern thickness, throughput, and requirement of suitable molds need to be addressed (Gates et al. 2005; White et al. 2011). Photolithography remains a leading technique to fabricate structures of high complexity over large areas quickly and reproducibly (Geissler and Xia 2004). However, the acidity of PEDOT:PSS tends to adversely affect the crosslinking or decomposition of conventional acid-sensitive photoresists (Nie and Kumacheva 2008), requiring intricate, and multi-step processes (Jensen et al. 2013; Ouyang et al. 2015), including orthogonal solvents for non-damaging patterning, (Taylor et al. 2009) or direct crosslinking (Khong et al. 2007). High temperature is often required, making them unsuitable for high-resolution biomedical applications (Ouyang et al. 2014). The development of benign and biomolecule friendly fabrication processes for CPs such as PEDOT:PSS is therefore highly desirable for harnessing them in OEs and biosensors.

We address this problem using a water-based conductive ink composed of a 'conducting polymer-carrier protein'. This composite is based on silk proteins, with unique mechanical and optical properties, biocompatibility, and controllable degradation (Altman et al. 2003; Kundu et al. 2014; Wu and Payne 2004). Our group earlier demonstrated biochemically modified silk proteins: fibroin and sericin, with photocrosslinkable methacrylate pendant groups that are photoreactive (Kurland et al. 2013; Kurland et al. 2014). We show that the water soluble "photo sericin" can be used as a matrix for water dispersible PEDOT:PSS to form a photoreactive sensing ink. This ink can be micropatterned using photolithography, and covalently attached to an underlying protein sheet to form a flexible, biodegradable biosensor in a scalable fashion. In contrast to dielectric substrates or thin films of conductive materials, the micropatterned electrodes and biocircuits are patterned on crosslinked silk fibroin sheets, resulting in a fully organic, freestanding device which can be proteolytically degraded in a controllable fashion (Cao and Wang 2009; Horan et al. 2005; Kurland et al. 2013; Taddei et al. 2006). The water-based lithographic process allows encapsulation of enzymes or other biomolecules leading to enzymatic sensors. The sensors are stable in air and liquid environments over several days, and

can be stored in both dry and wet condition, bent and twisted while retaining mechanical stability and importantly, electrochemical properties.

2. Experimental Section:

2.1. Synthesis and purification of photoactive silk proteins (fibroin and sericin): The extraction and purification of silk fibroin from silk cocoons of *Bombyx mori* (Mulberry Farms, Fallbrook, CA) was carried out as described elsewhere (Rockwood et al. 2011). Sericin was procured in pure form (Wako Chemicals, Richmond, VA) and used as received. Synthesis of photo-reactive conjugates of fibroin and sericin were performed as illustrated earlier (Kurland et al. 2013; Kurland et al. 2014) - referred to as sericin protein photoresist (SPP) and fibroin protein photoresist (FPP). Briefly, the proteins were separately solubilized in 1 M LiCl/DMSO (1% (w/v)) for 1 hour. The reaction used a stoichiometric amount of 2-isocyanatoethyl methacrylate equivalent to the total hydroxyl pendant groups present in the protein amino acids. The reaction was carried out at 60°C for 5 hours in a dry N_2 environment. The product with conjugated methacrylate groups was precipitated by pouring the solution into excess cold ethanol. Conjugates were washed in a mixture of cold ethanol/acetone, centrifuged 3x, and lyophilized for 48 hours to obtain the final product.

2.2. Surface functionalization: Micropatterns of the sericin protein photoresist SPP-PEDOT:PSS composite ink were fabricated on rigid glass substrates and flexible FPP films. To pattern on glass, the substrates were functionalized with acrylate moieties to enable covalent attachment of patterns. After cleaning the surfaces with piranha solution (3:1 98%H_2SO_4:30%H_2O_2) (*Caution*: Piranha solutions are extremely energetic and may result in explosion or burns if not handled with extreme care) for 30 min at room temperature. Functionalization with 3-(trichlorosilyl) propyl methacrylate (TPM) was accomplished by chemical vapor deposition in a desiccator for 12 hours at 0.4 bar. Treated surfaces were washed with hexane and water, and air-dried prior to use.

2.3. Formation of SPP-PEDOT:PSS patterns on FPP via photolithography: Dry re-dispersible pellets of PEDOT:PSS (Orgacon^{TM}, Sigma-Aldrich, St. Louis, MO) were dispersed in water and

ultrasonicated for 20 minutes. The mixture was then filtered through a 0.45 µm syringe filter. The solution was mixed with SPP, polyethylene glycol (PEG) (Alfa Aesar, Ward Hill, MA) (0.3 mg/mg of SPP), and dimethylsulfoxide (DMSO) (5% v/v in PEDOT:PSS stock solution) to form the conductive ink. Concentrations were varied as discussed above. Crosslinked films were prepared by dissolving FPP in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). Photoinitiator (Irgacure 2959, BASF) 0.6% (w/v) was added to the silk photoresist solution and mixed. The solutions were cast on poly dimethyl siloxane (PDMS) substrates and exposed to UV light (Lumen Dynamics OmniCure 1000 system) for 1 s at 20 mW cm^{-2} at 365 nm. Films were water annealed overnight. The composite SPP-PEDOT:PSS ink was mixed with photoinitiator (0.1µl/1mg of SPP) (Darocur 1173, BASF) and cast on the FPP film or glass, and left to air dry in a fume hood. After drying, the sample was exposed to UV for 1-1.5 s through a photomask to form patterns. Patterns were developed in water to remove any un-crosslinked material and form sensors.

