Development of 2D and 3D Conductive Biomaterial Composites for Use as Electromechanical Actuators

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Development of 2D and 3D Conductive Biomaterial Composites for Use as Electromechanical Actuators

By

Sean Severt

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Amanda Murphy

Dr. Janelle Leger

Dr. David Rider
MASTER’S THESIS

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Sean Severt
May 13, 2016
Development of 2D and 3D Conductive Biomaterial Composites for Use as Electromechanical Actuators

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Sean Severt
May 2016
ABSTRACT

Flexible and conductive biocompatible materials are attractive candidates for a wide range of biomedical applications including implantable electrodes, tissue engineering, and controlled drug delivery. Here we demonstrated that chemical and electrochemical polymerization techniques can be combined to create highly versatile silk-conducting polymer (silk-CP) composites with enhanced conductivity and electrochemical stability. Interpenetrating silk-CP composites were first generated via in situ deposition of polypyrrole during chemical polymerization of pyrrole. These composites were sufficiently conductive to serve as working electrodes for electropolymerization, allowing an additional layer of CP to be deposited on the surface. This two-step technique expanded the range of available polymers and dopants suitable for the synthesis of mechanically robust, biocompatible, and highly conductive silk-based materials. The sequential method was applied to 2D films, 3D sponge-like silk scaffolds, and electrospun silk fibers, allowing the fabrication of conductive materials with biomimetic architectures. The electrospun fibers were able to be prepared with a high degree of alignment and permeability that was conserved during modification with conducting polymers. These electrospun materials were utilized to fabricate electromechanical actuators. The mechanism, performance, and efficacy under extended cycling of the actuator devices were analyzed in biomimetic electrolyte solutions.
ACKNOWLEDGEMENTS

I am grateful for financial support from Western Washington University, Research and Sponsored Programs at WWU, a grant to WWU’s Advanced Materials Science and Engineering Center (AMSEC) from the M.J. Murdock Charitable Trust, and the National Science Foundation (DMR-1411292). I am also grateful for technical support from Charles Wandler, Erin Macri, Jim Mullen, Nathan Bradshaw, Nicholas Ostrovsky-Snider, and Carly Fengel.
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## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATR-FTIR</td>
<td>Attenuated total reflectance Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>CP(s)</td>
<td>Conducting polymer(s)</td>
</tr>
<tr>
<td>ChP</td>
<td>Chemical polymerization</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>DECP</td>
<td>Degradable conducting polymer</td>
</tr>
<tr>
<td>DBS</td>
<td>Dodecylbenzenesulfonate</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>EP</td>
<td>Electropolymerization</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>Iron(III) chloride</td>
</tr>
<tr>
<td>IPN</td>
<td>Interpenetrating network</td>
</tr>
<tr>
<td>NaDBS</td>
<td>Sodium dodecylbenzenesulfonate</td>
</tr>
<tr>
<td>PAni</td>
<td>Poly(aniline)</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PEDOT</td>
<td>Poly(3,4-ethylenedioxythiophene)</td>
</tr>
<tr>
<td>PEDOT-OH</td>
<td>Poly(hydroxymethyl-3,4-ethylenedioxythiophene)</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PEO</td>
<td>Polyethylene oxide</td>
</tr>
<tr>
<td>PPY</td>
<td>Poly(pyrrole)</td>
</tr>
<tr>
<td>PSS</td>
<td>Polystyrene sulfonate</td>
</tr>
<tr>
<td>p-TSA</td>
<td>para-Toluene sulfonic acid</td>
</tr>
<tr>
<td>SCEP</td>
<td>Sequential chemical and electrochemical polymerization</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope or scanning electron microscopy</td>
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</table>
CHAPTER 1: INTRODUCTION

1.1 Conducting Polymers. Biocompatible materials that conduct electricity have the potential for numerous biomedical applications. Conducting polymers (CPs) are a group of organic polymers able to conduct electricity through a conjugated π-system composed of an extended network of alternating carbon π-bonds (Figure 1). Numerous studies have shown a high degree of biocompatibility between many CPs and native tissues.\(^1\)\(^-\)\(^3\) CPs such as poly(pyrrole) (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) have been studied for use in drug delivery,\(^4\)\(^-\)\(^8\) neurological interfaces,\(^3\)\(^9\)\(^-\)\(^12\) tissue engineering,\(^13\)\(^-\)\(^15\) and electromechanical actuators.\(^14\)\(^,\)\(^16\)\(^-\)\(^18\) However, the poor mechanical properties of CPs present a major hurdle in the development of practical biomedical devices utilizing this class of polymer.\(^19\) Bulk CPs are typically stiff, brittle, and insoluble due to the extended conjugated π-system, leading to poor durability and complicating the fabrication of three-dimensional constructs.

![Figure 1. Structures of common conducting polymers: polyacetylene (PA), poly(pyrrole) (PPy), poly(aniline) (PAni), poly(3,4-ethylenedioxythiophene) (PEDOT), and poly(hydroxymethyl-3,4-ethylenedioxythiophene) (PEDOT-OH).](image-url)
1.2 **Doping Effects.** Dopants are molecules added to modify the properties (e.g. electrical or optical) of a material. For conducting polymers, dopants are generally anions, such as dodecylbenzene sulfonate (NaDBS), \(p\)-toluene sulfonic acid (\(p\)-TSA), perchlorate, or polystyrene sulfonate (PSS), added to stabilize and facilitate the formation of positive charges along the aromatic backbone during polymer oxidation (Figure 2A,B).\(^{17}\) Oxidizing the polymer creates holes in the valence band which act as charge carriers, making these materials \(p\)-type semiconductors.\(^{20}\) Dopant type and loading affects the end conductivity and mechanical properties of the polymer.\(^{21}\) Larger anionic dopants that are physically entrapped in the composite are desired to minimize dopant leaching and extend the electroactive lifetime of the polymer. Trapped dopants can also encourage cation exchange in the films, which can be advantageous when used as actuation devices.\(^{22}\)

![Figure 2](image-url)

**Figure 2.** Reversible redox reaction of poly(pyrrole) (A) and the structures of common anionic dopants (B).
1.3 Conducting Polymer Composites. Two of the major set-backs to the use of conducting polymers in biomedical applications are their rigidity and poor processability. To improve the mechanical properties of CPs, methods to create blends or composites of CPs with a variety of non-conductive polymers with mechanical properties more suited to the desired application have been published.\textsuperscript{23-26} Synthetic polymers and naturally occurring biopolymers including polylactide, polycaprolactone, polyurethane, chitosan and collagen have been investigated for use in conducting polymer composites.\textsuperscript{27-31} While the mechanics of the resulting conducting composite can be greatly improved compared to a pure CP, the conductivity is lowered due to the incorporation of an insulating polymer. Typically, conductivity of the polymer is lowered from 10-10\textsuperscript{3} S/cm to the order of 10\textsuperscript{7}-10\textsuperscript{1} S/cm.\textsuperscript{25,27-31}

Different fabrication methods for composite manufacture have been utilized to improve the processability of CPs. One method is to form hydrogels by blending the conducting polymer with a degradable conducting polymer substrate.\textsuperscript{23} However, almost all developed degradable conducting polymers are only soluble/stable in organic solvents, which are avoided for biomedical applications. Another common method is to swell a dried polymer film in CP monomer solution and then chemically polymerize the CP with an oxidant. Chemical polymerization has varied success with full incorporation of the CP into the polymer substrate, and can cause indiscriminate precipitation of the CP on the fiber surface with risk of CP delamination.\textsuperscript{25,32}

In order to maintain a balance between the mechanical properties of the substrate and the conductivity of the CP, an interpenetrating network (IPN) between the two polymers is
desired. By first chemically modifying a natural biopolymer support, our group has recently established methodology to fabricate IPN composite materials composed of silk fibroin and PPy with excellent incorporation of the CP.33

Silk is a naturally occurring biopolymer generated by spiders and Bombyx mori larvae among other insects. Due to industrial production for use in textiles, fibroin is both cheap and widely available. Fibroin has been used medically for centuries as a suture material, and has been extensively researched for its in vivo biocompatibility and low cytotoxicity.34–36 As a polypeptide, fibroin can biodegrade into non-toxic products, and the degradation rate of the material can be tuned based on how it is processed.34,37,38 Fibroin contains two main domains: a flexible hydrophilic domain and a highly crystalline hydrophobic domain.39 The combination of the two domains makes fibroin both flexible and strong. Another advantage of fibroin is that it can be denatured and dissolved into an aqueous solution, allowing the fabrication of a wide variety of material architectures including films, scaffolds, hydrogels, and nanofibers.34,36,40,41

To facilitate the integration of PPy with silk, our group developed a method to covalently modify the tyrosine residues on the surface of the silk films using a diazonium coupling reaction (Scheme 1A).42 Attachment of sulfonic acid groups using this chemistry produced a negatively charged silk surface, and increased the hydrophilicity of the silk. After this ‘acid-modification’ step, an in situ chemical polymerization of pyrrole was performed in the presence of silk films (Scheme 1B). The covalently bound anionic sulfonic acid groups helped
draw in the oxidized, cationic CP to form an interpenetrating network, resulting in durable composites with sheet resistivities on the order of $10^2$-$10^3$ Ω/sq (Figure 3).^{33}

**Scheme 1.** ‘Acid-modification’ of silk substrate by a diazonium coupling reaction (A) and chemical polymerization of pyrrole in the presence of acid-modified silk (B).

