Silk-based stretchable and functional devices

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**SILK-BASED STRETCHABLE AND FUNCTIONAL
DEVICES**

CHEN GENG

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2019

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**SILK-BASED STRETCHABLE AND FUNCTIONAL
DEVICES**

CHEN GENG

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for
the degree of Doctor of Philosophy

2019

Statement of Originality

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Authorship Attribution Statement

This thesis contains material from a paper published in the following peer-reviewed journal in which I am listed as an author.

Chapter 4 is published as Chen, G.; Matsuhisa, N.; Liu, Z.; Qi, D.; Cai, P.; Jiang, Y.; Wan, C.; Cui, Y.; Leow, W. R.; Liu, Z.; Gong, S.; Zhang, K.-Q.; Cheng, Y.*; Chen, X.* “Plasticizing Silk Protein for On-skin Stretchable Electrodes” *Adv. Mater.* 2018, 30, 1800129.

The contributions of the co-authors are as follows:

- Prof Chen provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The manuscript was revised by me, Dr Matsuhisa, Dr. Liu, Dr. Qi, Dr. Leow and Dr. Liu.
- I co-designed the study with Prof Chen and performed all the laboratory work at the School of Materials Science. I also analyzed the data.
- All microscopy, including sample preparation, was conducted by me in the Facility for Analysis, Characterization, Testing and Simulation.
- Dr Cheng constructed the model for molecular dynamics simulation and I conducted the simulation.
- Dr. Gong provided the mold of skin mimic and provided the ideas of skin-conformal structures observation.
- Dr. Pingqiang Cai and Dr. Zhiyuan Liu helped conducting the cross-sectional SEM images.
- Ms. Jiang and Ms. Cui has helped to prepare the samples and discussed the experimental optimization. Prof. Zhang provided the raw silk fibers from China.
- Dr. Wan helped to analyze the electrophysiological signals using MATLAB.

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Abstract

Silk fibroin is a type of biomaterials which attracted a lot of research efforts due to its nature biocompatibility and unique mechanical strength. The application of silk fibroin in flexible electronics has rendered excellent conformability, good flexibility and dielectric property. However, there is a lack of stretchable silk fibroin-based electronics which possess skin-like softness and stretchability, also the multifunctionality was not considered.

In this thesis, silk fibroin has been modified into soft and stretchable by two methods. One is by addition of CaCl_2 and the other is addition of glycerol. Both can realize high stretchability ($>250\%$) and skin-like softness ($<4\text{ MPa}$). In the first case, by adding various ratio of CaCl_2 , silk fibroin can obtain a tunable Young's modulus under various relative humidity. This plasticization was further explained by molecular dynamics simulations which revealed that the CaCl_2 and incorporated water would decrease the strength of crystallites and increase the ratio of extensible secondary structures. This plasticized silk fibroin films were further integrated with wrinkled gold surface which naturally formed at ambient environments. The feasibility of silk-based electrodes with high skin conformability can improve the signals fidelity of on-skin electrophysiological signals.

Beside planar silk fibroin thin film, mesh-structured silk fibroin possesses high gas permeability and low evaporative resistant was fabricated. The nanomesh structures were prepared by electrospinning of silk solution and followed by a stabilization with glycerol, which equipped the fiber mats with solution processability. After that, a solution coating process of PEDOT: PSS with 15 vol% glycerol which plays the role to conduct electrical signals. The silk electrode is highly stretchable with good breathability (low thermal insulation, low evaporative resistance, and high water-vapor transmission rate) compared with commercial gel and polymer electrodes. In sweaty status after exercise, our silk electrodes show better electrocardiography (ECG) signals quality than commercial gel electrodes, and they did not disturb the heat dissipation during sweat evaporation.

Furthermore, although on-skin electrodes have been realized based on plasticization of silk fibroin and mesh structures, there is missing point of multifunctionality. A mechanically heterogeneous silk fibroin film was proposed to act as the integration platform for hybrid-integrated sensors so that the multifunctionality can be obtained based on traditional complementary metal oxide semiconductor (CMOS) technology. This mechanically heterogeneous silk film was achieved based on the plasticized silk fibroin and selective methanol treatments at specific patterns. The contrast of modulus can be tuned by varying the ratio of CaCl_2 as well. Stretchable electrodes/circuits ($>120\%$) were screen printed at the soft region and rigid active devices were placed at the rigid region. Both experimental results and finite-element analysis (FEA) proved that the mechanically heterogeneous silk fibroin film can protect the rigid devices from mechanical failure, thus improving the stretchability of whole system ($>50\%$). Based on this, a wireless powered sensor and passive RFID were integrated to achieve communication and data processing functions, which is beyond single sensor unit.

To conclude, silk fibroin-based stretchable electronics pave the way for next-generation sustainable and environment-friendly electronics.

Lay Summary

Biomaterials are naturally biocompatible that their integration with electronics can be environment friendly and biocompatible. Silk fibroin is a well-studied biomaterial due to the mass production, solution processability and unique mechanical properties. Our study to utilize silk fibroin in stretchable electronics can provide more insights of biomaterials-based electronics, which provide better human-machine interface. Silk protein is not limited in consumer markets, but they can be smart textiles and smart skin-tattoos which can have intelligent electronics functions such as health monitoring.

The study of silk protein into on-skin electronics involves in materials science and engineering that more methods of tuning silk protein have been unveiled. Also, integration with electronic materials engages with interface between them, which further push the boundary of interdisciplinary research fields. Further, the study of on-skin electrophysiological signals based on silk fibroin can promote the data collection of related biosignals, which broaden the scope of wearable healthcare.

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Figure 7.3 A schematic of silk fibroin-based electronics for better human machine interface with sustainability and environmental friendliness.

Abbreviations

A	Alanine
ATR	Attenuated Total Reflectance
CMOS	Complementary metal-oxide semiconductor
E	Young's modulus
ECG	Electrocardiogram
EEG	Electroencephalogram
EES	Epidermal electronic system
EMG	Electromyography
FEA	Finite elements analysis
FET	Field-effect transistor
FFT	Fast Fourier transform
FPCB	Flexible printed circuit board
FTIR	Fourier Transform Infrared Spectroscopy
G	Glycine
HRP	Horseradish peroxidase
HRS	High resistance states
IC	Integrated circuits
LED	Light-emitting diodes
LRS	Low resistance states
NPT	Isothermal-isobaric ensemble
NVT	Canonical ensemble
P3HT	Poly(3-hexylthiophene)
PDMS	Polydimethylsiloxane
PEDOT: PSS	Poly (3,4-ethylenedioxythiophene): polystyrene sulfonate
PEO	Polyethylene oxide
PET	Polyethylene terephthalate
PI	Polyimide
PME	Particle mesh Ewald
PU	Polyurethane

PVA	Polyvinyl alchhol
RFID	Radio-frequency identification
RH	Relative humidity
RMSD	Root-mean-square-deviation
RSF	Regenerated silk fibroin
SEBS	Poly[styrene-b-(ethylene-co-butylene)-b-styrene]
S	Serine
SEM	Scanning Electron Microscopy
SEI	Secondary Electron Images
SNR	Signal-to-noise ratio
SWCNT	Single walled carbon nanotubes
TEM	Transmission Electron Microscopy
TIP3	Transferable intermolecular potential with 3 points
TEWL	Transepidermal water loss
UV	Ultraviolet
VMD	Visualized molecular dynamics
VNA	Vector network analyzer
WVTR	Water vapor transmission rate
XRD	X-ray Diffraction
Y	Tyrosine

Chapter 1

Introduction

This chapter introduces the background of bioelectronics, followed by the research development into materials engineering of silk. Silk, as a biomaterial, is suitable for its nature biocompatibility, solution processability and mechanical robustness. How silk protein has been utilized and modified for the application of bioelectronics is illustrated. Finally, the research motivation and objectives as well as the flow of this thesis are depicted.

1.1 Background

The field of bioelectronics are emerging for next-generation wearable devices which can continuously monitor the status of human. The devices can be divided into wearable on-skin electronics, and minimally invasive/implantable devices. As they must interface with biological tissues, several criteria for materials of bioelectronic devices have been proposed. Firstly, biocompatibility should be considered that these bioelectronic devices should not induce harmful immune response of human.^[1] Secondly, the mechanical properties should be similar to that of biological tissues, which includes Young's modulus, ultimate stretchability and elasticity, etc.^[2-5] For example, skin is the biggest organ of human whose Young's modulus is at the range of several hundred kPa and maximum stretchability is 75% at elbow skin.^[6] On-skin devices should at least be able to achieve such softness and sustain the strain as high as skin. Thirdly, bioelectronic devices should be multifunctional so that related bioinformation can be recorded such as mechanical strain, electrophysiological signals, temperature, humidity, etc.^[7, 8] To achieve these criteria, research in passive substrates and functional has flourished to next-generation devices.