2.4. Electrical measurements: The sheet resistance of the conductive ink was measured by a conventional four point probe technique using an HP 4156B precision semiconductor parameter analyzer (Agilent Technologies). SPP-PEDOT:PSS films (with and without DMSO) were formed on glass slides. To prevent damage, adhesive strips of copper tape of 1 mm thickness were used to provide ohmic contact between the sharp probe and sample. Film thickness was obtained by SEM imaging following cryo-fracturing (Jeol JSM LV-5610) (**Figure S1**). Resistivity and conductivity were calculated from sheet resistance and thickness data. I-V characteristics were measured on a Keithley 4200 semiconductor characterization system (Keithley Instruments, Cleveland, OH). All samples were fabricated in triplicate and at least 3 points were tested for each sample.

2.5. Electrochemical measurements: Cyclic voltammetry (CV) measurements were performed on a CHI 401(CH Instruments, Inc., Austin, TX). PBS buffer (0.1M, 7.4 pH) was used as the electrolyte. A standard three cell configuration was used - Ag/AgCl as the reference electrode, platinum as counter electrode, and the as-prepared samples as the working electrode. The working electrode was swept at a potential range of -1.0 to 1.6 V and scan rate of 100mV/s. Charge Storage Capacity (CSC) was calculated by calculating the area of the cyclic

voltammogram using Origin Pro (Origin Lab), and normalizing by scan rate and sensor area exposed to the electrolyte. Electrochemical Impedance spectroscopy (EIS) was performed on a CHI 660 workstation (CH Instruments, Inc.). The impedance spectra of the as-prepared electrodes was determined in 0.1M PBS buffer (pH 7.4) at a frequency range of 10^{-2} Hz to 10^{+4} Hz with a 5 mV AC amplitude.

2.6. Sensing experiments: Chronoamperometric response was measured for electrochemical sensing of glucose, dopamine hydrochloride (DA) and ascorbic acid (AA). A 10 ml of stirred solution of PBS (0.1M PBS buffer pH 7.4) was used with a polarization potential of 0.3 and 0.6 V for SPP/PEDOT:PSS patterns on FPP. For glucose sensing experiments, the conducting ink was loaded with 200 U of glucose oxidase (from *A. Niger*, Sigma-Aldrich). 0.1 mM ferrocenyl methyl trimethyl ammonium iodide (Strem Chemicals, Newburyport, MA) was added to the buffer to shuttle electrons from the enzyme active site to the conducting polymer. Fructose, sucrose and galactose were used as a negative controls for the glucose sensor. 0.1 M glucose, 0.1 M fructose, 0.1 M sucrose, 0.1 M galactose, 0.01 M ascorbic acid, and 0.01 M dopamine stock solutions were freshly prepared for each experiments. After stabilization of the background current, increasing amounts of analytes were added to the stirred solution at regular intervals to obtain I *vs.* t response curves. Limit of detection (LOD), limit of quantitation (LOQ), and sensitivity were calculated per ICH guidelines (Shrivastava and Gupta 2011):

$$LOD = \frac{3.3 \times S_{y/x}}{m}\ \mu\text{M}\ ,\ LOQ = \frac{10 \times S_{y/x}}{m}\ \mu\text{M and Sensitivity} = \frac{m}{A}\ \mu A/\mu M.cm^2$$

Where, $S_{y/x}$= Standard error at y-intercept, m= slope of the calibration curve (A/µM). Both values were obtained from the regression analysis of calibration curves. A= area of sensor exposed to electrolyte (cm^2). 3-4 different sensors were tested for each experiment.

2.7. Glucose oxidase leaching: Enzyme loss from sensor films was monitored over a duration of three days in a buffer solution. Three different sensor samples with and without GOx were soaked in 4 ml of PBS buffer. Using UV-Vis (BioSpec-mini, Shimadzu, Japan), the surrounding buffer was sampled each day over 3 days. Protein concentration was measured at 280 nm

assuming an extinction coefficient of 16.7 (1 cm path length cuvette) to calculate the total mass of protein lost in each sample.

2.8. In vitro proteolytic degradation and imaging. To demonstrate biodegradability of the devices, enzymatic degradation was followed over time. Devices (4 mg of protein total) were incubated in 10 mL of protease (1 U/mg of protein) at 37°C (Protease XIV from *S. Griseus,* Sigma-Aldrich). The enzyme solution was replaced every third day to preserve activity. As negative controls, films were incubated in PBS containing no enzyme. At different time intervals, samples were removed from solution, washed with deionized water, and dried for further study. The films were imaged using scanning electron microscopy (SEM) to observe the changes in the surface morphology. Optical and scanning electron microscopy (SEM) images were taken on a Nikon Eclipse and Jeol (JSM-5610 LV) instruments respectively. The patterns and films for SEM were first sputter coated in 20 Å platinum Denton vacuum V cold sputtering system (Moorestown, NJ).

2.9. Bending of sensors: To demonstrate the stability of material under mechanical stress, sensing and electrochemical experiments were carried out under static or cyclic bending. To obtain a static bend, the sensor was fixed on a 0.005″ thick PTFE tape, and used first without any bending, and subsequently was pinched to achieve a 30° stable bend. For cyclic bending the samples were applied to 45° bending and releasing cycles. This process was mechanically repeated ~150 times.