---

**Figure 3.** Formation of a silk-PPy interpenetrating network.
1.4 Conducting Polymer Actuators. Electromagnetic actuators are devices which transduce electrical energy into physical energy. Conducting polymers, when oxidized and reduced, transport ions/solvent in and out of the polymer network which causes a volume change. This change in volume can be observed either as movement or the generation of force, depending on device architecture and confinement (see Chapter 4 for further discussion). Conducting polymers such as PPy have been studied extensively as artificial muscles due to their biocompatibility in vitro and in vivo, long-term tissue compatibility during implantation, and ability to generate stresses at or above mammalian muscle tissues.\textsuperscript{17} Conducting polymer actuators are currently in industrial development for biomedical applications such as microanastomosis connectors (blood vessel sutures), myringotomy tubes (relieve ear drum pressure), microvalves for the prevention of urinary incontinence, and coatings for cochlear implants.\textsuperscript{17,43}

While proof-of-concept experiments have shown that conducting polymers can function as actuators, they are still limited by their poor mechanical properties, as described above. Therefore, conducting polymer composites are of particular interest for actuation fabrication. Polymer substrates can be used to form three-dimensional morphologies, such as porous scaffolds or nanofibers, otherwise unavailable to traditional CP synthesis methods.\textsuperscript{28,44,45} These advanced architectures are anticipated to be used for development of actuators with optimized alignment, surface area, mechanics and biological interactions.
CHAPTER 2: SEQUENTIAL CHEMICAL & ELECTROCHEMICAL POLYMERIZATION

2.1 Introduction. While the properties of the first-generation silk-PPy composites were sufficient for a variety of the aforementioned biomedical applications in section 1.1 such as tissue engineering or drug release, applying this chemical method to the synthesis of composites with CPs other than PPy has had limited success. The ability to incorporate thiophene-based CPs, particularly PEDOT derivatives, is highly desirable as they have been shown to have higher redox stability in biological environments, and the reactive β-positions are blocked, minimizing backbone degradation of the CP. Attempts to deposit PEDOT, PEDOT-OH or PANi using chemical methods resulted in either non-selective, poorly adhered polymer coatings precipitated on the surface or much higher sheet resistivities ($10^3 – 10^5 \, \Omega/\text{sq}$) than can be obtained with PPy. We hypothesize that these more hydrophobic monomers have difficulty absorbing into the outer layer of the silk films, leading to poor IPN formation. In addition, larger dopants such as NaDBS or PSS that cannot be readily absorbed into the silk network were also found to interfere with the IPN formation, and the CP preferentially formed in solution rather than depositing into the silk network.

Electropolymerization typically generates CPs with higher conductivities than chemical polymerization techniques, as the reaction conditions can be tightly controlled to minimize over-oxidation. The technique also gives improved control over the morphology and thickness of the deposited layer. However, electropolymerization must be performed on a conductive surface and is usually limited to rigid 2D electrodes. Here, a two-step CP deposition method was developed where silk-PPy IPNs were first generated using the
chemical polymerization techniques described above,\textsuperscript{33} giving a moderately conductive material. These composites were then used as the working electrode in a three-electrode electrochemical cell, and a second layer of CP was electrochemically deposited on the surface. A similar method employing sequential chemical and electrical deposition of PPy and PEDOT on a non-biodegradable PVdF membrane has been previously employed to make actuator materials.\textsuperscript{48,49} However, the range of polymers, dopants, and substrates for such applications has been very limited to date, creating opportunity for further toolset expansion.

This two-step deposition method was utilized to form silk composites with varied conducting polymers and anionic dopants. The morphological and electrochemical properties of the silk-CP composites were analyzed and compared. Composites with lower electrical resistivity and higher electrochemical stability than previous generations of silk-CP materials were obtained.

2.2 Preparation of Chemically Polymerized Silk-PPy IPN Composite Drop-Cast Films. Silk-PPy IPN films were synthesized as previously described.\textsuperscript{16,33} Briefly, aqueous silk solutions were cast onto a silicone baking mat, dried, and then treated with 70% v/v ethanol to render them insoluble in water. These silk films were then exposed to a buffered aqueous solution containing the diazonium salt of sulfanilic acid, which reacted with the tyrosine residues in silk (~280 per protein), producing ‘acid-modified’ silk films. Consistent with previous experiments, the diazonium reagent penetrated ~30-60 μm into the surface of the films, increased the hydrophilicity of the silk surface, and facilitated the infiltration of pyrrole monomers.\textsuperscript{16,33} When these acid-modified films were soaked in 50 mM pyrrole, 5 mM p-TSA
and 15 mM iron (III) chloride, an IPN of silk and PPy was formed that extended ~15-25 μm into the silk surface. These chemically polymerized films had average sheet resistivities on the order of $10^2$-$10^3$ Ω/sq. As seen in previous studies, the surface morphology of the films was not significantly affected by the PPy deposition.\textsuperscript{16,33}

2.3 Secondary Electropolymerization on Silk-PPy IPN Composite Drop-Cast Films. As stated above, chemical methods for synthesizing silk-CP IPN composites have been of limited use for CPs other than PPy. Therefore, to increase the versatility of these composites without losing the strong adhesion between the silk and the CP, a second layer of CP was deposited onto the surface of silk-PPy IPN composite films via electrochemical polymerization. Copper tape was used to make electrical contact to the silk-PPy films, which then served as the working electrode in a 3-electrode cell. Kapton\textsuperscript{®} tape was used to insulate the copper contact from the electrolyte solution.

To find the optimal potential for electropolymerizing pyrrole, EDOT and EDOT-OH, a series of polymerizations were performed at potentials ranging from 0.7-1.4 V for 20 min on strips with a 36 mm\textsuperscript{2} area exposed to solution. The results showed that final sheet resistivity of the film was not determined by the potential placed across the electrodes but the total charge passed through the system (data not shown). This indicates that the end sheet resistivity is directly related to the molar amount of CP deposited in accordance with Faraday’s laws of electrolysis, $m = \left(\frac{Q}{F}\right)\left(\frac{M}{z}\right)$, where m is the mass of the analyte deposited, Q is charge passed, F is Faraday’s constant, M is the molar mass of the analyte, and z is the number of electrons transferred per ion. With the other variables held constant in our system,
charge passed (in C) is directly proportional to the mass of the analyte deposited, in this case our monomer. As shown in Figure 4, films that were electropolymerized to a charge density of at least 2.0 C/cm² had optimal sheet resistivities for all monomer/dopant combinations. All polymerizations for which the charge passed exceeded 2.0 C/cm² had a negligible decrease in sheet resistivity. Sheet resistivity probes the resistivity in the lateral direction parallel to the surface, so the measurements are minimally dependent on film thickness. However, the values can be affected by surface morphology or a heterogeneous surface. Therefore, the leveling out of the sheet resistivity values above 2 C/cm² can be attributed to the fact that the surface becomes completely coated with the EP polymer at this point (see SEM discussion below).

**Figure 4.** Plot of final device sheet resistivity vs. charge passed per area for the given monomer/dopant combinations. Error reported as standard deviation, n = 3.
While the sheet resistivity levels out above 2 C/cm², a steady increase in current was observed in the cyclic voltammetry plots as the charge passed increased (Figure 5). However, the composites became quite brittle and difficult to handle as the EP layer became thicker at higher levels of charge passed. For these composites, our goal was to maximize the conductivity of the films while minimizing adverse effects on the mechanical properties of the underlying silk substrate. Therefore, polymerization conditions utilizing a charge passed of 2 C/cm² was selected for further studies.

**Figure 5.** Representative cyclic voltammograms of composites after chemical polymerization (ChP) or sequential chemical and electrochemical polymerization (SCEP) performed with increasing amounts of charge passed. Polymer/dopant compositions were A) PPy/pTSA and B) PEDOT-OH/NaDBS. Potentials between -1.0 V and +1.0 V vs. Ag/AgCl were scanned at 50 mV/sec in aqueous PBS (3rd cycle plotted).

By using this sequential chemical and electrochemical polymerization (SCEP) method, silk-based composite films were obtained at lower resistivities than previously seen (Table 1). This
process was successfully applied to deposit PPy, PEDOT, and PEDOT-OH. The incorporation of the small anionic dopant \( p \)-TSA and the large anionic dopant NaDBS was achieved for both PPy and PEDOT-OH. PEDOT deposition was only successful when using the surfactant NaDBS as a dopant due to the low solubility of the EDOT monomer in water. Previous attempts at optimizing chemical polymerization of PEDOT or PEDOT-OH onto silk substrates had resulted in composite films with sheet resistivity on the order of \( 10^4 \Omega \text{/sq} \), but here PEDOT and PEDOT-OH were successfully electropolymerized onto PPy/ silk substrates to give films with sheet resistivity on the order of \( 10 \Omega \text{/sq} \) (Table 1).