Natural biomaterials are ideal materials for bioelectronics due to their nature biocompatibility.^[9] Unlike synthetic materials, biomaterials are free of immune response. Among which, silk has attracted a lot of research efforts due to its superior mechanical strength, solution processibility and ease of modification, etc.^[10] Silk fibroin, the dominant protein, has been proved to be biocompatible *in vitro* and *in vivo*, showing successful application in tissue engineering, wound healing and scaffolds, etc.^[11-15]

The extraction of silk fibers into regenerated silk fibroin in solution equips silk fibroin solution processibility. The solution processibility has rendered silk fibroin the various formats including thin films, fibers, sponges, hydrogels and non-woven mats, etc.^[16] Specifically, the application of silk protein into flexible electronics is based on the integration with thin-film based process, realizing epidermal electronics, transient electronics, injectable optoelectronics, bio-memristor and so on.^[17-23]

Figure 1.1 Development timeline of silk fibroin for flexible electronics. Reprinted with permission from reference.^[17]

1.2 Hypothesis/Problem Statement

However, the robustness of silk fibroin can only provide flexibility but not stretchability. And the high Young's modulus of silk fibroin hinders the conformal contact with biological tissues unless silk fibroin was dissolved.^[19] Silk fibroin is a kind of semi-amorphous polymer that the microscale β -sheet crystallinity influence the macroscale mechanical properties.^[24, 25] Therefore, there is an urgent need for materials engineering of silk fibroin to achieve its application into bioelectronics. As mentioned before, except for intrinsic biocompatibility, mechanical match with biological tissue and integration with multiple electronic functions should be realized.

Therefore, two research challenges for silk fibroin into the application of stretchable electronics have come out. Firstly, materials engineering of silk fibroin should be targeted at stretchable, soft silk fibroin. Secondly, to integrate certain electronic function, proper rationale in structures for devices manufacturing should be solved.

In this thesis, on-skin applications based on silk fibroin was pursued. Specifically, on-skin bioelectronics should be able to achieve softness and stretchability of skin and detect the skin related bioinformation such as strain information, electrophysiological signals, temperature, humidity, etc.

Considering the hierarchical structure of silk, as illustrated in Fig 1.2, nanoscale structures can influence macroscale mechanical properties. There are two primary phases in microscale: one is the crystalline domain and the other is amorphous domain. Featuring repetitive consequence of $(\text{-Gly-Ala-Gly-Ala-Gly-Ser-})_n$, the chains adopted β -strand conformation, would transform into lamellae β -sheets and pack into three-dimensional structures of crystals.^[16, 26, 27] For amorphous phase, the amino acid sequence would not participate in β -sheet formation, which includes less-organized secondary structures such as helix, turns, and random coils.^[28] Due to the spatial packing of the crystallites and amorphous domain, it is believed that the crystallite domain mainly contributes to the high mechanical strength of silk while the amorphous domain is more responsible for the extensibility. Native silk fiber is very tough (Young's modulus is at the range of 10 GPa) and the stretchability is quite limited ($\epsilon < 10\%$).^[29] Therefore, we hypothesize firstly that a proper molecular interaction with the amino peptides can modify the macroscale mechanical properties of silk fibroin. In particular, how to link the microscale structures with macroscale stress-strain curve of silk is meaningful to further understand the fundamental theories of biological mechanics, molecular mechanics and so on.^[30] In this thesis, two methods were proposed to realize skin-like softness ($E = 0.2 \sim 2$ MPa) and stretchability more than 70%. Also, good gas permeability of silk materials realized by porous structures have been obtained.

Figure 1.2 Schematic of the hierarchical spider silk structure that ranges from nano to macro. Reprinted with permission from reference.^[24]

Secondly, ease of integration with functional materials such as conductive materials should be realized. For example, metal foils and conducting polymers can provide electronic conductivity that electrical signals can be transmitted. Thin film-based silk materials can be easily adopted for thin film deposition system. Also, solution processibility allows solution coating methods for functional materials. Therefore, on-skin stretchable electrodes for electrophysiological signals recording can be achieved with good skin adhesion, low interfacial impedance and good gas permeability, low thermal evaporative resistance and so on.

Lastly, not only the integration of single layer of functional materials with silk was achieved, systematic integration with sensing, communication and power functions have been gained based on structural design of silk protein. Materials engineering to achieve heterogeneous silk substrate do help to realize systematic devices performance of stretchability and good mechanical stability, etc.

To summarize, silk based functional stretchable devices system with good biocompatibility, skin-like softness and stretchability, and multiple healthcare functions can be realized through fundamental materials engineering, devices design and rational of systematic integration.

1.3 Objectives and Scope

This thesis describes how materials engineering of silk protein fulfill the requirement of mechanical properties for stretchable/flexible electronics, following mechanism study from microscale to macroscale. Also, the integration with functional layers including metals foils and conducting polymers was realized. Lastly, systematic integration with multiple electronic functions based on silk protein was proposed based on structural design and heterogeneous materials engineering. To emulate the exquisite objectives for the mechanical properties of silk protein, and subsequent integration with functional layers, and systematic electronic functions, the main objectives for this thesis are summarized below:

- 1). To design and modulate the mechanical properties similar to that of skin so that optimized silk materials can conform with skin intimately. As mentioned before, skin is soft and stretchable that our modified silk protein should be able to achieve similar mechanical properties in order to conform with skin. Also, the underlying mechanism from molecular scale should be understood from molecular or microscale. This thesis has proposed two methods of plasticizing silk protein to achieve skin-like softness and stretchability. Molecular dynamics simulation has been adopted in unveiling the microscale interaction
- 2). To integrate functional layer such as conducting layers on plasticized silk substrates. Not only silk substrates can sustain high stretchability, the functional materials should also maintain its functions under strain. In this thesis, metal foils and conducting polymers have been integrated with plasticized silk protein, achieving stretchable electrodes with high stretchability more than 100%. Also, skin adhesion and gas permeability are considered for on-skin electrophysiological measurements which require low skin interfacial impedance and long-term monitoring.
- 3). To systematically combine multifunctional electronic components such as CMOS-based processors and sensors. Integration with screen printing process and heterogeneous

structure engineering of silk substrate, a printed hybrid stretchable electronic device was realized. Based on this, a wireless identification and sensing function can be easily combined on stretchable silk substrates. This thesis aims to develop silk based electronic devices from materials engineering, architected structural designs and systematic integration.

1.4 Dissertation Overview

The thesis can be attributed to the following seven sections:

Chapter 1 provides a rationale for the research and outlines the goals and scope to develop silk based functional and stretchable devices. Background of flexible electronics was briefly introduced, and the key research challenges for silk protein into the application of flexible/stretchable bioelectronics have been summarized. Several criteria for mechanical properties and electrical performance to achieve on-skin electronics based on silk protein are discussed.

Chapter 2 introduces the state-of-art flexible electronics techniques and the application of silk in flexible electronics. Dominant strategy and achievement for stretchable electronics are discussed. Also, the advancement of silk protein in flexible electronics is summarized as active layer and passive layer.

Chapter 3 illustrates the experimental methodology. The underlying mechanism of process of silk protein and correlated mechanical properties is unveiled based on protein conformation. Fabrication processes and characterization of stretchable electrodes based on plasticized silk protein are elucidated. Methods to prepare silk fiber mat and solution coating process of conducting polymer are explained. Methods to prepare heterogeneous silk substrates to achieve stretchable electronics, together with screen printing techniques, are presented. Experimental setup for performing microscope measurement, thermal vapor resistance, electrical measurement under strain is illustrated. Methods for constructing the models and simulation for Molecular Dynamics Simulation are depicted.

Chapter 4 elaborates the successful plasticization of silk protein with skin-like softness and stretchability, and following stretchable electrodes with high stretchability for electrophysiological measurements. Underlying mechanism of CaCl_2 induced plasticization of silk fibroin is disclosed together with molecular dynamics simulation. Mechanical and electrical performance based on Au/plasticized silk is elucidated and on-skin electronics for conformal contact with skin has been achieved.

Chapter 5 gives details of gas permeable sensors based on silk fiber mat and conducting polymer. Gas permeability, high stretchability, good conformability with skin, and low thermal vapor resistance are obtained by glycerol plasticization and coating of PEDOT:PSS on porous silk fiber mat. Based on these, on-skin electrophysiological measurements for sweaty conditions can be achieved.

Chapter 6 depicts a systematically integrated stretchable hybrid electronics based on heterogeneous silk substrates. Screen printing technique has been used for stretchable interconnects and methanol treatment induced local toughening of silk can isolate the mechanical strain for rigid electronic components. A wireless powered stretchable sensor on silk is characterized with good stretchability and electrical performance.

Chapter 7 summarizes the conclusion of this thesis and provides perspective and recommendation for future efforts further promoting biocompatible and high-performance stretchable electronics based on biomaterials, from the view of materials science and engineering.

1.5 Findings and Outcomes/Originality

This research led to several novel outcomes by:

1. Establishing two methods to plasticize silk fibroin for on-skin stretchable electrodes.
2. Correlating microscale protein conformation with macroscale mechanical properties of silk fibroin from molecular dynamics simulation

3. Forming two methods for coating conducting layers for silk fibroin with high stretchability.
4. Proposing a new strategy for systematic integration of stretchable electronics on heterogeneous silk substrate.