3. Results and Discussion

Silk proteins have been shown for various applications like in drug delivery, nanostructured scaffolds, (Kim et al. 2012; Omenetto and Kaplan 2010) and as platforms for transistors, solar cells and photonic devices (Kim et al. 2009; Tao et al. 2012). Pioneering work has shown silk biopolymers as substrates for conductive materials such as Mg, Au/Cr, Si/SiO_2 can be used *in vivo* without generating noticeable immunogenic response (Kim et al. 2010; Tao et al. 2014). Here, we demonstrate an alternative strategy using a conductive polymer as the active material instead of metals, silicon or their oxides. We show that such microfabricated silk-protein based

biosensors are mechanically robust, flexible and can be formed at high resolution, while providing excellent electrochemical response.

3.1 Fabrication of flexible silk-PEDOT:PSS sensors

Photolithography is a relatively simple, rapid, high throughput and high resolution technique. The primary requirement is the use of a material containing photoactive moieties, such as a photoresist, which has typically precluded its application to proteins. As shown earlier by our group, the incorporation of methacrylate groups enables the two silk proteins – fibroin and sericin to be transformed into negative tone photoresist-*like* materials, referred to as fibroin protein photoresist (FPP) and sericin protein photoresist (SPP) (Kurland et al. 2013; Kurland et al. 2014). In the presence of a short duration UV exposure, these "protein photoresists" crosslink and become water-insoluble. Silk photopolymers can therefore be used in standard photolithographic processes to form mechanically robust, micro and nanoscale architectures. While lithography using FPP involves solvents such as HFIP, SPP is water soluble, which permits a fully aqueous process. These silk photopolymers provide unique matrices for the linking of non-photoactive polymers via co-polymerization to render them photopatternable (Pan et al. 2005). Here, we use PEDOT:PSS combined with SPP to form a photoreactive conductive sensing ink, termed SPP-PEDOT:PSS.

To form micropatterned biosensor devices, the conductive ink can be cast on a variety of substrates using drop casting or spin coating. Exposure of UV light through a photomask is used to crosslink the ink and form patterns. Un-crosslinked ink is washed off using water during the development step (**Scheme 1)**. The designs and complexity are restricted only by the nature of the photomasks, and virtually any kind of micropatterns can be scalably fabricated. Towards the goal of forming a flexible, degradable biosensor, the SPP-PEDOT:PSS ink was cast on an FPP film as shown in **Figure 1a**, wherein microscale patterning is demonstrated on a contact lens sized film. This process creates high fidelity structures as depicted in **Figure 1b**. SEM imaging shows the relatively smooth and highly defined patterns, with smallest feature sizes ~5 µm (**Figure 1c**). The thickness of the support film as well as patterns can be easily controlled by

simply modulating the total material in the solution. The protein patterns are flexible and mechanically robust, and can withstand bending and twisting while hydrated, without breakage of either the films (substrate) or patterns (conductive ink). Critically, no delamination of the patterns is observed owing to the *covalent linking* of the two components of the system. This addresses a major concern in the design of flexible systems, wherein the active layer and substrate tend to separate due to mechanical stress. The entire device can be stored either dry or wet (in buffer) for weeks without any appreciable reduction in mechanical integrity or function, as discussed further below. It should be noted that the entire process is carried out on a benchtop setup and involves all water based processing at ambient temperature. This gives rise to a facile, high throughput, green microfabrication process for CPs in comparison to earlier reported processes.

3.2 Electrical and electrochemical characterization

The electrical and electrochemical characteristics of the conductive composite ink were initially evaluated utilizing different techniques. The first involved optimizing the composition of the ink for desired applications. A two-point probe was used to obtain current-voltage (I-V) characteristics as a function of the PEDOT:PSS concentration. Initial observations suggested that the composite ink behaved like an ohmic conductor without any hysteresis, under application of a dual potential sweep in a voltage window from -2 to 2 V. Expectedly, the conductivity increased with an increase in the PEDOT:PSS/SPP ratio, implying that the conductivity of the composite depends on the density of the conductive polymer network inside the silk matrix. The silk sericin acts as an inactive insulator and does not trigger any instability. Repeated I-V measurements also suggested that the material was stable under the applied voltage range. At ~19% concentration the onset of a slight nonlinear behavior was observed. The µA range of current obtained with the material having 19% PEDOT:PSS provided a good conductivity for further applications (I-V curves are shown in the Supplemental Information **Figure S1**). Conductive AFM imaging was used to confirm that there is no nanoscale phase separation of the PEDOT:PSS in the silk matrix – **Figure S2**).

To understand the relative change in resistivity as a function of PEDOT:PSS concentration, and to observe the effect of secondary dopants, a four-point probe technique was used to determine sheet resistance, resistivity and conductivity. At least three sets of measurements were taken on three separately prepared films. SPP-PEDOT:PSS films were made at 11, 16 and 19% PEDOT:PSS concentration. A positive control of 50% PEDOT:PSS/50% PEG was used. The resistivity data indicated that the material is more conductive as the PEDOT:PSS concentration increases (**Figure 2a**). 5% (v/v) DMSO was mixed with the PEDOT:PSS stock solution keeping the other compositions constant in order to positively affect conductivity of PEDOT:PSS by inducing better packing of particles and reducing PSS content from the surface of the film (Thomas et al. 2014). The results indicate that DMSO improves the conductivity of the composite ink by ~2 orders of magnitude. DMSO might be shielding the non-conductive silk protein from PEDOT:PSS to obtain better packing and defining a better path for current flow. Overall, the investigations of electrical property of the material suggested that 15 to 20% concentration in the silk matrix provided an optimal PEDOT:PSS range for different applications.