Table 1. Sheet resistivity of drop-cast silk films after sequential chemical and electrochemical polymerization

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Dopant</th>
<th>Sheet Resistivity (( \Omega \text{/sq} ))</th>
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<tbody>
<tr>
<td>PPy</td>
<td>( p )-TSA</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>PPy</td>
<td>NaDBS</td>
<td>74 ± 8</td>
</tr>
<tr>
<td>PEDOT-OH</td>
<td>( p )-TSA</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>PEDOT-OH</td>
<td>NaDBS</td>
<td>6.3 ± 0.8</td>
</tr>
<tr>
<td>PEDOT</td>
<td>NaDBS</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

2.4 Analysis of Drop-Cast Film Morphology. Unlike the chemically polymerized layer that absorbed into the silk network, SEM images reveal that the electropolymerized (EP) polymer formed a distinct layer on the surface (Figure 6). To reveal the morphological differences between the composite surface before and after EP, the silk substrate shielded from
electropolymerization by Kapton® tape was uncovered by removing the insulating tape, which revealed the interface between the silk exposed to EP and shielded from EP. Before EP, the morphology of the silk composite was smoother, as seen in the lower half of Figure 6A. After EP, all of the films had nodular structures similar to those seen in pure conducting polymer films. For the more hydrophilic monomers pyrrole and EDOT-OH, using NaDBS as the dopant yielded EP films with a smoother morphology than those doped with $p$-TSA (Figure 6B-E). When the more hydrophobic EDOT monomer was used in conjunction with NaDBS, smaller nodules on the same scale as the other $p$-TSA doped films were produced (Figure 6F).

In all cases, the EP layers were tightly adhered to the surface. A modified ASTM procedure was utilized to confirm polymer adhesion. A piece of Scotch® tape was firmly adhered to the film surface and then peeled off. Little to no polymer was removed along with the Scotch® tape. No flaking or delamination was observed following sonication or extended redox cycling, further demonstrating that a robust interface was formed between the layers. We hypothesize that the strong adhesion can be attributed to the fact that the polymerization is initiated from the chemically-deposited PPy layer, resulting in some degree of covalent grafting of the second EP layer to the underlying silk-PPy IPN.
Figure 6. SEM images of the various silk-CP composites generated using SCEP. The interface between the underlying silk-PPy IPN and the EP layer is shown in (A). Surface morphologies of different electropolymerized polymer/dopant combinations as labeled are shown in (B-F). Scale bars represent 25 μm.

Homogeneity of the EP layers as a function of the distance from electrical contact was also studied. SCEP samples measuring ~1 cm x 1.5 cm were prepared using each monomer/dopant combination, and sheet resistivities and morphologies in three regions of increasing distance from the copper contact were analyzed (Figure 7). SEM images reveal that the surface morphology of the EP layer is fairly homogenous across each sample type, but a small decrease in nodule size was observed as the distance from the contact increased. However, we were unable to accurately measure the thickness of the EP deposited layer due to the variations in the thickness of the underlying silk film, and the difficulty in distinguishing the
exact interface between the silk-PPy IPN and the EP layer in the SEM images. Areas of the underlying silk-PPy surface could be protected by Kapton® tape to give an estimate of the EP film thickness, but removal of the tape caused lifting of the EP film edge, confounding thickness measurements. Sheet resistivity was consistently lowest in the region closest to the copper contact, but remained greatly improved for the entire sheet when compared to the sample before EP.

![Figure 7](image)

**Figure 7.** SEM images of surface coverage of electropolymerized polymer/ dopant combinations deposited on silk-PPy IPNs as a function of distance from the electrical contact. A) PPy/ p-TSA B) PPy/ NaDBS C) PEDOT-OH/ p-TSA D) PEDOT-OH/ NaDBS. ‘Top’ region refers to film within 2 mm of contact interface. ‘Middle’ region refers to film at half-distance from contact interface. ‘Bottom’ region refers to last 2 mm of film at furthest distance from contact interface. Scale bars represent 100 μm. Sheet resistivity measurements for each area are given in Ω/sq (n = 4).
2.5 Electrochemical Characterization. In order to study the electrochemical behavior of the SCEP composite films, cyclic voltammetry was performed. Composite samples were prepared as working electrodes by establishing electrical contact with copper tape and then covering the contact with Kapton® tape to prevent reactions from occurring at the metal-electrolyte interface. The electrochemical response was probed in aqueous solutions containing either PBS or NaDBS to evaluate the role of large and small anions in the redox processes.

Cyclic voltammograms of all electropolymerized strips showed a dramatic increase in current response when compared to chemically polymerized strips (Figure 8), indicating enhanced capacitance and ion exchange in the SCEP composites. The underlying silk/PPy IPN composite, while relatively conductive, contains insulating silk fibers in the CP matrix, diminishing electronic communication between PPy chains and hindering ion exchange. The increase in current after EP is likely due to both improved conductivity as there is no insulating silk present in this layer, as well as the augmented surface area due to the roughness of the EP layer which facilitates ion exchange with the electrolyte.
Figure 8. Cyclic voltammetry plots of various silk-CP composites in aqueous PBS (black) or NaDBS (red) solutions. Potentials between -1.0 V and +1.0 V vs. Ag/AgCl were scanned at 50 mV/sec with the 3rd cycle plotted. Polymer/dopant compositions were A) PPy/ \( p \)-TSA (no EP) with magnified insert, B) PPy/ \( p \)-TSA, C) PPy/ NaDBS, D) PEDOT/ NaDBS, E) PEDOT-OH/ \( p \)-TSA and F) PEDOT-OH/ NaDBS.

The redox potentials of the films also gave insight into the ion exchange taking place as the different polymer/ dopant combinations were oxidized and reduced. For \( p \)-TSA doped, electropolymerized strips (Figure 8B, E), two oxidation peaks were seen at approximately -0.25 and 0.5 V, and two reduction peaks were seen at -0.75 and 0 V when cycled in PBS (small cation, small anions), indicative of both cation and anion exchange. When the same films were cycled in NaDBS (small cation, large anion), only one set of oxidation/ reduction peaks were
observed at 0 and -0.75 V, respectively, suggesting only cation exchange is occurring. Likewise, for films doped with NaDBS (Figure 8C, D, and E) only one set of oxidation/reduction peaks was observed for both electrolyte solutions, indicating preferential cation exchange even in the presence of small anions available in solution.

Extended redox cycling of the materials by cyclic voltammetry demonstrated the stability of the SCEP materials (Figure 9). After cycling 300-400 times, no polymer delamination was observed for any film. The peak oxidation and reduction currents of each polymer/dopant combination exhibited a slight increase in potential over time.

**Figure 9.** Extended cyclic voltammetry plots of various SCEP composites in aqueous PBS solution. Scans were taken at 50 mV/sec from -1 V to +0.8 V vs. Ag/AgCl. Polymer/dopant compositions were A) PPy/ p-TSA (no EP), B) PPy/ p-TSA, C) PPy/ NaDBS, D) PEDOT/NaDBS, E) PEDOT-OH/ p-TSA and F) PEDOT-OH/ NaDBS.
The relative change in voltammogram area over the course of the experiments was calculated by integration of the current density versus time, and the resulting area of each voltammogram cycle was plotted relative to the 2nd cycle (Figure 10). The voltammogram area is proportional to capacitance, and gives insight into the level of the ion exchange that is occurring during redox cycling. Samples which had been chemically but not electrically polymerized had the most rapid and severe degradation in current response, with nearly no response after 400 cycles. SCEP films doped with p-TSA had small decreases in area after several hundred cycles (-2% for PPy and -34% for EDOT-OH), indicating some degradation was occurring. However, each of the films doped with NaDBS continued to increase in area over the course of the experiments (+53% for PPy, +46% for EDOT-OH, and +88% for EDOT), likely due to increased doping levels introduced during redox cycling in the PBS electrolyte. These results suggest that samples which had undergone SCEP are much more stable under repeated electrical cycling than those which had only been chemically polymerized. The dramatic increase in stability during CV indicates that SCEP samples are better candidates for devices designed for extended use.
Figure 10. Integrated areas of extended CV cycles relative to the area of the 2\textsuperscript{nd} cycle.