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Chapter 2

Literature Review

In this chapter, the state-of-art flexible bioelectronics advancement and the application of silk in flexible electronics are reviewed and discussed. Specially, dominant strategy and achievement for stretchable electronics are discussed. Also, the advancement of silk protein in flexible electronics as active layer and passive layer is summarized. Lastly, the bottleneck for developing fully biocompatible electronics from materials, devices designs, and system level is illustrated.

2.1 Bio-integrated electronics

Development of bioelectronics is benefited from the advancement in soft materials and stretchable devices with high sensitivity and good biocompatibility, mechanical designs, progress of manufacturing processes.^[1] With the soft nature, bioelectronic devices can conform with biological tissue, which eliminates the intrinsic mechanical mismatch between rigid electronic devices and soft/curvilinear tissues and curved substrates.^[2] Various elements for flexible/stretchable system have been developed including biosensors, transistors, actuators, amplifiers, light emitting diodes, energy harvesting devices, and integrated circuits.^[3-6] Therefore, a wide range of biosignals can be detected for biomedical functions including electrophysiological signals (e.g., electroencephalogram (EEG), electrocardiogram (ECG), electromyography (EMG)), physiological signals (e.g., pulse, temperature), temperature and biochemical signals (Figure 2.1).^[7] These bio-integrated systems can be divided into two categories: one is wearable bioelectronics and the other is implantable devices. For long-term applications, biocompatibility should take priority to consider that no toxicity and harmful side effects should be induced by the bioelectronic devices. Therefore, biocompatible constructive materials should be utilized. In the past decades, great achievement of bioelectronics have been witnessed by virtue of materials and devices engineering, which would be discussed in the following parts.

Figure 2.1 Scheme of bio-integrated flexible and stretchable system mounting across the whole human body for biomedical applications, which includes implantable devices such as electrophysiological sensing (EMG, ECG), and wearable devices for physiological sensing.

First principle for materials targeted in bioelectronics is the possess of similar mechanical properties with biological tissue. As we know, biological tissues are soft and even deformable. Therefore, the field of stretchable electronics emerged in order to conform well with affected organs to detect related biosignals. The definition of stretchable electronics refers to materials and devices which can accommodate mechanical strain (not only few percentages, which distinguish flexibility) and maintain the electrical functions. Typical mechanism for realizing stretchable electronics can be categorized into: elastic/plastic deformation, pre-stretched buckling, crack formation (Figure 2.2).^[8] For functional materials, such as semiconductors, metallic films, they are generally hard and not stretchable. A typical method is to combine with stretchable elastomers which play the role of stretchable matrix. The paring methods can be strategically patterning or randomly incorporation. Therefore, section 2.1.1 would briefly introduce several dominant methods to achieve stretchable composite such as serpentine/microwavy design, microcrack based stretchable materials, and intrinsically stretchable materials.

Figure 2.2 The classification of stretchable strategies and stretchability regime. Reprinted with permission from reference.^[8]

Continuing the stretchable materials, system-level integration is important for stretchable electronics as single elements can't possess a complete process including sensing, power, processing and communication. Also, multifunctionality is also the key challenge for bioelectronics as human body transmit various kind of information. Thus, recent advancement to achieve stretchable systems with multiple functions is discussed in section 2.1.2 based on architected structural designs.

In this section, stretchable conductors and stretchable system integration for bio-integrated devices are focused to discussed for conformal and stretchable electronics

2.1.1 Stretchable conductors

Stretchable conductor is the key elements for stretchable electronics since its transmission of electronic signals. It is indispensable for the stretchable electronics. High stretchability of conductors, high conductivity and low resistance change are three main factors that researchers pursued in recent decades. Stretchable conductors normally consist of stretchable elastomeric matrices and conductive layers/fillers. The combination is either bilayer structures through interfacial bonding or mixing. The softness and ultimate

stretchability are dominated by the stretchable matrices, with Young's modulus of several popular elastomers concluded in Figure 2.3.^[9]

Figure 2.3 Young's modulus of various elastomer matrices. Reprinted with permission from reference.^[9]

For conducting materials, metal foils, conducting polymers, nanomaterials the mainstreams. Among them, metals own the highest conductivity, but they are rigid and not deformable in bulk.

Since metal foils are not be able to elastically/plastically deformed, structural designs including coil-like or serpentine-shaped conductive stripes of metals on stretchable substrates, have facilitated the high-performance thin-film stretchable conductors (Figure 2.4).^[10] Further, pre-strained induced wavy or 3D buckling structures, also can reduce the surface strain on the conductors to minimize the mechanical fracture under external strain. As illustrated in Figure 2.5, thin film of gold layers would be deposited on pre-stretched substrates and wavy structures form.^[11] Therefore, when being stretched, the wavy structures would unfold, and electrical conductance would not decay until reaching the value of prestretch strain.

Figure 2.4 Microscope images of metal wires on elastomer before, during and after the critical strain with high, low and no amplitude. Scale bars are 100 μm . Reprinted with permission from reference.^[10]

Figure 2.5 Sample preparation process of pre-stretched gold electrodes. Reprinted with permission from reference.^[11]

The other method is formation of microcracks for metal thin films that the connected microcrack network can conduct the electrons through the connected pathways. Developed by Stephanie and Suo's group, thin films ($<100\text{ nm}$) of Au deposited on polydimethylsiloxane (PDMS) show no failure up to 20% strain which can sustain extensive cyclic loading (Figure 2.6).^[12] The gold film locally fractures into transversal crack pattern in microscale and form the electrical percolation path through interconnected

island. This stretchable cracks-based thin-film conductor show more compliance with thin-film based electronics as reliable interconnects.

Figure 2.6 SEM images of 50 nm Au on PDMS under strain at 1st cycle and after 35,000 cycle. Reprinted with permission from reference.^[12]

Liquid metals which is liquid under ambient have good electrical conductivity and stretchability. Dickey's group has progressed in utilizing gallium based liquid metals for stretchable wires embedded in stretchable matrix (Poly[styrene-*b*-(ethylene-co-butylene)-*b*-styrene] (SEBS) resin) so that the stretchability is as high as 800% (Figure 2.7).^[13] Composites based on microdroplets liquid metals incorporating silicone elastomer show high dielectric constant and large stain limit (Figure 2.7c & d).^[14] Further, the ease of integration with microfluidic pattern and self-healing properties, renders more fantastic functions for soft and stretchable electronics.

Figure 2.7 a) An ultra-stretchable conductive fiber with cross-section of liquid metal, b) showing stretchability up to 800% without failure; c) Scheme of composites of liquid metal embedded elastomers, and d) photos demonstrating the patterning and stretchability of silicone LMEEs from 0% to 250% to 500% strain. Reprinted with permissions from reference.^[13, 14]

Nanomaterials such as nanoparticles, nanowires can form percolation network that the conductivity can be maintained under strain is also a good candidate to achieve stretchable conductors. High aspect ratio of nanomaterials, such as carbon nanotubes and metallic nanowires, can form a conductive network with less junction points. For example, single walled carbon nanotubes (SWCNT) is a representative conductive material for stretchable conductors.^[15] The formed bundles would slide under strain that the electrical stretchability is >150% while showing a transparency of 79% (Figure 2.8a). AgNWs show better

conductivity (sheet resistance of 0.11 Ohm/sq^{-1}) and higher aspect ratio can achieve higher stretchability and high transparency being embedded in elastomer (Figure 2.8b).^[16] Benefiting from the advanced synthesis, stretchable electronics based on nanomaterials as conducting materials and semiconductors opens up more opportunities for next-generation high-performance electronics.

Figure 2.8 a) Schematic and SEM images of SWCNT on elastomer before and after stretch. b) SEM image of AgNWs embedded in elastomer. Reprinted with permissions from reference.^[15, 16]

Stretchable conducting polymers are regarded as intrinsically stretchable materials as the stretchability is profited from the molecular structures. They possess the advantages of solution processibility and ease of tuning the mechanical and electrical properties. Further, their mixed transportation of ions and electrons can greatly decrease the interfacial impedance with biological tissues. Bao's group has reported highly stretchable and conductive poly (3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT:PSS) films by incorporating ionic additives (Figure 2.9), with conductivity higher than 4100 S/cm under 100% strain and a fracture strain of 800%.^[17] This intrinsically stretchable conductors can work as the interconnects of stretchable electronics with rigid island-to-interconnect strategy. It is believed that through rational molecular design, highly stretchable and conductive polymers broaden the scope of materials science and engineering in the field of stretchable electronics.

Figure 2. 9 Intrinsically stretchable conductive polymer based on poly(3,4-ethylenedioxythiophene): poly(styrenesulfonate) (PEDOT: PSS). Reprinted with permission from reference.^[17]

2.2.2 Stretchable integrated systems

Stretchable electrodes function as the interconnects of stretchable electronics, beyond which a wearable sensor system should contain multiple sensors with functional electronic components such as transistors, diodes, etc. For wearable on-skin electronic devices, physical signals such as pressure, strain, temperature, etc., and physiological signals such as EMG, ECG and EEG are supposed to be collected as critical clinical hints to evaluate the functionality of body and detect the manifestation of various diseases.