For an optimal electroactive biomaterial, it is crucial that it is not only electrically conductive, but also stable in an electrode-electrolyte interface at physiological pH. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed to characterize electroactivity and electrochemical stability. Pure SPP patterns (no PEDOT) on FPP films were used as a negative control. It may be noted that films with only PEDOT:PSS (no sericin matrix) on FPP were not stable, and tended to delaminate as there is no chemical linkage to attach the PEDOT:PSS to the underlying substrate. A wide stable potential window of -1.0 to 1.6V without water splitting was observed (**Figure 2b**), which might therefore be useful to realize detection of various biomolecules having different peak potentials.

The electroactivity of the material increased with an increase of PEDOT:PSS concentration. Films with 19% PEDOT:PSS ink (+DMSO) showed a good charge storage capacity (CSC) of ~74 mC/cm^2, while films without DMSO had CSC ~55mC/cm^2 (scan rate of 0.1 V/s) (**Figure 2b, d**). The electrochemical stability was monitored via two different time duration studies. In the

first study, samples were soaked overnight in 0.1 M PBS solution (pH 7.4). 200 redox cycles at a potential window of -1.0 to 1.6 V and scan rate of 0.1 V/s were performed. A 90.5 % retention of electroactivity was observed. In another study, 10 cycles of CV were taken each day for 3 days while keeping the samples soaked in PBS. The retention of electroactivity was found to be ~80 % (**Figure S3**). The CSC measures obtained are comparable, or better than reported values where PEDOT:PSS is within a non-conductive matrix such as a hydrogel or even in flexible materials without a conductive support (Guo et al. 2014; Patton et al. 2015).

Following stability analysis, the impedance of the material was assessed. An important source of noise can arise due to high impedance from the electrode (Cogan 2008). Conductive polymers have the potential to reduce impedance in addition to their high charge storage capacity, when used as an interface between soft tissue and metal electrodes (Green et al. 2008). At the neuronal level, the frequency of release of dopamine and other neurotransmitter is around 1 kHz region. Therefore, the flat impedance regime of impedance spectra of the film containing 19% PEDOT:PSS is important to obtain a high signal to noise ratio which may result in sensitive biosensing of neurotransmitters. Films approached a near-resistive region below 1 Hz as the phase angle approaches zero, indicating that the resistance arises from the solution (Cogan 2008). These results suggest that the SPP-PEDOT:PSS composite has very low impedance (13.6 kΩ) at 1 kHz.

Finally, to consider the viability of the composites for electrochemical activities in flexible systems, the effect of mechanical stress on the performance of the conductive patterns was investigated. The conductive patterns were bent to ~30° degrees and characterized using EIS. The bend caused a slight increase in impedance of the material but the order remained the same (**Figure 3a**). Separately, conductive patterns were subjected to repeated cycles of bending (30^{o}) and release. The CSC values at increasing stress cycles showed only a very slight decrease to 150 cycles (<1.5% loss). SEM images of the surfaces showed no damage to the composite or delamination (**Figure 3b**), indicating a high stability under stress, and the ability to sustain electrical and electrochemical properties under mechanically challenging conditions.

3.3. Proteolytic degradation

Being mechanically strong yet intrinsically biodegradable, silk biomaterials are desirable in implantable bioelectronics and biosensor devices (Hwang et al. 2013). Various groups including ours have shown that silk proteins can be proteolytically degraded (Horan et al. 2005; Pal et al. 2015). As our system is comprised of silk proteins and PEDOT:PSS, we expect it to be degradable. Silk-PEDOT films (SPP-PEDOT:PSS on FPP) were incubated in a PBS solution at 37°C with and without protease for several weeks, and protease solution was changed every 3 days to retain the enzymatic activity of protease. SEM and AFM images were taken at various time points to morphologically observe the composite film surface (Images are presented in supplemental **Figure S7** and **S8)**. The composite film has a smooth surface on Day 0. Both the films (protease and without) have comparable surfaces at Day 6. After 21 days, while the control sample is minimally affected, the sample in protease solution is significantly degraded. Quantitatively, the roughness of the films in PBS buffer changes by ~15% in comparison to ~90% for the protease solution. Following a period of ~4 weeks, FPP film and SPP matrix degrade completely in the enzyme solution and preclude imaging. It should be noted that even though PEDOT:PSS is biocompatible, it is not biodegradable. In the case of micropatterns of conductive ink, the samples under proteolytic degradation lost PEDOT:PSS in the form of fine fibrous strands, which is intuitive given that the PEDOT:PSS is blended with SPP. Thus, by controlling the film crosslinking, it is possible to fabricate a device that is programmably degraded over a specified period. As shown above, the PEDOT:PSS does not escape from the crosslinked matrix, but when the carrier and support degrade, they break down as well.