2.6 Preparation of Highly Conductive 3D Scaffolds. The SCEP method reported here was further expanded to produce highly versatile 3D conductive silk architectures. Sponge-like silk scaffolds were fabricated using a previously described salt-leaching technique.\textsuperscript{36} Briefly, sodium chloride crystals of a defined size (500-600 µm) were added to an aqueous silk solution, and left to allow the silk to crystallize and become insoluble in water. Silk sponges with an interconnected porous structure could then be obtained following dissolution of the salt. The chemical modification techniques described above for the films could be directly translated to these 3D structures to produce acid-modified and chemically-deposited PPy composites (Figure 11A). Even after the incorporation of conducting polymer, scaffolds were robust and could be compressed reversibly (Figure 11B).
Sequential electropolymerization could also be performed to further improve the conductivity and stability of the scaffolds. Accurate surface area measurements could not be obtained for the scaffolds, so EP was performed for 40 min for PPy/ $p$-TSA reactions and 120 min for PPy/ NaDBS reactions. Pyrrole reactions doped with NaDBS tended to hold a lower steady-state current during electrolysis, hence the longer reaction time. The scaffold EP set-up requires a large volume of reaction solution ($\geq$ 30 mL), so reactions were not attempted with the more expensive EDOT-OH monomer for these proof-of-concept experiments. Following EP, no changes were visually apparent, and the scaffold maintained the ability to be compressed and quickly regain its shape. Sheet resistivity measurements, cyclic voltammetry, and scanning electron microscopy were then performed on the scaffolds in order to demonstrate CP incorporation.
Direct four-point probe measurements were successfully obtained on scaffolds that had been thoroughly sonicated and washed in water. Prior to the measurement, excess water was removed by blotting, and scaffolds were left to dry briefly in the air. The probe head was lowered into the sample until a stable reading was obtained, resulting in some compression of the scaffold. As seen with silk films, the addition of an electropolymerization step lowered the sheet resistivity by three orders of magnitude (Table 2). Importantly, minimal changes in sheet resistivity were observed for the SCEP scaffolds before and after seven rounds of full compression (Table 2; additional data given in Figure 12), further evidence that a robust composite is formed with the SCEP technique.

Table 2. Sheet resistivity before and after compression of silk scaffolds subjected to chemical polymerization (ChP) or sequential chemical and electrochemical polymerization (SCEP).

<table>
<thead>
<tr>
<th>Monomer/Dopant</th>
<th>Polymerization Method</th>
<th>Sheet Resistivity Before Compression (Ω/sq)</th>
<th>Sheet Resistivity After Compression (Ω/sq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPy/ p-TSA</td>
<td>ChP</td>
<td>40,000 ± 10,000</td>
<td>34,000 ± 6,000</td>
</tr>
<tr>
<td>PPy/ p-TSA</td>
<td>SCEP</td>
<td>29 ± 3</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>PPy/ NaDBS</td>
<td>SCEP</td>
<td>170 ± 30</td>
<td>210 ± 40</td>
</tr>
</tbody>
</table>
Figure 12. Sheet resistivity values for scaffolds before and after seven full compression cycles. A) ChP scaffold prior to EP deposition (scale in kilohms), B) SCEP scaffolds with an EP layer of PPy/ p-TSA or PPy/ NaDBS.

The change in electrochemical behavior of the scaffolds was also monitored by CV (Figure 13). For both dopant types, a substantial increase in current response was observed following electropolymerization. The change in current response, as seen with the SCEP films, can be attributed to an increase in conductivity and surface area leading to higher capacitance and ion exchange.
Figure 13. Cyclic voltammetry plots of chemically polymerized silk scaffolds before (red) and after electropolymerization (black). Monomer/dopant combinations for electropolymerization were PPy/ p-TSA (A) and PPy/ NaDBS (B). Scans were taken from 1.0 V to -1.0 V vs. Ag/AgCl at 10 mV/sec in PBS with the 2nd cycle plotted.

The incorporation of additional conducting polymer during electropolymerization was also confirmed by SEM (Figure 14). Macroscopically, there was no discernable difference between an acid-modified, chemically-polymerized, and SCEP silk scaffold (Figure 14A-C). At smaller scales, there was still very little difference between acid-modified and chemically polymerized scaffolds (Figure 14E, F), as previously seen with silk films.30 However, after SCEP, an increase in surface roughness was observed as the nodular electropolymerized layer was formed (Figure 14G). Higher-magnification images show the surface morphology of the scaffolds (Figure 14D, H) to be similar to films made with comparable SCEP conditions (Figure 6B, C).
Figure 14. SEM images of silk scaffolds after: acid-modification (A,E), chemical polymerization of PPy/ p-TSA (B,F), SCEP with PPy/ p-TSA (C,D,G) and SCEP with PPy/ NaDBS (H).

Taken together, this data suggests that SCEP is a very effective method to produce highly conductive sponge-like scaffolds from silk. These composites hold great promise for use in tissue engineering, where electrically-active scaffolds with biomimetic 3D architectures are highly sought-after.23,45
2.7 Conclusion. Here we presented optimized sequential chemical and electrochemical polymerization (SCEP) methods to deposit PPy, PEDOT or PEDOT-OH doped with either para-toluenesulfonic acid (p-TSA) or sodium dodecylbenzene sulfonate (NaDBS) on silk-PPy IPNs. In all cases, the second layer of electrodeposited CP was found to lower the sheet resistivity by 2-3 orders of magnitude (6-70 Ω/sq as compared to the initial 10^2-10^3 Ω/sq), and increase the stability during extended redox cycling. All electrodeposited layers exhibited remarkable adhesion to the silk, presumably since the polymerization is initiated from the surface of the silk-PPy composite. Films with selective ion exchange properties were synthesized by varying the dopant used during electropolymerization. Three-dimensional scaffolds were successfully modified and were able to maintain their electrical conductivity extended compressions. In addition to the set of monomers and dopants evaluated in these proof-of-concept studies, the SCEP technique is anticipated to be compatible with any CP/dopant combination desired, allowing facile integration of drugs,^50 adhesion peptides,^2 growth factors,^51 etc. into conductive silk composites.
CHAPTER 3: ELECTROSPINNING SILK

3.1 Introduction. An important aspect in the development of implantable biomaterials is the mechanical interaction between the material and the native tissue. Any material implanted into the body will elicit a foreign body response.\textsuperscript{52,53} The severity of the foreign body response determines what level of inflammation, macrophage formation, and scar tissue occurs around the material. In order to minimize the foreign body response, a material should mimic the mechanical properties of the target native tissues and their surroundings as much as possible.\textsuperscript{54}

The extracellular matrix (ECM) is a general term for the intercellular material in a tissue. The majority of the ECM is comprised of nanofibrous protein structures and provides a medium for cell interaction, migration, and differentiation.\textsuperscript{55} Materials that mimic the fibrous nature of the ECM promote cell adhesion, proliferation, and differentiation \textit{in vitro} and \textit{in vivo}.\textsuperscript{56,57} Electrospinning has recently emerged as a production method for such nanofibrous materials. Electrospinning is a polymer processing technique that can fabricate nano- to micro-sized polymer fibers of homogenous diameter. Electrospun fibers have high surface area per unit mass, tunable fiber width, high permeability, and are regarded as one of the most promising substrates for tissue engineering due to their resemblance to native ECM.\textsuperscript{58-63}

Electrospinning is carried out by first loading a polymer solution into a syringe attached to a syringe pump. The solution is slowly passed through an emitter needle that is connected to a high voltage (HV) power supply (Figure 15). Once an adequate potential is applied, the electrostatic attraction between the charged polymer solution and the grounded collector
plate is able to overcome the surface tension of the solution. The polymer drop at the end of the emitter needle elongates and forms what is referred to as a Taylor cone. From the tip of the Taylor cone, a liquid jet of polymer solution is emitted and travels through the air from emitter to collector. As the polymer jet travels through the air, it begins to dry which whips and elongates the jet, thinning the diameter of the deposited fibers. By rotating the grounded collector target during deposition, fibers with high degrees of alignment may be obtained.

Figure 15. Diagram of an electrospinning apparatus.

An additional benefit of the electrospinning process is the wide variety of materials that can be utilized. A broad range of both synthetic polymers and biopolymers such as poly(methyl methacrylate), poly(ethylene oxide), chitosan, collagen, silk and poly(ε-caprolactone) have been used.\textsuperscript{62,64–67} Polymer solutions must be concentrated enough for adequate polymer entanglement.\textsuperscript{68} To prevent droplet formation, solutions must have low enough surface tension and sufficient charge density and viscosity.\textsuperscript{69}

To create nanofibrous conductive polymer composites, groups have used polyurethane, poly(vinylidene fluoride), nylon, collagen or other spinnable polymer supports that are either combined with a solution of conducting polymer (typically PANi) before electrospinning, or
deposited with CP by in situ polymerization. These composites typically have conductivity in the range of $10^{-7}$ to $10^{-1}$ S/cm, with a maximum achieved conductivity of ~2 S/cm. While the solubility of PANi helps overcome processing issues present for other conducting polymers, the conductivity of PANi is pH dependent and adversely affected by operation at biological pH, making it a poor candidate for biomedical applications. CPs such as PPy, PEDOT, and PEDOT-OH operate normally at biological pH, making them better candidates as biomaterials.

Here, we set out to create a nanofibrous silk substrate for a new generation of conducting polymer/silk composites. By taking advantage of our established SCEP protocol, we hoped to improve the conductivity of nanofibrous CP composites while expanding the available polymer/dopant toolset. Fibers were spun based on established protocols, and modified according to the SCEP protocol outlined in Chapter 2. Fibers with high degrees of alignment, large surface area to volume ratios, and high electrical conductivities were successfully prepared.