A conventional method to achieve stretchable electronics is so called “rigid islands” and stretchable interconnects.^[18] The idea is to place the high-performance but rigid electronic components on top of the “rigid islands” so that the mechanical stress would be isolated upon mechanical strain (Figure 2.10a). The key advantage of this strategy is the high-performance electronic processors for data processing and communication. Most of

research efforts have been paid for stretchable sensors and stretchable conductors while the processing and communication functions are still realized based on traditional complementary metal-oxide semiconductor (CMOS) technology. However, the stress concentration at the interface between rigid to soft is the bottleneck issue,^[19] which also induce a lot research attention to deal with it.

A fully stretchable circuit (Figure 2.10b) with all the constitutes stretchable can improve the mechanical durability and softness of the whole system. This strategy proposes challenges in developing high-performance stretchable conductors, stretchable semiconductors and stretchable dielectric layers, which requires novel approach and materials designs.^[20] Advanced materials engineering in nanomaterials and manufacturing optimization are in highly demands. It can be foreseen that our goal is to achieve fully stretchable electronic device while the “rigid islands” based strategy provides solution for upcoming integration with traditional electronics. Therefore, in the following part, research progresses towards these two directions would be explained separately.

Figure 2.10 A schematic of a) “rigid islands” and b) fully stretchable strategy for stretchable electronics. Reprinted with permission from reference.^[18]

A systematic integration of electronics including electrodes, electronics, sensors, power supply and communication components into ultrathin, low-modulus, lightweight and stretchable membranes was reported as epidermal electronics.^[21] With the dissolvable

substrates of polyvinyl alcohol (PVA), an integrated circuit on a thin sheet of polyester (Young's modulus ~ 60 kPa), with multifunctional sensors (temperature, strain, and electrophysiology), microscale light-emitting diodes (LEDs), elements (transistors, diodes, and resistors), RF coils and wireless power coils for power and communication, can be transferred into skin. Due to the softness of the substrates, the circuits can conform well with skin like tattoo while the skin electrophysiological signals (EEG), strain information, and temperature can be detected intuitively. In this case, the rational for materials and mechanics has enabled a high-performance epidermal electronic system, which demonstrates fully skin-conformable electronics.

Figure 2.11 a) Schematic and b, c) optical images of epidermal electronics with multifunctional sensors, c) showing good adhesion with skin that under deformed status. d) Also, combination with commercial tattoo can replace the substrates. Reprinted with permission from reference.^[21]

Stretchable active electronic elements like transistors, are important for electronic applications, playing the role of signal amplification and processing. Someya et.al have developed printable single-wall carbon nanotubes (SWCNTs) conductor inks incorporating a driving-cell matrix based on organic transistors.^[22, 23] The electrical conductivity of the stretchable conductors can be maintained until the strain of 120% and the transistor characteristics can be maintained until 70% strain. It can be integrated with two-dimensional array of pressure sensors that electronic skin with sensing capability can be obtained.

Figure 2.12 A large-area stretchable active matrix consisting of stretchable conductors and a 19-by-37-cell organic transistor. Reprinted with permission from reference.^[23]

With all the components soft and stretchable, Wang et. al have developed a highly conformable matrix as a multi-sensory electronic skin which can detect temperature, planar strain, relative humidity (RH), magnetic field, pressure, ultraviolet (UV) light and proximity.^[24] The multilayer configuration with proper insulation has enabled highly dense sensor arrays and simultaneous detection. Each layer of the sensors is compatible with planar thin film deposition technology with proper selections of active layers. For example, the temperature sensors are realized based on resistive metals, platinum and constantan alloy (45% Ni, 55% Cu). At the same time, constantan alloy can play the role of strain sensors as well with a high gauge factor. Relative humidity can be detected through a capacitor-based sensor and UV photodetector is obtained based on ZnO. Giant magnetoresistive-sensing elements is consisted of Co/Cu multilayers. At last, dual-mode capacitor sensors enable the pressure (contact) and proximity (non-contact) sensing capability. This work has demonstrated a fully stretchable and conformable sensor matrix

while all the signals would not interfere with each other, which pave the way for multifunctional and intimate human-machine interface.

Figure 2.13 a) A schematic and b) an optical image of the skin-inspired stretchable and conformal sensor networks. Scale bar is 5 mm. c) SEM images of the networks and the meandering interconnects (scale bar: 500 μm). d) Schematic layout of the interlayer configuration and e) optical images of bending, wearing on skin, and stretching (scale bar: 1 cm). Reprinted with permission from reference.^[24]

Hydrogels based electronics is of great interest to realize bioelectronics due to the extreme softness, tunable toughness and good biocompatibility, which is suitable for soft machines and interfacial medium with biological tissues.^[25] However, the water-embodied hydrogels suffer from dehydration and poor adhesion with other materials, which hinder the application under ambient and as the platform for hybrid integration.^[26] Therefore, many

research efforts have been paid to realize strong bonding with elastomers though ultraviolet-initiated polymerization and surface treatment for robust interfaces.^[27]

Figure 2.14 a) A schematic and b) an optical image of the hydrogel electronic skin. Reprinted with permission from reference.^[28]

Martin et.al has reported a dispersion of synthetic adhesives which would polymerize to form instant tough bonding between a tough hydrogel and a single-network elastomer, hydrogel or polymer.^[28] In this case, the strong bonding makes hybrid electronics based on hydrogel untethered without delamination issues and prestretch method is utilized to realize stretchable power supply. Being benefited from tough interfacial bonding, a hydrogel smart electronic skin containing power supply, control, readout and communication units fabricated on $1.4\ \mu\text{m}$ PET foils can be well bonded to hydrogel being pre-stretched. At the same time, excellent tissue conformability of hydrogels can provide advantageous biotic/abiotic interface with an autonomous smartphone app realizing controllable drug delivery.

Figure 2.15 A schematic of self-healing electronic system based on PDMS-MPU_{0.4}-IU_{0.6} with a strain sensor, an ECG sensor and LEC array. Reprinted with permission from reference.^[29]

One systematic-level multi-functional self-healable electronic skin has been demonstrated by Bao et.al.^[29] They have utilized self-healing polymer (PDMS-MPU_{0.4}-IU_{0.6}) and 1D nanostructured network (CNTs) to recover the conductivity, mechanical strength and functionality of the system after damage at room temperature. CNTs here can form percolated network on self-healable matrix, realizing mechanically and electrically healable electrodes. Similarly, AgNWs was chosen as the self-healable interconnects owing to its high conductivity. Further, a light emitting capacitor was prepared based on Cu-doped ZnS nanoparticles embedded in the self-healing polymer. Therefore, a multi-functional stretchable and self-healing electronic skin has been realized integrating with strain sensors and ECG sensors (Figure 2.15). In this case, the LEC pixel can visualize the heart beat by blinking at each ECG peaks and the 5 LEC pixels can be in accordance with experienced strain values. Since human skin is self-healable, electronic components and system based on self-healing polymers undoubtedly shed a light to realize unbreakable and robust electronics with multiple functions.

To conclude this section, system-level integration of multifunctional electronics on soft and stretchable substrates is more beyond individual devices, sensor and electrodes. The issue lays on the interfacial bonding and compatibility of fabrication process. We can foresee that in the future, with mature advancement, a fully stretchable and adaptable electronic device would provide a distinct insight into the human-machine interface.

2.2 Application of silk in flexible electronics

Silk fibroin is a well-studied biomaterial which has been applied widely for biomedical applications due to the solution processibility and controllable degradability.^[30] Therefore, the aqueous processing enabled materials formats, such as hydrogels, tubes, sponges, fibers and films can be obtained.^[31] Further, the extreme toughness, good optical properties and unique electrical properties are valuable for next-generation biocompatible electronics.^[32] This section would specifically focus on the application of silk fibroin into the field of flexible electronics, benefiting transient electronics, lightweight electronics, etc, dividing into passive functions and active functions.

2.2.1 Passive layer

Flexible and stretchable electronics can conform well with curved surfaces so that it is promising to acquire biosignals with high signals fidelity.^[33] Silk fibroin is an appealing material as the substrates for biointegrated electronics due to the controllable biodegradability and bioresorbability.

Even silk fibroin possesses an ultrahigh Young's modulus of 4~10 GPa, when its thickness has been reduced into microscale, according to the following equation,^[34] the flexural rigidity is at the range similar to that of polyethylene terephthalate (PET) and polyimide (PI) with same thickness.

$$D = \frac{Eh}{12(1 - \nu^2)}$$

Here, E is the Young's modulus of bulk materials and h is the thickness of the thin films. Thin-film based manufacturing techniques including physical vapor deposition, roll-to-roll printing and transfer printing have contributed to realize flexible electronic devices based on silk fibroin. Specifically, richness of hydroxy and carboxy groups in the molecules of silk fibroin renders it good adhesion with surfaces of various organs, which is suitable for biomedical application.