3.4. Electrochemical sensing

Wearable and conformable electronics, electronic skin and tissue-integrated sensing or actuation systems are some evolving fields where such degradable silk/PEDOT:PSS devices might find utility. To demonstrate functional aspects, we explored the electrochemical sensing capabilities of the conductive composite micropatterned films. Initially, the devices were explored for *non-specific* sensing of biomolecules, specifically ascorbic acid (AA) and dopamine (DA), which are

of physiological importance. AA, widely known as vitamin C, plays an important role in the formation of collagen, an active material for the development of bones, muscles and blood vessels (Rawat et al. 2015). DA, from the catecholamine family, is a vital neurotransmitter that helps to control the reward and pleasure centers of the brain. Various neuronal diseases like Parkinson's, schizophrenia and attention deficit hyperactivity have been linked to dysfunction in the dopamine system (Hsu et al. 2012). These two analytes are commonly found together and have been the focus of several reports, but still remain a challenge from the perspective of microfabricated or miniaturized sensors (Cai et al. 2014).

In separate experiments, 0.01 M DA and AA were added to a 10 ml PBS buffer system at various intervals after stabilization of background current. SPP-PEDOT:PSS (on FPP films), Ag/AgCl and platinum were used as working, reference and counter electrode respectively in a chronoamperometric setup at 0.3 V constant potential. Each experiment was replicated using at least 3 different sensors. The lowest detectable concentration was 15.21 µM and 15.47 µM of DA and AA respectively, whereas the limit of quantitation was 46.1 µM and 46.87 µM of DA and AA respectively, calculated from linear regression of the calibration curves. The amperometric response curve of the dopamine and ascorbic acid reveal a linear range from 10 µM to 200 µM and 600 µM respectively, with a sensitivity of 45.9 nA/(µM.cm^2) and 256.5 nA/(µM.cm^2)(**Figure 4**). The response time (time taken to reach 95% of steady state current) for DA and AA sensors is ~30 seconds. The data are presented for 3 sensors, starting at the same baseline. Another example of sensor response for each analyte is presented in **Figure S9**, showing the reproducibility of these sensors. The linear and dynamic ranges are comparable with reported sensors, for instance a flexible graphite-paper sensor (Cai et al. 2014). Notably, the patterns contain only PEDOT:PSS (19%), and neither the sensor nor the electrolyte solution contain any other electroactive species to enhance the electrochemical response of the sensor. With the addition of suitable biocompatible electroactive species, it is possible to enhance sensitivity and detection limits.

3.5. Enzyme encapsulation and glucose sensing:

The benign nature of the photolithography process at ambient temperature and using only water, coupled with a natural silk protein matrix further provide the possibility to encapsulate and stabilize enzymes, therapeutics and other active biomolecules (Mottaghitalab et al. 2015; Pritchard et al. 2012). The patterned silk-CP sensors were subsequently demonstrated as a biosensor specific to glucose via the encapsulation of the enzyme, glucose oxidase (GOx) (Wang 2008). To date, most conductive polymer based enzymatic biosensors have shown that the conductive polymer helps transduce electrons from analyte recognition enzymes to the active electrode. Here, the glucose sensor is demonstrated by immobilizing the GOx directly in the SPP-PEDOT:PSS composite ink, to work as both the transducer and active electrode. The fully water-based processing enables dissolution of the enzyme to form a photopatternable enzyme ink. 0.1 M glucose solution were added to sensors in PBS, under continuous stirring at different intervals. The amperometric response of the glucose sensor is shown in **Figure 5a**. The sensor shows a rapid linear response throughout the dynamic range (0 to 10 mM), which covers physiologically relevant blood glucose concentrations. The selectivity of the sensor was tested by adding solutions of galactose, fructose, and sucrose. It is clear from Figure 5 that the sensors do not detect any other sugar except glucose. The limit of detection and quantitation of the sensor is 1.16 mM and 3.52 mM respectively, with a sensitivity of 7.57 $\mu A/mM.cm^2$. The average response time of the sensor (time taken to reach 95% of steady state current) is ~15 seconds. Sensor response was also tested as a function of ionic strength using 0.2x and 0.5x PBS as the electrolyte. The sensors were still linear with comparable metrics as those reported above for 1x PBS (Figure S10).

The encapsulated GOx sensors were extremely stable over time and could be used 2 weeks after fabrication. Samples with entrapped GOx and without enzyme in the SPP-PEDOT:PSS matrix were soaked in a PBS buffer for 3 days to study possible loss of the enzyme due to leaching. Aliquots of buffer collected and analyzed using UV/Vis absorbance (A_{280}) showed that both sets of films showed a negligible amount of protein leaching (<0.5%). Encapsulation of enzymes without any leaching can therefore lead to multifunctional devices; for instance, where drug delivery can be integrated with sensing of different targets. To the best of our knowledge, this is the first study showing a functional fully organic biosensor device formed by a green

microfabrication technique that can also be degraded. Such biosensors may be potentially used on skin (**Figure 5b**) and display conformability, with variations in the degree of crosslinking and thickness of films to precisely modulate the degradation. This gives an opportunity to realize organic bioelectronic devices, which can be programmed to specific levels of transience.

4. Conclusions

The confluence of bioinspired materials with synthetic conducting polymers provides a platform to realize unique properties and advanced functionality in biosensors and bioelectronics. We show a strategy to form all organic and degradable microfabricated devices using natural silk proteins and PEDOT:PSS in the form of a carrier protein/substrate matrix. Water-based photolithography is utilized to fabricate complex and precise microstructures on fibroin sheets that are optically transparent, flexible, biocompatible and biodegradable. This system is electrochemically active and stable for ~1 month, and can withstand mechanical deformation. Microfabricated devices formed are successfully shown as nonspecific and sensitive biosensors for dopamine and ascorbic acid *in vitro*. By encapsulating enzymes such as glucose oxidase at biological pH and ambient temperature, a highly sensitive and selective glucose biosensor is demonstrated. The composite material is shown to degrade over 4 weeks under enzymatic action. The mechanical, electrical and electrochemical properties can be easily tailored by varying the content and crosslinking of materials used. With outstanding mechanical properties and tunable biodegradability, these silk-based devices are excellent candidates for 'implant and forget' kind of applications. We envision that, such materials can be employed to develop diverse electrochemical, bioelectronic and optoelectronic devices for implantable or smart skin applications.