3.2 Preparation of Electrospun Fibroin Fibers. Submicron fibroin fibers were successfully deposited by the electrospinning process. Aqueous silk fibroin was mixed with a high molecular weight polyethylene oxide (PEO) solution to improve the processability of the protein solution and deposited using an applied potential of 0.6 kV/cm distance between the emitter and the ground. By changing the rotational speed of the grounded stainless steel drum, fibers of varied alignment were easily obtained.
Early in the fabrication process, an issue with polymer ‘beading’ during deposition was discovered. Beading is a phenomenon where the deposited fiber has a non-uniform diameter that varies by several degrees of magnitude, causing the fiber to resemble a chain of beads (Figure 16A). This phenomenon has been indicated to be caused by high humidity which prevents the spinning fibers from drying uniformly.\textsuperscript{41} To combat this issue, a polycarbonate box to house the electrospinning apparatus was fabricated in-house. Valves were installed on opposing sides of the box, one on the top edge and the other on the bottom edge. The bottom valve was attached to a cylinder of compressed nitrogen and the top valve was left open (Figure 16C). The humidity of the chamber was lowered by purging with nitrogen gas while monitoring the relative humidity (RH) by hygrometer. By lowering the relative RH of the chamber from atmospheric (~50% RH) to 20-30% RH, highly uniform fibers were easily obtained (Figure 16B).

![Figure 16. SEM images of electrospun silk fibers at 50% RH (A) and 25% RH (B), and a diagram of the nitrogen purged electrospinning set-up (C). Scale bars represent 20 µm.](image-url)
In order to remove the PEO used to aid the electrospinning process, the fibroin was first rendered water-insoluble by curing in methanol. The samples were then submerged in water and gently rocked at 37 °C for 48 h to completely dissolve the PEO. IR spectroscopy was performed to confirm the removal of PEO (Figure 17). Samples of drop-cast PEO, drop-cast fibroin, electrospun PEO/fibroin before curing, and electrospun fibroin after PEO removal were compared. Samples containing the high molecular weight PEO (Mₐ ~ 900,000) had pronounced C-O stretching vibrations from 1175 to 1000 cm⁻¹. The sample of pure, drop-cast PEO had a peak at 1094 cm⁻¹, corresponding to the C-O-C stretching vibrations from the crystalline phase of PEO. The sample of pure, drop-cast fibroin contained a small, C-O stretch at 1060 cm⁻¹. The electrospun, uncured PEO/fibroin sample had a moderately strong C-O stretch with a maximum at 1105 cm⁻¹, indicating the presence of PEO. After curing and dialysis, the electrospun fibers had a small C-O singlet with a maximum at 1066 cm⁻¹, indicating the complete removal of PEO. At 950 cm⁻¹, C-H bends were observed in the pure PEO and spun PEO/fibroin samples, but neither the pure fibroin or spun fibroin after treatment, also indicating the removal of PEO.
Figure 17. ATR-FTIR spectrum of PEO/fibroin films. Samples were scanned from 4000 cm\(^{-1}\) to 525 cm\(^{-1}\), with the 1200 cm\(^{-1}\) to 900 cm\(^{-1}\) region plotted for comparison.

3.3 Modification of Electrospun Fibroin Fibers. The resulting fibers were modified by our established methodology for creating SCEP composites described in Chapter 2. Similar to drop-cast films, these samples by and large kept their original surface morphology (Figure 18A-D) through the modification procedures. After hydration and acid-modification (Figure 18B, C) the fibers compacted to a much higher degree, likely due to the increased hydrophilicity of the materials. After chemical polymerization (Figure 18D), the fiber hydrophilicity decreased and reduced fiber-to-fiber adhesion. A minor increase in fiber diameter was observed post chemical polymerization. Unlike drop-cast films, the electropolymerization step did not obscure the substrate morphology by depositing a distinct film over the surface of the silk-based electrode (Figure 18E, F). Instead, polymerization occurred at the surface of each fiber. The fiber diameters swelled after EP, increasing from
0.6 ± 0.1 µm after chemical polymerization to 1.47 ± 0.09 µm after SCEP modification with PPy/NaDBS. This result further supports our hypothesis that electropolymerization is initiated from the silk-PPy surface and is likely covalently grafted onto the sample surface. Samples that were SCEP modified with PPy/NaDBS (Figure 18E) had smooth fiber surfaces, whereas samples SCEP modified with PEDOT-OH/NaDBS (Figure 18F) had rougher fiber surfaces covered in small nodules (Figure 18E).

**Figure 18.** SEM images of electrospun silk fibers after: no cure (A), cure and dialysis (B), acid-modification (C), chemical polymerization with PPy/p-TSA (D), SCEP with PPy/NaDBS (E), and SCEP with PEDOT-OH/NaDBS (F). Scale bars represent 10 µm.

The electrical properties of the fiber mats were tuned during both the chemical polymerization and electropolymerization stages. For chemical polymerization, a monomer to oxidant ratio of 3:1 had been previously used for drop-cast film and scaffold modification described in Chapter 2. Here, a higher monomer to oxidant ratio of 1:1 was chosen for the
modification of electrospun fibers, as it lowered the sheet resistivity of the samples from $10^3$ to $10^2$ Ω/sq with less deviation across the surface. Sample substrates with higher, more consistent conductivity were desired for a more even distribution of the electropolymerized layer down the fiber surfaces. Similar to the drop-cast films, the conductivity of the fiber mats after EP was affected by the density of charge passed (Table 3). Unlike the drop-cast films, the fiber mats contained no insulating layer of polymer, thereby facilitating thickness measurements and allowing the calculation of sample conductivity. Samples polymerized with the optimized density of charge passed for drop-cast films (2 C/cm$^2$) had a conductivity of ~3 S/cm. By increasing the charge density during EP, sample conductivities were raised to over 40 S/cm. These conductivities are on the same order or greater than other nanofibrous conducting polymer composites. 70-76

**Table 3.** Sample conductivities of fiber mats after various degrees of PPy/p-TSA electrodeposition.

<table>
<thead>
<tr>
<th>Charge Passed Density (C/cm$^2$)</th>
<th>Sheet Resistivity (Ω/sq)</th>
<th>Sample Thickness (µm)</th>
<th>Conductivity (S/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>31 ± 6</td>
<td>117 ± 6</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>12 ± 3</td>
<td>110 ± 10</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>7 ± 3</td>
<td>107 ± 6</td>
<td>13 ± 6</td>
</tr>
<tr>
<td>8</td>
<td>5 ± 1</td>
<td>117 ± 6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>20</td>
<td>2.3 ± 0.1</td>
<td>103 ± 6</td>
<td>42 ± 3</td>
</tr>
</tbody>
</table>
The sheet resistivity of the devices did not plateau as the drop-cast devices did. This can be partially attributed to inaccuracies in the calculation of the initial surface area. For both drop-cast films and fiber mats, the surface dimensions were measured by digital calipers and the area was calculated assuming a flat surface. This measurement type may have been reasonably accurate for the smoother drop-cast films, but was a gross-underestimation for the highly faceted fiber mats. The measurements were useful for sample-to-sample consistency, but the charge passed density between substrate types cannot be directly compared. High amounts of charge passed density qualitatively embrittled the samples, so a density of 6 C/cm\(^2\) was chosen for further use, as it achieved high conductivity without becoming too brittle.

Figure 19. SEM images of PPy/p-TSA SCEP fiber mats after passing a charge density of 2 C/cm\(^2\) (A), 6 C/cm\(^2\) (B), and 20 C/cm\(^2\) (C). Scale bars equal 10 µm.

3.4 Fabrication of Electrospun Fiber Coils. Devices designed for linear actuation (see Chapter 4) were prepared by coiling electrospun fibers. First, silk/PEO fibers were electrospun onto a grounded stainless steel barrel rotating at 3000 RPM to create fiber alignment. After deposition, the fibers were coiled together by gently rolling the fibers together with their alignment parallel to the length of the coil (Figure 20A). The coiled fibers were cut from the
barrel and the PEO was removed in the same manner as for the fiber mats. Fiber alignment was noted to have been maintained throughout the surface (Figure 20B) and the bulk of the sample (Figure 20C). While the surface fibers were able to be misaligned by handling or other abrasive forces, the bulk alignment of the material was always conserved.

**Figure 20.** Coiling of electrospun silk fibers on rotating steel barrel target after deposition (A). SEM images of uncured PEO/silk fiber coils viewed from the surface (B) and the cross-section (C).
The mechanical properties of the coiled fibers were analyzed at various steps of the SCEP modification by uniaxial tensile tests (Table 4). As expected, the incorporation of conducting polymer stiffened the fibers. After chemical polymerization, the Young’s modulus of the fibers increased by over 2000% from ~7 MPa to ~160 MPa, and electropolymerization further increased the modulus by about 250% to ~400 MPa. The ultimate strength of the material also increased with incorporation of conducting polymer. The ultimate strength of the silk fiber coils increased from ~5 MPa (pure silk) to ~8 MPa (ChP) to ~13 MPa (SCEP modification). Inversely, the elongation at rupture decreased with conducting polymer incorporation from over 200% (pure silk), to ~70% (ChP), all the way down to ~4% (SCEP modification).