Transfer printing is a unique method to avoid damage on silk fibroin while complicated configuration can be fabricated on silicon wafer first and then transfer into silk substrates. By transferring the intricate electrode array embedded in ultrathin polyimide films into silk substrates, a conformal interface between human brain and outer electronic wiring can be constructed by dissolving the substrates (Figure 2.16).^[35] Here, silk fibroin plays the role to support the ultrathin mesh electrodes (2.5 μm) first and consequently form a conformal interface after dissolution so that the evoked response can be measured and mapped precisely for brain-computer interface.

Figure 2.16 Image of an electrode array with a mesh design on dissolvable silk substrates, realizing conformal contact with brain model after dissolving silk (inset). Reprinted with permission from reference.^[36]

Besides utilizing dissolvable silk fibroin, a fully degradable device was achieved by combining with dissociable metals and dielectric, form the concept of “transient electronics” for a silicon-based complementary metal oxide semiconductor technology.^[37] In this case, integrated sensors, actuators, power supply and wireless control have all been considered, showing a set of materials, manufacturing and theoretical designs to achieve a programmable biointerface. The crystallinity of silk fibroin can be regulated through vapor annealing that it would not be dissolved instantly, which serves the function of encapsulation. And based on this, the degradability can also be coordinated for various implantation cases that require different time scale to function. More research works based on silk substrates have been demonstrated on sensors, actuators, solar cells and wireless antennas, wireless heater, etc.^[38, 39] By virtue of the modifiable crystallinity and degradation, several modes of transience can be adjusted, and further modification can provide stimulus-response degradation, which further enrich the class of green electronics for bioelectronics.^[40]

Figure 2.17 A scheme of transient electronics showing key materials, devices components and reaction mechanisms. Reprinted with permission from reference.^[37]

Carbonization of silk fibroin is not discussed in detail as it is no longer the protein formats. But it has decent research works to realize energy storage devices and strain sensors utilizing silk fibroin as carbon sources.^[41, 42] To conclude this part, great success has been obtained based on flexible silk substrates for conformal, transient electronics. They have showed insurmountable merits in bio-integrated devices and are pending for more creative application of this ancient materials.

2.2.2 Active layer

Besides as the passive substrates, silk fibroin has exhibited a good dielectric property,^[43] which is possible for optical devices, organic filed effect transistors and resistive switching memory devices.^[39, 44]

Solution processing of silk fibroin is process-compatible with thin-film based transistors with good flexibility. Transistors are believed to be the base of modern electronics. A flexible organic field effect transistor based on pentacene has been reported using silk fibroin as the dielectric layer, showing a high mobility of $23.2 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ and a low working voltage of -3 V .^[45] This transistor has showed an outperforming characteristic which was hypothesized as the result of the better crystallization of pentacene and low interface scattering on silk fibroin dielectric. The other report shows that the silk fibroin/Poly(3-hexylthiophene) (P3HT) interface can reduce traps and defects, thus enhancing the carrier mobility, which is the result of highly ordered crystals of silk fibroin in nanoscale.^[46] A high carrier mobility of $0.21 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$, a low operating voltage ($<3 \text{ V}$) and a low threshold voltage of -0.77 V have been achieved. Several works demonstrated that the transistors with silk fibroin as the dielectric show higher mobility and low operating voltage compared to the transistors with traditional dielectric layers.^[47, 48] Further, silk fibroin can be freestanding so that the whole device can be fabricated on silk substrates, replacing rigid substrates.^[49]

Figure 2. 18 Flexible organic field-effect transistors taking silk fibroin as the gate dielectric layer. a) The scheme of the FET configuration. B) The optical image of the flexible OFET. c) Output characteristics and d) transfer characteristics of the flexible FET. Reprinted with permission from reference.^[45]

Besides utilizing silk fibroin as the dielectric layer of transistors, the excellent dielectric property of silk fibroin can also be applied into resistive switching memory devices, which has attracted a lot attention as it is promising for next-generation high-density, large-scale and low-power consumption data storage. The simple configuration of metal-insulator-metal like a capacitor is ease of fabrication and allows higher density, which can be realized through simply spin coating. Therefore, silk fibroin was sandwiched by Ag and Au, and shows threshold resistive switching behavior.^[44] There are two resistive states, distinguishing “0” and “1” through judging the high resistance states (HRS) or low resistance states (LRS). So, if the compliance current is below 10^{-6} A, the resistive switching is volatile, which is so-called threshold RS. When the compliance current (I_{cc}) is higher than that, the volatile resistive switching would turn into non-volatile states, showing good feasibility of operation modes. The high ON/OFF ratio ($>10^7$) and long retention time (>4500 s) show important advantages for practical applications. Further, by incorporating gold nanoparticles, the resistive switching memory performance can be modified to achieve a lower set/reset voltage (± 2 V), which would tune the conductive filaments inside silk fibroin.

Therefore, with the great space for tunable resistive switching behaviors and performances, silk fibroin is an ideal candidate for next-generation high-density data storage devices. The transformation from volatile resistive switching into non-volatile states is also of great importance to solve the sneak path problems for highly dense cross-bar structures. By replacing the metals into Mg and alternate the silicon substrates into silk fibroin, ultralight memory devices comprised of all silk fibroin can dissolve in water, shedding lights on sustainable electronic storage devices.^[39]

Figure 2.19 a) The configuration of silk fibroin based resistive switching memory devices. b) The SEM images of the top electrodes. c) The resistive switching characteristics of threshold RS and memory RS. d) The statistical study of ON/OFF ratios. Reprinted with permission from reference.^[44]

The dielectric property of silk fibroin can also be integrated with radio-frequency identification (RFID) for biosensing application.^[50] Utilizing silk fibroin as the substrates, the biosensors based on silk fibroin can conform well with any curvilinear surfaces of food and fruits for quality monitoring. If the food went bad, the reflections of the sensors would be shifted, indicating the food quality continuously and conformally. There is report showing that with proper coating of silk fibroin, food can be longer kept due to the compact filming of silk fibroin and controllable crystallinity.^[51]

Further integration with certain enzymes can further improve the selectivity of silk fibroin-based biosensors, showing good mechanical flexibility and biodegradability for environment-friendly electronics. For example, merging glucose oxides enzyme with silk fibroin gated FET, a flexible glucose sensor can be realized at the range of 0.1- 10 mM, which further broaden the horizon of silk fibroin-based electronics.^[52]

Figure 2.20 Conformal food sensors based on silk fibroin. a) Optical image of silk fibroin-based RFID on various food and b) reflection spectra of the sensors. Reprinted with permission from reference.^[50]

2.3 Summary and status

To summarize this chapter, recent advancement of flexible and stretchable electronics haven been introduced, and the application of silk fibroin in this field as active and passive component. Although recent year witnessed the successful application of silk fibroin in flexible devices, there is a missing point of proper stretchability of silk fibroin-based electronics as well as multi-functionality. Hydrogels based silk protein is soft but is not compatible with traditional CMOS technology. Therefore, there is a high demand to develop highly stretchable silk materials with electronic functions, which complement the rest of stretchable electronics based on biomaterials.

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Chapter 3

Experimental Methodology

This chapter illustrates the experimental methodology. The underlying mechanism of process of silk protein and correlated mechanical properties is unveiled based on protein conformation. Fabrication processes and characterization of stretchable electrodes based on plasticized silk protein are elucidated. Methods to prepare silk fiber mat and solution coating process of conducting polymer are explained. Methods to prepare heterogeneous silk substrates to achieve stretchable electronics, together with screen printing techniques, are presented. Experimental setups for performing microscope measurement, thermal vapor resistance, electrical measurement under strain, on-skin electrophysiological signals are illustrated. Methods for constructing the models and simulation for Molecular Dynamics Simulation are depicted.

3.1 Rationale for selection

Silk is an ancient material which has been used for textile for centuries. In recent years, the feature that regenerated silk fibroin in solution can be processed into multiple formats such as thin films, fibers, gels, etc., greatly flourish its application.^[1] As protein, the amino acids in silk sequences can be modified through molecular engineering, thus modifying the macroscale mechanical properties. Further, the degradability of silk protein can be controlled by various processing methods and corresponding levels of crystallinity. Numerous hydroxyl groups inside amino acids can contribute to the good adhesion between silk and other layers. Therefore, silk fibroin protein is an ideal materials candidate of biomaterials for flexible and stretchable system. However, silk is famous for its superior mechanical properties that raw silk fiber is tough and non-stretchable (Table 3.1).^[2] Therefore, materials engineering of silk fibroin to make it fulfill the requisite in mechanical properties and electrical performance is highly desired.

Table 3.1 Mechanical properties of several pristine silk fibers.

Material	Form	Elastic modulus (GPa)	Ultimate stress (MPa)	Ultimate strain (%)
B. mori silk	Ultrathin films	6-8	100	0.5-3.0
B. mori silk	Fiber mats	1.6	510-650	~32
Spider silk	Fiber	4	-	35

The following three methods address how silk fibroin is processed to achieve tunable mechanical properties for on-skin applications. Following the preparation of silk fibroin, the stretchable electrodes and integrated electronic system are realized.