Supplemental Information

Additional data on electrochemical characterization of the conductive ink and device are presented including film composition, SEM images of degradation, conductive AFM, I-V and CV characteristics and sensor response.

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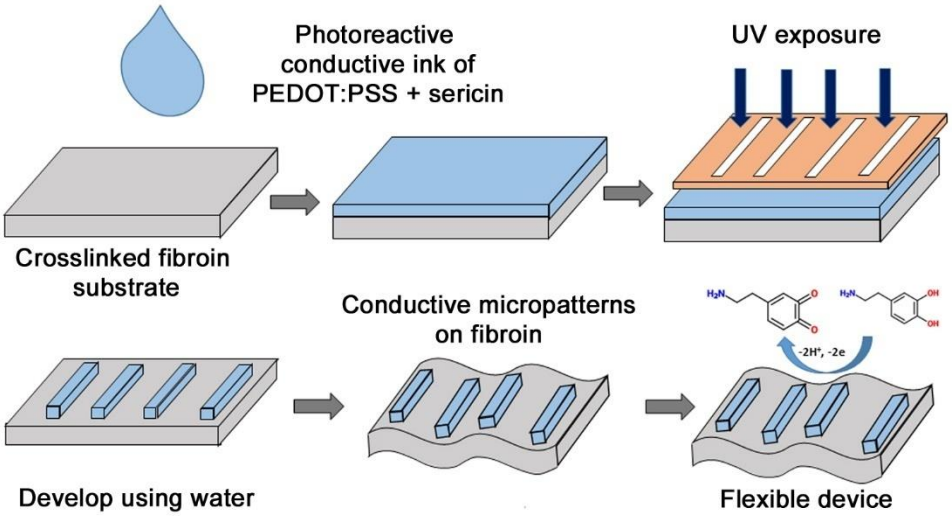

Scheme 1: Schematic diagram showing the different steps to fabricate conductive micropattern on a flexible substrate.

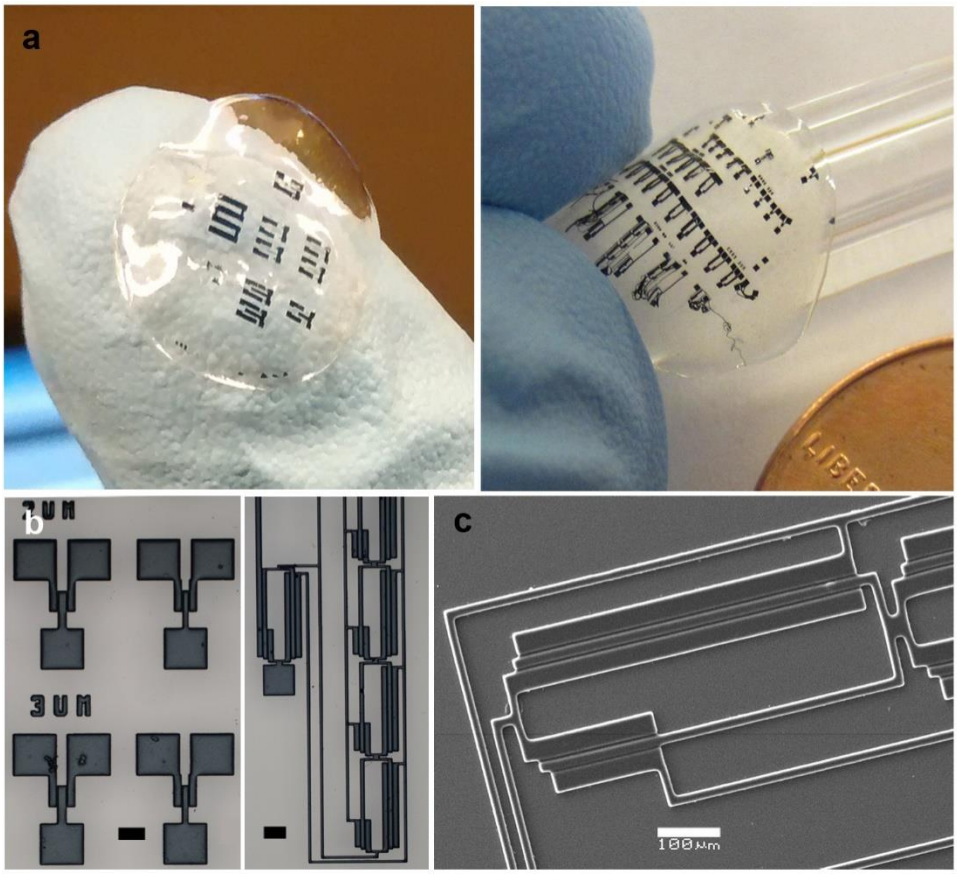

Figure 1: Formation of complex microstructures via photolithography. (a) Large area micropatterns of PEDOT:PSS can be formed on flexible and conformable silk fibroin sheets, (b) Optical micrographs and (c) SEM images of PEDOT:PSS micropatterns on glass. Scale bars = 100 µm.