Table 4. Mechanical properties of silk fiber coils at various stages of SCEP processing. Tests performed in submersion chamber filled with RT water.

<table>
<thead>
<tr>
<th>Fiber Coil Type</th>
<th>Young’s Modulus (MPa)</th>
<th>Elongation at Rupture (%)</th>
<th>Ultimate Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncured Silk/PEO</td>
<td>430 ± 160</td>
<td>49 ± 8</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Cured Silk</td>
<td>7.1 ± 0.9</td>
<td>250 ± 96</td>
<td>4.7 ± 0.7</td>
</tr>
<tr>
<td>ChP Silk (PPy/p-TSA)</td>
<td>160 ± 50</td>
<td>70 ± 10</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>SCEP Silk (PPy/NaDBS)</td>
<td>400 ± 90</td>
<td>4 ± 2</td>
<td>13 ± 4</td>
</tr>
</tbody>
</table>

*a Tests run in dry chamber due to sample solubility.  
*Charge passed density = 6 C/cm².
3.5 Conclusion. Electrospinning was used to fabricate submicron silk fibroin fibers with high homogeneity and surface area to volume ratios. A rotating steel barrel was used to yield a high degree of fiber alignment. The sequential chemical and electrochemical polymerization processing methodology presented in Chapter 2 was successfully applied to this new silk substrate. The electrochemical properties of the fibers were easily tailored at the electropolymerization step which allowed for the incorporation of different polymers/dopants and varied levels of conducting polymer deposited. After SCEP processing, fiber samples had conductivities on the range of 2-42 S/cm. As expected, the incorporation of conducting polymer into the silk network stiffened the devices, raising the Young’s modulus and the ultimate tensile strength while lowering the elongation at rupture. The incorporation of the electropolymerization step had the most drastic effect on the elongation at rupture and the Young’s modulus. The fiber alignment and high degree of permeability were conserved across each step of the SCEP process. Our fibers achieved higher conductivity than the large majority of other conducting polymer nanofiber composites, while maintaining lowered mechanical moduli closer to that of the silk substrate than pure conducting polymer.
CHAPTER 4: ACTUATION

4.1 Introduction. Electromechanical actuation is a general process where electrical charge is transduced into mechanical force or movement. Conductive polymers are able to operate as actuator devices by taking advantage of the ion/solvent flow during oxidation and reduction of the aromatic backbone.\textsuperscript{17} Actuators made from conductive polymers generally operate under one of two mechanisms: cation or anion exchange.

A cation exchange mechanism can occur when a large anionic dopant is incorporated into the CP network (Figure 21A). During reduction, the anionic dopant is unable to leave the polymer and the CP is in a neutral state, so cations are taken into the network to balance the electrostatic charge. This ion/solvent intake swells the device, causing it to expand/relax. During oxidation, the anionic dopant and the cationic CP backbone balance each other’s charges. The cations that were taken in during reduction are expelled, causing the polymer to contract.

An anionic exchange operates under an opposite mechanism (Figure 21B). During oxidation, there are no trapped anionic dopants to stabilize the cationic CP backbone, so anions from solution are taken into the polymer network for charge balance, relaxing the device. During reduction, the anions are no longer electrostatically stabilized by the polymer and are repulsed out of the network, contracting the polymer.
Figure 21. Diagram of volume change in conducting polymers under cation exchange (A) and anion exchange (B) mechanisms. NaDBS used as an example of an entrapped anionic dopant for cation exchange.

Linear actuator devices can be made by immersing a homogenous piece of conducting polymer in an electrolyte solution. By changing the applied potential, the conducting
polymer is oxidized or reduced, causing its volume to change and generate linear movement. Linear actuation inherently generates more stress than bending actuation, and most instruments designed to measure force operate in a linear direction.17

Here, we set out to take advantage of both the SCEP process developed in Chapter 2 and the electrospun, nanofibrous silk substrate developed in Chapter 3 for the preparation of linear actuation devices. Like native musculature, our devices were composed of bundles of individual, active fibers. Ideally, we want to generate stress and strain on the same order of magnitude of native muscles, which typically generate 0.1-0.5 MPa stress and up to 40% strain. The addition of an electopolymerized layer of CP was performed to not only raise the conductivity of the device, but to also incorporate previously unavailable dopants such as NaDBS for cation-dominant exchange. By using a nanofibrous silk substrate, we aimed to increase the surface area-to-volume ratio to facilitate ion exchange, and use the fiber alignment to increase linear actuation performance.

4.2 Electromechanical Actuation of Electrospun Fiber Coils. Electrospun silk fiber coils were modified by the SCEP procedure outlined in Chapter 2. In short, the fibers were first ‘acid-modified’ to covalently bind sulfonic acid groups to the silk. Chemical polymerization was performed to form a silk-PPy IPN. This conductive substrate was attached as the working electrode in a three-electrode cell, and an additional layer of conducting polymer and dopant was deposited. The performance of the fiber coil actuators was evaluated using a dual-mode muscle lever system. Briefly, the sample was fixed on one side by a clamp and attached on the other side to a piezoelectric arm. The arm was held either at a set length (length-controlled
mode) or a set force (force-controlled mode). When the actuator changed dimensions, the piezoelectric arm compensated either its force or length to hold the other steady, and output the resultant change. Mechanical stress was calculated by holding the device in length-controlled mode to give force output under actuation, then dividing the force by the cross-sectional area of the device. Mechanical strain was calculated by holding the device in force-controlled mode to give change in length, then dividing the change in length by the initial length.

The standard actuation protocol consisted of two phases: an initial ‘cure’ phase, and a main phase. The cure phase was used to help increase device performance based on past group work.\textsuperscript{16,80} The cure phase consisted of a ±2 V square wave at 1 Hz run for 50 cycles, and the main phase consisted of square waves (±2 V) held for 1/30 Hz for reduction and 1/10 Hz for oxidation. In order to make the samples taut and help align the polymer networks, the loaded samples were ‘crept’ for 30 min before actuation by applying ~1 MPa of stress in length-controlled mode and allowing the sample to relax. Despite the creep phase, samples had dramatically larger relaxations for the first few reduction periods, seen as 0.2-1.0 MPa drops in stress. All calculated stress values and highlighted regions are shown for after the relaxation period. All runs were performed in PBS solution in order to achieve similar osmolality to biological conditions (280-315 mOsm/kg for PBS, 285-295 mOsm/kg for human plasma).

Fiber coils chemically polymerized with PPy/p-TSA generated stress during both the reduction and oxidation phases (Figure 22A, B). During reduction, ~70 kPa stress was
generated and then the device held steady. During oxidation, an additional ~20 kPa stress was generated, which was immediately followed by a ~90 kPa relaxation. The generation of stress during both redox phases is indicative of competing cation and anion exchange mechanisms. Because the stress generated during reduction was nearly 4X greater in magnitude than that during oxidation, the device is anion exchange dominant. The small stress generated during oxidation from cation expulsion was quickly overwhelmed by the relaxation from anion intake, whereas the peak stress during reduction was held relatively steady.

Figure 22. Mechanical stress under actuation for silk fiber coils chemically polymerized with PPy/p-TSA (A, B) and SCEP modified with PPy/p-TSA (C, D).
SCEP modified PPy/p-TSA fiber coil actuators behaved similarly to chemically polymerized samples of the same polymer/dopant combination (Figure 22C, D). By incorporating the more conductive electropolymerized layer, the peak stress was increased by ~580%. During reduction, ~410 kPa stress was generated, followed by a slow relaxation of ~100 kPa. During oxidation, ~85 kPa stress was generated which was immediately followed by a ~400 kPa relaxation. The stress generated during reduction no longer reached a plateau as seen in the devices without the SCEP layer. The relaxation during reduction is indicative of increased cation movement into the actuator during reduction.

To simplify the operating mechanism of the actuators, new devices were fabricated using sodium dodecylbenzenesulfonate (NaDBS) anion as the dopant during electropolymerization. Previous work indicated that films with this dopant primarily undergo cation exchange, as the large size of the anion facilitates its physical entrapment in the polymer network, preventing its loss during reduction.\textsuperscript{81-83} Extended CV studies performed on drop-cast films also suggested that doping with NaDBS helped extend device lifetime (Figure 9).

Stress testing confirmed that actuators employing NaDBS operated only on a cation exchange mechanism (Figure 23A-D). Oxidation expelled cations, generating stress, whereas reduction incorporated cations, swelling the polymer and relaxing the device. The polymer electrodeposited with the dopant had no effect on the resulting exchange mechanism; both PPy and PEDOT-OH coated samples doped with NaDBS gave similar stress curves (Figure 23B, D). During reduction, PEDOT-OH coated samples relaxed at a 2-3X faster rate than those
coated with PPy. PEDOT actuators have previously been shown to have faster response times than PPy actuators due to higher porosity and lower rigidity. Both samples generated stress with a similar order of magnitude, with ~200 kPa generated for PPy/NaDBS and ~280 kPa generated for PEDOT-OH/NaDBS.