3.2 Plasticized silk protein for on-skin stretchable electrodes

3.2.1 *B. mori* silk fibroin structure

Raw silk fibers (*B. mori* silkworm) contains two protein: one is silk fibroin (70%) and the other is sericin as the glue (25-30%). Sericin would be removed through degumming process of boiling in Na_2CO_3 solution.^[3]

Silk fibroin consists of a light chain (~26 kDa) and a heavy chain (~390 kDa) in a 1:1 ratio which are linked by a single disulfide bond. The amino acid sequence mainly consists of glycine (Gly) (43%), alanine (Ala) (30%) and serine (Ser) (12%). As mentioned, crystalline domain of silk fibroin contributes to the mechanical strength and amorphous, which consist of Gly-X repeats, while X includes Ser, Ala, tyrosine (Tyr). Each domain is made of hexapeptides including GAGAGS, GAGAGY, GAGAGA and GAGYGA with the approximate ratio of 32:9:2:3.^[4] Coming to the polymorphs of silk, several secondary structures have been reported.^[5] Silk I is the water soluble structure prior to crystallization and would transform into β -sheet secondary structures (silk II). Silk III is a kind of air/water assembled silk with a helical structure. In aqueous solution, the silk I structure is more common and would tend to form into a β -sheet structure when exposed to methanol.^[6] There is strong hydrogen bonds as well as van der Waals forces in β -sheets so that the highly packed silk II can exclude water and can't be soluble in some solvents.^[7] Therefore, proper processing of silk fibroin can control the mechanical properties.

3.2.2 Plasticization of silk fibroin by CaCl_2

Raw silk fibers are from China, reeled from cocoons. By boiling the silk fibers in 0.02 M Na_2CO_3 (Sigma-Aldrich Pte Ltd) solution for 1 hour and rinsing thoroughly with deionization water, sericin is removed. Dried silk fibers can be obtained after drying in the oven at 60° C for 2 days.

Silk fibroin and CaCl_2 are mixed in 20 grams formic acid (Sigma-Aldrich Pte Ltd, reagent grade $\geq 95\%$) with a weight ratio of 85:15.^[8] Stirring and ultrasonic bath are needed to get a well dispersed solution. All experiments related to formic acid were done in the fume hood since the volatility, irritant smell and toxicity of formic acid. The dispersed CaCl_2 -FA-silk solution was casted into three polystyrene petri-dish (diameter 90 mm) in the fume hood and let the formic acid evaporate. After the evaporation overnight inside the fume hood at room temperature and 70% relative humidity, three silk fibroin- CaCl_2 films can be achieved. (Figure 3.1)

Figure 3.1 Dissolution process of silk fibroin to achieve plasticized silk protein.

After obtaining the plasticized silk films, stress-strain curves were obtained by a mechanical tester (C42, MTS Systems Corporation) with environmental chamber (Bionix Environbath, MTS Systems Corporation) to control the relative humidity (Figure 3.2). The relative humidity was tuned by placing saturated salt solution (KCl , NaCl , KI , NaBr , K_2CO_3) in the chamber overnight, and the relative humidity values were estimated according to the partial pressure of saturated salt solutions. The measurement of ambient humidity was realized using commercial humidity meter HI 9565 (HANNA Instruments). Tensile tests were conducted for more than three samples for each of the conditions at a speed of 2 mm/min. The Young's modulus was calculated using the first 0.5% strain region.

Figure 3.2 The Bionix Environbath, MTS C42 for mechanical tests under controlled relative humidity.

Infrared Spectroscopy (FTIR) measurements: All the FTIR spectra were obtained using the FTIR spectrometer (The PerkinElmer FrontierTM IR) with Attenuated Total Reflectance (ATR) accessories from the wavenumber of 4000 cm^{-1} to 1000 cm^{-1} . The silk films with different CaCl_2 weight ratios were cut into 1 cm^2 to be tested. The absorption intensities were normalized according to the peak of random coils at 1646 cm^{-1} . And the peak of β -sheets was analyzed at 1625 cm^{-1} .

3.2.3 Fabrication and characterization of stretchable electrodes

Prior to the metal depositions, silk films with CaCl_2 were dried in the oven at 60°C for 1 h to remove the water. Au and Ag thin films were formed by a vacuum thermal evaporator (Nano 36, Kurt J. Lesker) under 2×10^{-6} Torr at a deposition rate of $0.3\text{ \AA/s}$. The Au and Ag sources were purchased from Kurt J. Lesker and the purity was 99.99%. Pt was sputtered by JFC-1600 auto fine coater for 240 s at 40 mA. After the depositions, samples were hydrated under 60% relative humidity for overnight. As a control, PDMS films were prepared by mixing the precursor and cross linker at 10: 1 ratio, spin coating on top of fluorinated silicon wafer at 1000 rpm and curing under 60°C for 4 h. All the metal films were deposited simultaneously on both silk films and PDMS films.

Measurements of resistance change under strain: All the tests were performed under 60% relative humidity, and the sample sizes were united to 2 cm (length) $\times$ 1 cm (width). The resistance was measured by a semiconductor parameter analyzer (Keithley 4200-SCS,

Tektronix) while strains were applied by a manual tensile stage. For the measurement of resistance under cyclic strains, 20% strain was applied using a mechanical tester at a speed of 2 mm/min while the resistance was recorded by a semiconductor parameter analyzer (Keithley 4200-SCS, Tektronix).

LED integration with plasticized silk electrodes: A plasticized silk electrode and chip LED (5050, 3MTM) were attached on a finger. A double-side tape was used to fix the LED. The plasticized silk electrode was connected with the LED and power generator (SPS-3610, GW Instek) using copper wires and Ga-In eutectic (Sigma-Aldrich). In order to light up the LED, a voltage of 2.7 V was applied from the power generator. The wiring setup is as figure 3.2.

Figure 3.3 a) The photograph of LED connected with power source without applying voltage by our on-skin silk electrodes. b) The photograph of LED connected with power source applying 2.7 V voltage by our on-skin silk electrodes.

90° peeling tests of the adhesion strength between our plasticized electrodes and skin: The methods followed and modified the previously reported procedure.^[9] The Au side of our plasticized electrodes were glued with a polyimide sheet (RS Components, Singapore) by using super glue in order to eliminate the effect of substrate deformation. The widths were 1 cm. Water mists were then applied to our forearm skin, and the electrodes with polyimide sheets were attached to skin with one end released. The released end was gripped to a mechanical tester (C42, MTS Systems Corporation) to detect the peeling force at a speed of 6 mm/min. The adhesion strength was calculated dividing the force by the width of the electrodes.

Cross sectional SEM observation: The skin replica was fabricated by casting PDMS onto the forearm texture mold (P&G Singapore LTD.). Microstructured surface was prepared by casting PDMS onto the Si pyramidal mold which was fabricated by reported anisotropic etching process on silicon wafer.^[10] These surfaces were treated with short O₂ plasma to improve the hydrophilicity and were sprayed with water mist before laminating silk electrodes. Then, the cross section was obtained by cutting whole the layers using sharp razor blades. SEM images were obtained through Zeiss Supra 55 SEM at an acceleration voltage of 10 kV under secondary electron image (SEI) mode.

Skin interfacial impedance measurement and EMG signals recording: The interfacial impedance was measured by laminating pairs of electrodes with a circle shape (diameter: 2 cm), and a center-to-center distance of 5 cm on forearm skin. As the electrodes, plasticized silk electrodes and commercial gel electrodes (Large muscle electrodes, Backyard Brains) were used. The interfacial impedance measurements were conducted using electrochemical workstation (ZAHNER ZENNIUM) from 1 Hz to 1 MHz with a voltage of 100 mV. EMG signals were obtained while bending the wrist towards the body at 90° for 3 s and the signals were recorded by a toolkit for EMG recording (Muscle Spikerbox, Backyard Brain). The test was approved by Institute of Review Board, Nanyang Technological University (approval number: IRB-2017-08-035). The toolkit includes amplifier and 50/60 Hz filter. SNR were obtained through the equation (1):

$$SNR = 10 * \log_{10}(P_{signal} - P_{noise})/P_{noise} \quad (1)$$

P_{signal} , and P_{noise} denote the signal intensity, and background noise level, respectively. They were calculated from the obtained signals by the previously reported algorithm.^[11]