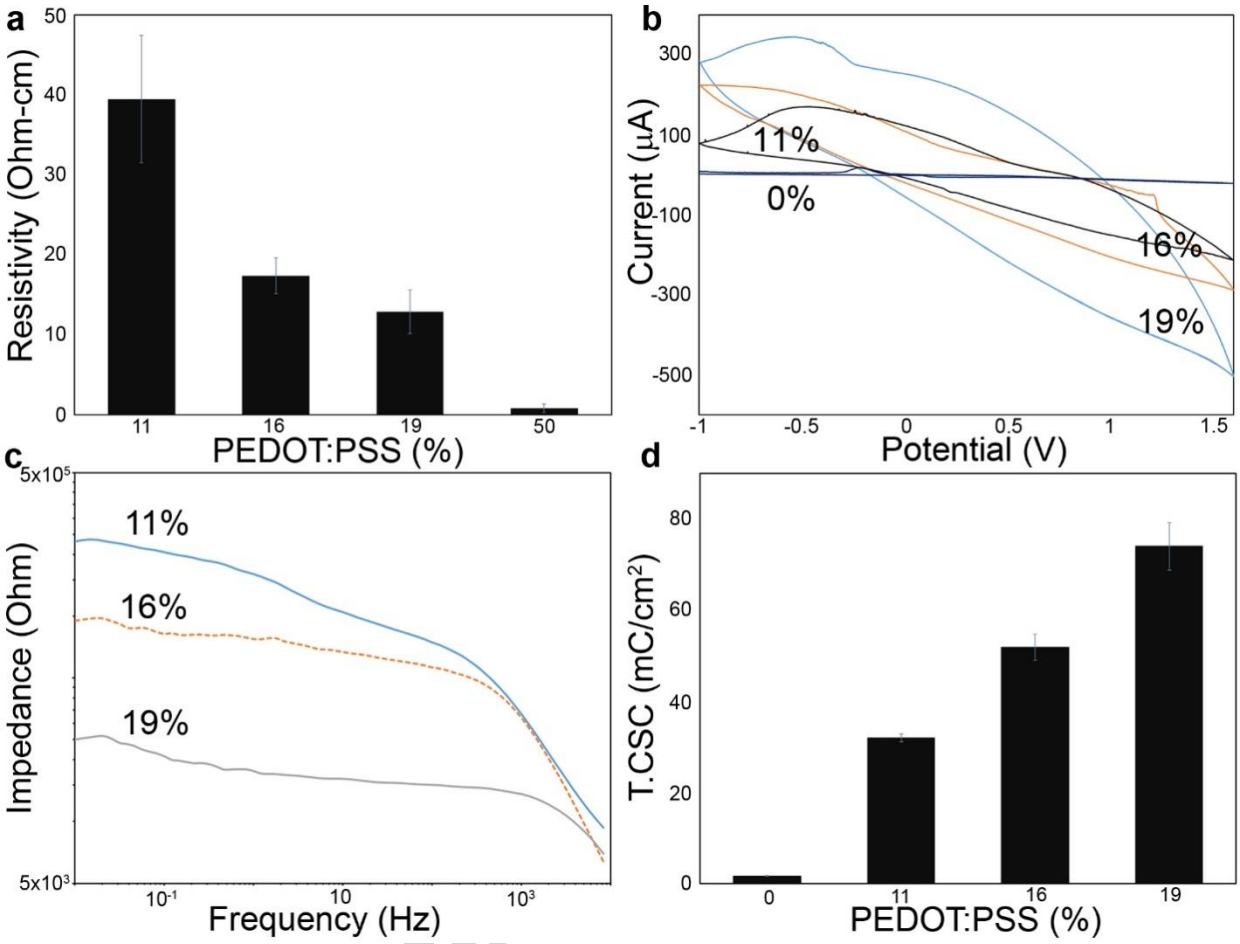

Figure 2: Electrical and electrochemical characterization of the conductive composite ink as a % of PEDOT:PSS: (a) Resistivity, (b) Cyclic voltammogram, (c) Impedance spectra, (d) Charge storage capacity. Scan rate= 100mV/s in 0.1 M PBS (pH 7.4) solution.

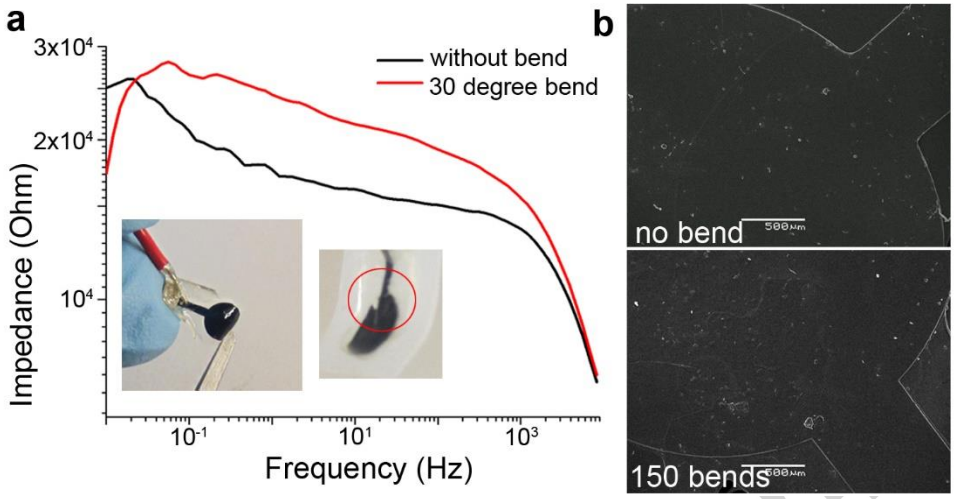

Figure 3: Stability under mechanical stress: (a) Mechanical bending and corresponding effect on impedance after 150 bending cycles. The inset (red circle) shows the region of highest stress during bending that is further explored using (b) SEM images showing the film integrity is retained without any cracks or defects after 150 bending cycles (scale bar = 500 µm).