Figure 23. Mechanical stress under actuation for silk fiber coils SCEP modified with PPy/NaDBS (A, B) and PEDOT-OH/NaDBS (C, D).

In order to test the actuator lifetime, a 24 h (2160 cycle) run was performed with a SCEP modified, PPy/NaDBS fiber coil (Figure 24A-D). Due to software limitations, only seven sets of stress data were collected during this 24 h period. The software controlling the potential and monitoring the current response had no time limitation and gave a continuous data set.
Figure 24. Mechanical stress under extended actuation for silk fiber coils SCEP modified with PPy/NaDBS (A) with expanded views shown at 1 h (B), 11 h (C), and 24 h (D) into run.

After running for 24 h, the actuator device was still fully operational and working under a cation exchange mechanism (Figure 24D). The stress generated went from ~200 kPa/cycle in the first hour (Figure 24B), to ~130 kPa/cycle in the 11th hour (Figure 24C), to finally ~40 kPa/cycle after 24 h (Figure 24D).

The lifetime of a SCEP modified PEDOT-OH/NaDBS fiber coil was tested as well (Figure 25A-D). While the device generated ~20% more stress during the first hour of actuation (Figure 25B), the device lifetime was greatly reduced compared to the PPy/NaDBS sample. Although current response was not directly proportional to stress generation, the steep
A decrease in current response between 400 and 500 min was related to where the device failed (Figure 25A). Stress generation dropped ~40% from ~260 kPa/cycle in the first hour (Figure 25B) to ~155 kPa/cycle in the sixth hour (Figure 25C). By the 11th hour, the stress dropped drastically by ~97% to ~8 kPa/cycle (Figure 25D). While the device failed more quickly and with greater magnitude than the PPy/NaDBS analog, the mechanism stayed as cation exchange throughout the entire run, even at the 11th hour (Figure 25D magnified insert).

**Figure 25.** Mechanical stress under extended actuation for silk fiber coils SCEP modified with PEDOT-OH/NaDBS (A) with zooms at 1 h (B), 6 h (C), and 11 h (D, with magnified insert) into run.
Strain was analyzed on a PPy/NaDBS fiber coil (Figure 26A, B). Dramatic relaxations in the coil were observed during the first few cycles, similar to what was seen during stress testing. This reduction in applied stress was enough to change the offset mode of the instrument from length-controlled to force-controlled (Figure 26A). After about 200 s, the sample had equilibrated enough to remain in length-controlled mode, allowing strain analysis. The sample operated with an average of 1.5% strain per cycle. Consistent with the stress data, films elongated during the oxidation phase due to an influx of cations and contracted during reduction due to expulsion of cations (Figure 26B).

**Figure 26.** Mechanical strain under actuation for silk fiber coil SCEP modified with PPy/NaDBS (A,B zoom)
4.3 Conclusion. Utilizing the fabrication methods outlined in Chapter 3, silk fibers were modified with conducting polymers for use as electromechanical actuators. A rotating steel collector was used to create a high degree of electrospun fiber alignment, and the fibers were coiled together for easy handling. The enhanced attributes of these materials were used to increase performance and lifetime of actuator devices made from silk-CP composites. Actuators chemically polymerized with PPy/p-TSA generated peak stresses of ~70 kPa. The addition of an electropolymerized layer of PPy/p-TSA increased the peak stress to over 400 kPa. While these devices generate stresses several orders of magnitude below the maximum achieved by pure conducting polymers, this increase put our devices well into the stress generation range of native muscles (0.1-0.5 MPa). Devices doped with p-TSA exchanged both anions and cations, with anion exchange being the dominant mechanism.

Doping the CPs with NaDBS during electropolymerization allowed the fabrication of cation-only exchange actuators. While a slight decrease in peak stress was observed (~200-300 kPa), stress generation was observed solely in the oxidation phase. Devices modified with PPy/NaDBS had the longest lifetime; after 24 h of operation and ~2200 redox cycles the actuators still performed at ~20% efficiency. The PPy/NaDBS devices generated ~1.5% strain per cycle. This linear strain generation is on par with other nanofiber conducting polymer composites which exhibit 1-2% strain.

Electrospinning and SCEP processing were successfully utilized to create actuator devices with greatly improved lifetime compared to past group publications. Previous generations of devices generated maximum stress during the cure phase, and saw reduction in stress
production with each successive cycle of the main phase. The maximum stress generated by
the drop-cast and fiber-based devices is on the same order of magnitude, however our new
fiber devices generate ~10X more stress during the main phase. Our new generation of
actuators were able to operate under a ±2 V potential range, lower than the ±3 V potential
used in the first generation. Devices capable of anion or cation dominant exchange were
prepared, allowing different devices to generate stress at opposite potentials.
CHAPTER 5: MATERIALS & METHODS

5.1 Materials and Instrumentation. All chemicals were purchased from Sigma-Aldrich, Alfa Aesar, or Sarchem Laboratories (EDOT-OH, New Jersey). The Bombyx mori silk cocoons were purchased from Mulberry Farms in Fallbrook, CA or Oregon Silkworms in Bend, OR. Electrospinning was performed utilizing a Spraybase® electrospinning instrument with a rotating drum collector by Profector Life Sciences Ltd. Electropolymerizations and cyclic voltammetry experiments were carried out using either a Pine Research Instrumentation WaveNow potentiostat or WaveDriver bipotentiostat, and data was recorded by a computer workstation running AfterMath software. Counter and reference electrodes were purchased from Bioanalytical Systems, Inc. Electrical measurements were made using a Lucas Labs Pro-4 four-point probe equipped with a Signatone SP4-40045TBY tip and powered by a Keithley 2400 SourceMeter. Scanning electron microscopy was performed using a Vega TS 5136 MM. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was performed with a Thermo Scientific Nicolet™ iS10 Spectrometer with Smart iTR™ Attenuated Total Reflectance Sampling Accessory. Samples were analyzed dry and at room temperature.

5.2 Preparing Aqueous Silk Solutions for Films. Silkworm cocoons were cut into pieces and then boiled in aqueous Na$_2$CO$_3$ (0.02 M) for 1 h. The fibers were transferred to boiling DI water for 10 min, rinsed three times in room temperature DI water (10 min each), and then left to dry in a fume hood overnight. The fibers were added to a beaker and aqueous LiBr (9 M) was added to make a 20% w/v solution, covered with foil, and placed in a 60 °C oven for 1 h. The resulting solution was transferred to hydrated dialysis tubing (Fisherbrand™, 3.5k
MWCO) and dialyzed against DI water for at least 24 h, changing the water four times. If needed, the pH of the silk solution was adjusted to pH 7 by dialyzing against aqueous HCl (0.1 mM). Optionally, the resulting solution was concentrated by dialysis against aqueous polyethylene glycol (PEG, 10% w/v, 10k MW) or the dialysis bag was laid on dry PEG powder (10k MW) until the desired concentration (6-8% w/v) was reached. The silk solution was then passed through a syringe filter (Millex®-SV 5.00 μm) and stored under refrigeration. The concentration of silk solution was found by dividing the dried mass of an aliquot of solution by the original volume of solution, giving the w/v fraction in g/mL.

5.3 Preparing Drop-Cast Silk Films. Silk sheets were drop-cast with 1 mL of solution dispensed over a 3 x 4 cm area on a silicone baking mat from a commercial retailer and dried in a laminar flow hood. The dried films were immersed in ethanol (70% v/v in water) for 18-24 h to render them insoluble in water, and then transitioned into nanopure water gradually over 1 h.

5.4 Preparing Aqueous Silk Solutions for 3D Scaffolds and Electrospinning. Silkworm cocoons were cut into pieces and then boiled in aqueous Na₂CO₃ (0.02 M) for 30 min. The fibers were transferred to room temperature DI water for 10 min, rinsed three additional times in DI water (10 min each), and then left to dry in a fume hood overnight. The fibers were dissolved and dialyzed as described above. The silk solution was then centrifuged to remove any particulate and stored under refrigeration.

5.5 Preparing Silk Scaffolds. Silk scaffolds were prepared as previously described.36 Silk solution (6% w/v) was added to a polyethylene sample vial (5 mL, Fisherbrand™). Sodium
chloride (500-600 μm sieved crystals) was then gradually added to the silk (2 g NaCl per 1 mL of silk solution). The container was sealed, the salt-silk solutions were allowed to solidify at room temperature for 2 d, and then the containers were submerged in DI water to leach out the salt. After 1 d, the scaffolds were removed from their containers and soaked in fresh water for at least another day. The resulting scaffolds were cut into uniform sizes with a 10 mm biopsy punch or a razor blade. The samples were stored either hydrated in nanopure water for short-term storage or dry for long-term storage.