3.2.4 Molecular dynamics simulation

Molecular dynamics simulations of crystallites domain: All the simulations for crystallites and amorphous domain were conducted by the software package GROMACS 4.5.5. using the force field set of Amber 03^[12]. First, the models for crystallite domain were built, which are three anti-parallel β -sheet crystallite units (GAGAGA, GAGYGA, and GAGAGS)

representing the crystal domains of *Bombyx mori* silk. They contain repeating 3 layers and 5 chains as a typical crystallite. The initial structure of GAGAGA crystallite was obtained from Protein Data Bank Entry (2slk),^[13] the initial structures of GAGYGA and GAGAGS were mutated through Chimera^[14] by replacing the A to S or Y on the first layer. For the simulation of the crystallite units in water, the systems were solvated in a water box with a dimension of $3.6 \times 3.6 \times 2.8 \text{ nm}^3$. A model of transferable intermolecular potential with 3 points (TIP3P) was adopted for the water.^[15] In addition, energy minimization was carried out to obtain the equilibrium status both in vacuum and in water. Finally, molecular dynamics simulations were performed to the pre-equilibrated systems under 100 ns of Canonical ensemble (NVT) at a temperature at 300 K and 100 ns of Isothermal-isobaric (NPT) ensemble at a temperature of 300 K and pressure of 1 bar both in vacuum and water.^[16] The boundary condition was set to periodic. The time step to collect data was 0.002 ps. The long-range electrostatic interactions were calculated by particle mesh Ewald (PME) method.^[17] The criteria for judging whether there is a hydrogen bond between a hydrogen atom and a hydrogen bond acceptor was set to that the distance is within 0.35 nm and the bonding angle is less than 30° . The interchain energies were calculated from two chains in the edge of the outmost layer of crystallites as the representative pair (described by two-direction arrows in Figure 4.8). Representative snapshots for protein configuration were produced using software visualized molecular dynamics (VMD) Molecular Graphics Viewer.^[18]

Molecular dynamics simulations of amorphous domain: The initial structures for amorphous domain were built based on the silk fibroin sequence according to the Linker 6 as published by Zhou et al: (GAGAGAGAGAGTGSSGFGPYVANGGYSGYEYAWSSSEDFGTGS).^[19] The initial structure of the amorphous domain consisted of 12 fully-extended amorphous chain. These amorphous chains were arranged in an antiparallel manner with an initial distance of 5 Å between adjacent chains, followed by energy minimization to the initial structure. After that, 500 ns of stochastic dynamics simulation in implicit solvent was carried out on the structure to obtain the pre-equilibrated amorphous domain. The following molecular dynamics simulation was carried out based on this pre-equilibrated amorphous domain in

vacuum and in water with Ca^{2+} ions, respectively. For the simulation of the amorphous domain in water with Ca^{2+} ions, the systems were first solvated in explicit water box with a dimension of $4 \times 4 \times 11.2 \text{ nm}^3$. A model of transferable intermolecular potential with 3 points (TIP3P) was adopted for the water. Then, 18 Ca^{2+} ions were added to replace some of the water molecules to neutralize the system. In addition, another energy minimization was carried out to obtain the equilibrium status both in vacuum and in water with Ca^{2+} ions. Finally, molecular dynamics simulation was performed to the pre-equilibrated systems under 100 ns of Canonical ensemble (NVT) at a temperature of 300 K and 100 ns Isothermal-isobaric (NPT) ensemble at a temperature of 300 K and pressure of 1 bar both in vacuum and water with Ca^{2+} ions. Secondary structures of protein were analyzed using the STRIDE algorithm,^[20] which is a built-in module in the VMD Molecular Graphics Viewer. All the representative snapshots for protein configuration were produced using VMD Molecular Graphics Viewer.^[18]

3.3 Gas permeable and conformal silk electrodes

3.3.1 Silk fiber mats electrodes preparation

Silk fibers were boiled in aqueous solution of 0.5 wt% Na_2CO_3 for 60 min and then rinsed thoroughly with distilled water to remove the glue-like sericin. The degummed silk fibers were dried in air and then directly dissolved in formic acid which contains 6 wt% CaCl_2 yielding 3 wt% silk solution. After magnetically stirring for 4 h, the silk solution was casted in petri dish and dried in fume hood overnight to evaporate formic acid. After that, the resulting silk fibroin films were immersed in water for 24 h to remove the CaCl_2 and residual formic acid, and finally the regenerated silk fibroin films were dried in oven for later usage.

Regenerated silk fibroin films were dissolved in 98% formic acid to prepare 12 wt% spinning solution. A small amount of polyethylene oxide (PEO, 1000000 g/mol) (~0.24 wt%) was added to promote fiber formation. The electrospinning procedure of silk solution was performed with an applied voltage of 15 kV and solution flow rate of 0.8 ml/h. The

distance between the needle tip and metallic frame collector was kept at 20 cm. To prepare silk mats with aligned fibers, two paralleled strip electrodes separated by a gap of 3 cm was used as a collector.^[21]

The electrospun silk fiber mats were stabilized by immersing in glycerol for 1h at 60 °C and then rinsed in water overnight to remove glycerol and PEO.^[3, 22] After that, the silk mats were dried in air at ambient temperature. The coating solution was prepared by simply mixing PEDOT: PSS aqueous solution (Clevios PH 1000) with various mounts of glycerol and 1 wt% Triton X-100. To prepare silk electrode, the glycerol stabilized silk mats were firstly immersed in the above coating solution for 15 min. After taking out and slightly removing the redundant liquid, they were dried in fume hood for 2-6 h according to the glycerol content. Finally, the dried fiber mats were thermally annealed at 120 °C for 15 min and then kept at ambient conditions for 24 h to reach the balance of moisture absorption. The glycerol content in PEDOT: PSS coating solution varied from 0, 2, 5, 10, 15, 20 to 40 wt%, and the final silk electrodes were accordingly labeled as Silk-P-0G, Silk-P-2G, Silk-P-5G, Silk-P-10G, Silk-P-15G, Silk-P-20G, and Silk-P-40G, respectively.

3.3.2 Gas permeability, thermal insulation and thermal resistance measurements

Measurement of WVTR. The water-vapor transmission rates of electrodes at 20 °C and 37 °C were evaluated based on standard ASTM E96-95. The tests were performed by periodically measuring the weight loss of water in a bottle where the opening was covered by the samples. The WVTR is calculated by the following equation:

$$WVTR = \frac{G/t}{A}$$

where G is the weight change, t is the time during the weight change, A is the test area for the sample.

Measurement of thermal insulation and evaporative resistance of electrodes: Here, a 34-zone “Newton” thermal manikin (Measurement Technology Northwest, Seattle, WA, USA)

equipped with sweating function was used (as shown in Fig. 3a). Each zone contains a heating element and temperature sensors within the “skin” of the manikin, and the individual surface temperature or heating power could be controlled at a normal human body state. Moreover, a water circulation system is connected to the manikin and small pores over the surface of manikin are spaced to uniformly and controllably deliver water to the skin surface after wearing a removable wicking fabric skin, which can mimic the sweating of real human body. The temperature, sweat rate, and heat flux generated during experiment at each body part were monitored by ThermDAC® software.

The measurement of thermal insulation followed the ISO 15831 2004 standard with some modifications. The manikin was placed in a climatic chamber (ESPEC Inc., Osaka, Japan) where the constant air temperature, air speed, and relative humidity was set as 20 °C, 0.4 m/s, and 50 % respectively. The constant temperature mode was used, and the manikin surface temperature was controlled at 34 °C during experiments. All samples were cut into rectangle and reconnected together to totally cover the zone 15 of manikin without gaps between samples. After starting the test, the system was equilibrated for at least 30 min to reach steady-state conditions, and data were recorded at 30s intervals. The thermal insulation was calculated by the following equation:

$$R_t = \frac{(T_m - T_a)A}{H_i}$$

Where R_t is the local thermal insulation at the used segment of manikin (°C·m²/W); T_m and T_a are the manikin surface temperature of the corresponding segment and the air temperature, respectively (°C); A is the sweating fabric skin surface area of the corresponding segment (m²); and H_i is the measured dry heat loss at the corresponding segment (W).

The evaporative resistance was measured based on the procedures in standard ASTM F2370-10. The tests were performed in an isothermal condition where the air temperature and relative humidity were controlled at 34 °C and 60 % respectively. Typically, the water flow rate was set as 400 mL·m²/h and a pre-wetted polyester fabric “skin” was used before attaching samples to simulate sweating of human skin. The other procedures were similar

to those of the thermal insulation measurement as mentioned before. The thermal insulation was calculated by the following equation:

$$R_e = \frac{(P_s - P_a)A}{H_e}$$

Where R_e is the evaporative resistance at the used segment of manikin ($\text{Pa} \cdot \text{m}^2/\text{W}$); P_s is the corresponding segmental water vapor pressure on the wet fabric skin (Pa); P_a is the water vapor pressure in the air (Pa); A is the sweating fabric skin surface area of the corresponding segment (m^2); H_e is the local segmental evaporative heat loss (W).

3.3.3 Mechanical and electrical characterization of silk fiber mats electrodes

Measurements of mechanical and electrical properties: Stress-strain curves were obtained by a mechanical tester (C42, MTS Systems Corporation) at a speed of 1 mm/min in ambient humidity. Typically, the relative humidity is at the range of 60–70% in the lab.