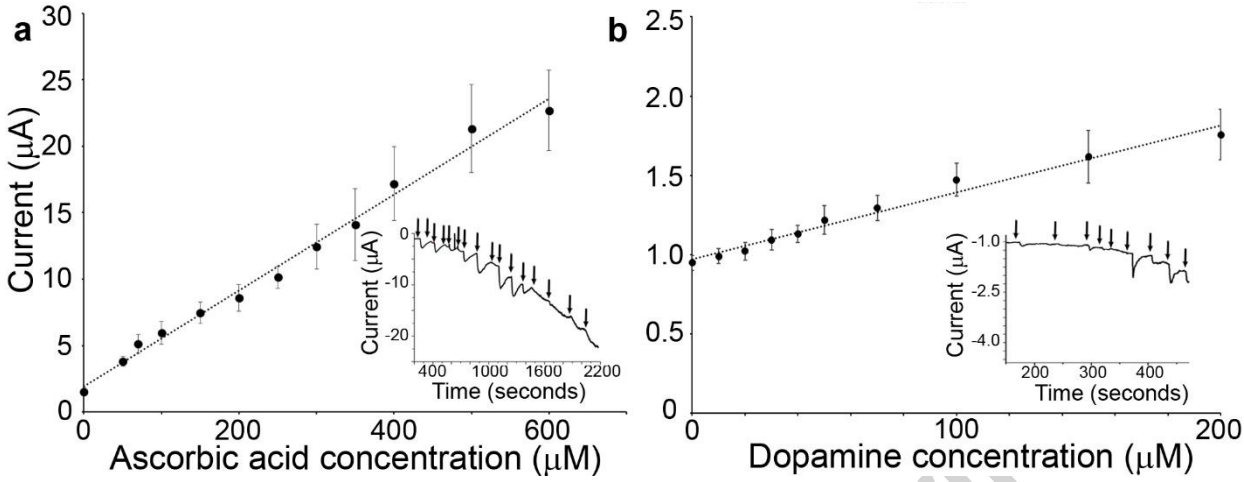

Figure 4: SPP-PEDOT:PSS enzyme biosensor: Non-specific sensing of (a) ascorbic acid and (b) dopamine biosensing, showing the linear ranges of both sensors (R^2 = 0.989 and 0.979 respectively with n=3 sensors). The insets show the chronoamperometric response with addition for one sensor as an example. Both sensors start from the same baseline current.

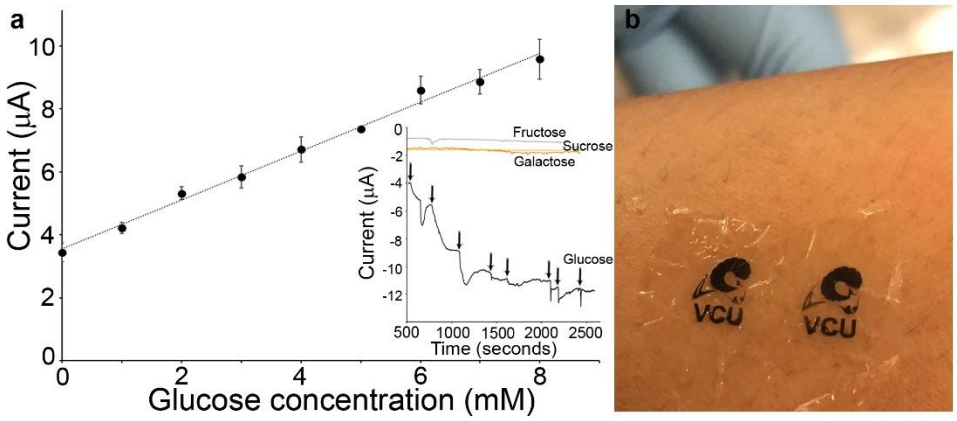

Figure 5: SPP-PEDOT:PSS enzyme biosensor: (a) Glucose sensing shown by immobilizing glucose oxidase (GOx) in the microfabricated sensor (R^2 = 0.992 with n=3 sensors). The inset shows *I vs. t* response for one sensor and the specificity control experiment showing that it does not detect other sugars. **(b)** The flexible micropatterned sensors may be placed on skin. The dark patterns are formed from the conductive ink (SPP-PEDOT:PSS) on the underlying crosslinked fibroin sheet.

Highlights

We show a facile and high resolution photolithographic process for patterning conducting polymers such as PEDOT:PSS using a silk protein carrier.

The unique conductive photoink is patterned on flexible silk fibroin sheets to form an all organic, degradable bioelectronic device

Highly sensitive biosensors are shown with and without enzymes.

The sensors are mechanically robust under flexure and retain structure and electrochemical properties.