5.6 Electrospinning Silk Fibers. Aqueous silk solution (8% w/v, 4 mL) was combined with aqueous polyethylene oxide (PEO, 900k MW, 5% w/v, 1 mL) under slow stirring for 10 min. The silk/PEO solution was transferred to a 5 mL syringe attached to several feet of PTFE tubing with an 18 gauge stainless steel emitter needle fitted to the end. The syringe was attached to a syringe pump and the emitter needle was connected to the high voltage socket of the instrument. A flow rate of 8-24 μL/min was distributed by the pump while a 9-12 kV potential was applied to the emitter needle to induce a Taylor cone in the solution. The target was a grounded rotating steel barrel spun at 0-4000 RPM located 16-20 cm from the emitter. The entire instrument was contained in a polycarbonate chamber. The humidity of the chamber was monitored by a hygrometer, and could be controlled by purging with N₂ gas. Unless noted, the chamber was kept at 20-30% relative humidity. After deposition (1-3 mL solution for sheets, 0.5 mL for coils), the fibers were either removed from the barrel whole and cut into sheets, or rolled together to form coils. The fibers were immersed in methanol (MeOH, 90% v/v in water) for 10 min to render the silk insoluble in water. The PEO was dialyzed out.
of the fibers by soaking them in 37 °C water for 48 h under gentle rocking. To aid in fiber cohesion, fiber sheets were dried overnight at ambient temperature on a silicone mat before rehydration and further processing.

5.7 Acid-Modification of Silk Structures. The diazonium reaction used to modify the surface of the films or scaffolds with sulfonic acid groups was carried out as previously described.21 Silk films, scaffolds, or fibers were placed in a Petri dish, immersed in borate buffer (8 mL, 100 mM sodium borate, 137 mM NaCl, pH 9.0-9.5), and placed on ice. Sulfanilic acid (34 mg, 0.20 mmol) and p-TSA (152 mg, 0.88 mmol) were dissolved in DI water (2 mL) and placed on ice for 10 min. An aqueous solution of sodium nitrite (4 M NaNO₂, 64 μL, 0.26 mmol) was mixed with the other reactants and returned to ice for 15 min. The reaction mixture was added to the Petri dish containing the silk films or scaffolds and left to react for 40 min on ice. The solution was decanted and the films were rinsed 2-3 times with DI water then stored in nanopure water.

5.8 Chemical Deposition of PPy on Acid-Modified Silk Structures. Acid-modified silk structures (films, scaffolds, or fibers) were immersed in a solution containing p-TSA (5 mM) and pyrrole (50 mM). Immediately following the addition of pyrrole, an aliquot of aqueous FeCl₃ solution was added to give a 15 mM final concentration for films and scaffolds, 45 mM for fibers. The polymerization was left to proceed for 2 h. The solution was decanted and silk structures were washed with DI water and stored in nanopure water for temporary storage or dry for long-term storage.
5.9 Electropolymerization on Silk Structures. Silk substrates (drop-cast films or electrospun fibers) that had PPy chemically deposited on their surface were fashioned into an electrode for further electrodeposition. Adhesive copper tape (3M™) was affixed to one end of the substrate. The copper tape was covered with Kapton® tape at the contact region, leaving at least half of the silk-PPy substrate exposed. For scaffolds, electrical contact was made by clamping several mm of one edge between two pieces of gold foil. Contact was maintained by securing the gold/ scaffold edge together using a Delrin™ clamp fabricated in-house. Alligator clips were then used to connect the copper tape or gold foil to a three-electrode electrochemical cell, where the silk film/scaffold served as the working electrode, a polished stainless steel plate as the counter electrode, and Ag/AgCl (in 3 M NaCl) as the reference electrode.

The electrochemical cell was filled with an aqueous solution containing 0.1 M of either pyrrole, EDOT or EDOT-OH as the monomer, and either p-TSA (0.25 M) or NaDBS (0.1 M for films, 0.25 M for scaffolds) as the dopant. The cell potential was held at 1.0 V for PPy electropolymerizations and 1.4 V for PEDOT and PEDOT-OH electropolymerizations. A total charge of 2.0 C/cm² was passed for each drop-cast film polymerization Unless otherwise noted, a total charge of 2.0 C/cm² was passed for each drop-cast film polymerization and 6.0 C/cm² was passed for electrospun fiber polymerization. PEDOT was only able to be polymerized with NaDBS as a dopant due its poor solubility in water. Since accurate surface area measurements could not be obtained for the scaffolds, those electropolymerizations were performed for 40 min when p-TSA was used as the dopant and 120 min with NaDBS.
5.10 Adhesion Tests. A modified version of “Measuring Adhesion by Tape Test” (ASTM D3359) was used. A piece of Scotch® brand invisible tape was placed firmly on the deposited polymer film, pressed gently to achieve even contact between tape and film, and then carefully peeled off. The visible quantity of polymer removed by the tape from the surface of the silk film was used to compare the relative adhesion of the samples. Due to the fragility of the electrospun fiber samples, this test was only able to be performed on drop-cast films.

5.11 Sheet Resistivity and Conductivity Measurements. Electrical measurements were made using a four-point probe. The sheet resistivity was calculated using the equation $Rs = \frac{V}{I \times 4.532}$ where $Rs$ is the resistivity per area ($\Omega$/sq), $I$ is the current applied (A), and $V$ is the resulting potential (V). The constant 4.532 takes into account the relative area measured by the probe heads. At least three measurements were taken on different areas of the sample and averaged for each data point. Conductivity was calculated using the equation $\sigma = \frac{1}{(Rs \times t)}$ where $\sigma$ is the conductivity (S/cm), $Rs$ is the resistivity per area ($\Omega$/sq), and $t$ is the sample thickness (cm). Sample thicknesses were calculated by measuring three areas with digital calipers and averaging the set with standard deviation as error.

5.12 Cyclic Voltammetry. Drop-cast films were cut to 10-15 mm lengths and 5-10 mm widths and fiber coils were cut to 10-15 mm with 0.5-1 mm diameters. Copper tape (3M™) was adhered to one end to make electrical contact. The copper contact was completely covered by Kapton® tape leaving 5-10 mm length of the strip exposed to the electrolyte solution. Contact to the scaffolds was established using the same methods described for electropolymerization. A 3-electrode electrochemical cell was used where the film/scaffold served as the working
electrode, a platinum wire as the counter electrode, and Ag/AgCl (in 3 M NaCl) as the reference electrode. The electrolytes used for CV were PBS (0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2) and NaDBS (0.1 M). The potential was swept from -1.0 V to +0.8 V or from -1.0 V to +1.0 V at a scan rate of 10, 50, or 200 mV/s. Areas of the voltammograms were calculated using integration by the trapezoidal rule in an Excel spreadsheet.

5.13 Mechanical Characterization. Uniaxial tension tests were performed on the fibers using a single column universal testing machine (ADMET eXpert 7601). Hydrated samples were cut into ~2 cm lengths and loaded into the instrument cell. The chamber surrounding the cell was filled with room temperature DI water and the samples were allowed to fully hydrate for several minutes. The instrument was run with a constant extension rate of 0.5 cm/min, and the resulting load versus distance was converted to stress vs. strain and plotted. Elastic Young’s modulus, ultimate tensile strength, and elongation at rupture were calculated and averaged for each set.

5.14 Stress and Strain Testing During Actuation. Samples were loaded into an Aurora Scientific 300C-LR Dual-Mode Muscle Lever System equipped with a 600A digital controller. Electrical contact was made by fixing one of the sample ends with a copper lead with the copper covered from solution by Kapton tape. Actuation was performed in PBS (0.1 M sodium phosphates, 0.15 M NaCl, pH 7.2) using a two-electrode chemical cell with the silk/CP sample as the working electrode and a 25 x 25 mm platinum mesh as the combined reference/counter electrode. Alternating potentials were applied by a Keithley 2400 Sourcemeter controlled by
a custom LabView program. Sample cross-sectional areas and initial lengths were measured with digital calipers.

Samples were crept for 30 min before actuation by applying ~1 MPa of stress in length-controlled mode and allowing the sample to relax. The actuation protocol consisted of an initial ‘cure’ phase (±2 V square wave at 1 Hz for 50 cycles) followed by an actuation phase consisted of square waves (±2 V) held for 1/30 Hz for reduction and 1/10 Hz for oxidation.

5.15 Scanning Electron Microscopy. Samples were mounted onto stainless steel stubs using carbon tape and dried under vacuum for 24 h. Cross-sectional samples were prepared by fixing the sample to a vertical mount with carbon tape before fractioning off the sample top while submerged under LN₂. To prevent sample dissolution due to condensed water after removal from LN₂, uncured silk cross-sectional samples were prepared by cutting with a fresh razor. For homogenous surface conductivity, samples were sputter coated with ~10 nm of a gold/ palladium mixture. Fiber thicknesses were calculated from SEM images using ImageJ software with at least 20 points analyzed and averaged with error reported as standard deviation.
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