The conductivity of silk electrodes was measured by Keithley 2450 source-meter using a four-point method. Four electrical probes with an equal spacing of 5 mm between probes were used to avoid the changes of contact resistance on samples. The resistance change under cyclic strains were measured by a semiconductor parameter analyzer (Keithley 4200-SCS, Tektronix) while strains were applied by a mechanical tester at a speed of 1 mm/min. The effective stretch area of samples was kept at 1 cm (length) $\times$ 0.5 cm (width).

Infrared Spectroscopy (FTIR) measurements: The FTIR spectra of all samples were collected using the FTIR spectrometer in attenuated total reflection (ATR) mode (The PerkinElmer FrontierTM IR) from the wavenumber of 4000 cm^{-1} to 1000 cm^{-1} . For each measurement, 32 scans with a nominal resolution of 4 cm^{-1} were co-added to yield spectra. Scanning electron microscope (SEM) observation and fast Fourier transform (FFT) analysis: The SEM images were obtained by Field Emission Scanning Electron Microscopy (JEOL JSM-7600F) at an acceleration voltage of 5 kV under secondary electron image (SEI) mode. Before observation, the samples were stretched to a certain strain and glued to a silicon wafer. A 2D FFT image analysis was applied to quantify the

fiber alignment.^[23] SEM images were squarely cropped and converted to 8-bit grayscale at first. Then FFT computation was adopted to the images by open-source ImageJ software. The FFT images were then analyzed with an “Oval Profile Plot” plugin, in which the radial intensity was summed at the acquisition angle from 0° to 180°. All the data was normalized to a baseline value of 0 for easy comparison.

3.3.4 On-skin electrophysiological signals recording for sweaty conditions

Skin interfacial impedance measurement: The interfacial impedance was measured by laminating pairs of electrodes with a circle shape (diameter: 2 cm) on forearm skin, and the center-to-center distance was kept at 5 cm. As comparison, silk electrodes, Au mesh (depositing Au on as spun silk and then dissolving silk on skin), and commercial gel electrodes (Large muscle electrodes, Backyard Brains) were used. The interfacial impedance measurements were conducted using electrochemical workstation (ZAHNER ZENNIUM).

ECG signals measurement: For performance evaluation of the silk electrode and comparison with commercial gel electrode, ECG signals were measured both in normal state before exercise and sweaty state after exercise. The ECG signals were acquired by a commercial portable ECG monitor (model PC-80B, Heal Force Bio-Meditech, Shanghai, China), and a three-lead system was used. Two electrodes were fixed on the left and right sides of the ribcage, and the reference ground was located in their lower right abdomen. The experiments were performed outdoor on the playground (Sports & Recreation Centre, Nanyang Technological University). The resting-state ECG signals were measured first, and after running while wearing the electrodes for 1000 m within 3-4 min, the ECG signals were measured again. To visualize the influence of various electrodes on the temperature variation of skin after exercise, three types of electrodes (silk electrode, commercial gel electrode, and Au on SEBS polymer electrode) were pasted side by side on the middle of the ribcage. A thermal infrared camera (Ti450, Fluke Corp., USA) was used to record the skin temperature before and after running. It should be noted that excess exercise was avoided, because in this case the skin temperature will be extremely high and sweat

evaporation was unable to effectively lower down the skin temperature, as a result there will be no difference between the three electrodes. All experiments on human skin were approved by Institute of Review Board at Nanyang Technological University (approval number: IRB-2017-08-035).

3.4 Mechanically heterogeneous silk fibroin for hybrid stretchable electronics

3.4.1 Preparation of mechanically heterogeneous silk fibroin films and circuits printing

Preparation of plasticized silk fibroin films: The preparation of plasticized silk fibroin films was same as section 3.2.2. The main difference is the sample size that should fit with the electronic patterns and screen-printing process. In this project, the sample size is 10 cm × 10 cm.

Printing of the Ag traces on silk substrates: The screen-printing process (Figure 3.4) was finished in cleanroom at SIMTECH, Singapore. The ink for screen printing is LS-411AW Silver Paste from ASAHI CHEMICAL RESEARCH LABORATORY Co., LTD, with a sheet resistivity <40 mOhm/sq. and a viscosity of 200~300 ps at 25 °C. The designed mask would block the unwanted area and are only permeable to the ink at certain area. Firstly, the as prepared silk substrates with 15 wt% CaCl₂ and 20 wt% CaCl₂ were fixed by vacuum at the screen printer and printed with designed patterns. After the printing process, silk substrates were taken out and annealed in oven for 20 min at 80 °C.

Figure 3.4 a) The scheme and b) setup of screen printing.

Methanol treatment of silk fibroin films with various weight ratios of CaCl_2 : As-printed silk circuits were treated with methanol of a small volume of 5 μL , 10 μL and 20 μL at certain areas inside fume hood on the surface. Front treatment and back treatment for checking the side variation of methanol treatment were adopted for comparison for lateral test of resistance change under strain. After the treatment, rigid and functional components such as resistors, capacitors, and rectifier, etc, were attached and bonded using the same silver ink as printing process to the designed locations following annealing at 60° C for 20 min.

3.4.2 Mechanical, electrical characterization of mechanically heterogeneous silk fibroin films

Mechanical tests of mechanically heterogeneous silk fibroin films: Silk fibroin films with various weight ratio of CaCl_2 with and without methanol treatment were prepared as 2 cm (length) $\times$ 1 cm (width) to measure the stress-strain curves by a mechanical tester (C42, MTS Systems Corporation), whose slope were fitted to get the values of Young's modulus. The room temperature is 25 °C and relative humidity is 60 ± 2 %.

Scanning electron microscope observations: SEM images of Ag traces on top of plasticized silk fibroin film, mechanically heterogeneous silk film and PDMS were characterized by field effect scanning electron microscope with 5 kV voltage (JEOL JSM-7600F) at an acceleration voltage of 5 kV under secondary electron image (SEI) mode.

Electrical measurement of resistance: All the tests were performed under 60% relative humidity, and the sample sizes were united to 2.5 cm (length) $\times$ 2 mm (width) as the printed coils were confined to 1 mm width. The resistance was measured by a semiconductor parameter analyzer (Keithley 4200-SCS, Tektronix) while strains were applied by a manual tensile stage.

Mechanical FEA simulation for the mechanically heterogeneous silk fibroin films: The mechanical strain distribution of the mechanically heterogeneous silk fibroin films was analyzed by FEM when being stretched up to 50% principle strain. The thickness of the plasticized silk films was measured and assumed as 200 μm . The length and width of the plasticized silk film is set as the experimental setup, which are 2.5 cm $\times$ 2 mm. The dimension of the methanol-treated area is 2 mm width $\times$ 2 mm length $\times$ 50 μm thick. They are assumed to be embedded and adhere closely as they are *in-situ* methanol treatment of plasticized silk fibroin films. The boundary conditions are set as free in X and Z directions and nominal strain was applied in Y direction.

3.4.3 Electromagnetic measurements of wireless antennas

Electromagnetic coupling of stretchable antennas: The resonant frequency of the printed Ag coils was measured using vector network analyzer (VNA) (TTR503A, Tektronix) with a commercial reader coil (Figure 3.5).

Figure 3.5 Experimental setup of a vector network analyzer with a commercial reader coil.

For electromagnetic coupling under deformation, the stretchable coils were applied strain using the mechanical tester (C42, MTS Systems Corporation). The VNA was set in S_{11} magnitude (reflection) mode to measure the resonant frequency and bandwidth.

$$f_{resonant} = \frac{1}{2\pi\sqrt{LC}}$$

Where L is the inductance and C is the capacitance of the printed coils.

Q factor (defined as the quality factor) is an important factor to evaluate the efficiency of the coil. Experimentally, Q factor is related the sharpness of the resonant peak by dividing the $f_{resonant}$ by bandwidth with -3 dB. The values of Q factors of printed Ag coils were calculated based on experimental data. Theoretically, the Q factor can also be calculated through the equation.

$$Q = \frac{1}{R} \sqrt{\frac{L}{C}}$$

Where L is the inductance calculated by equation (2), R is the total resistance of the coils, and C is the capacitance of the circuits. R was measured through an ohmmeter whose value is $88 \, \Omega$. Based on the measured R and $f_{resonant}$, we can estimate the C and L values. Q factor in Figure 6.12 under 0% is 15.28. Therefore, the calculated C value is 6.5 pF and L value is 1.17 μH .

Measurements of AC and DC signals: The AC signals were sent out using the arbitrary waveform generator (33522A, Keysight Technologies) and measured using the digital phosphor oscilloscope (DPO 5054B, Tektronix).

3.4.4 Hybrid integration in mechanically heterogeneous silk fibroin films

Fabrication and characterization of flexible pressure sensors: The resistive pressure sensors are based on pyramidal PDMS elastomers with AgNWs as the active layer. The fabrication process of silica mold can be referred to Zhu et. al.^[10] The monomer and the crosslinker of PDMS (Sylgard 184) were mixed at a ratio of 10:1, stirred uniformly and degassed by a centrifuge process. The density of AgNWs (Sigma-Aldrich Pte Ltd) is diluted into 0.1 g/mL. After that, the solution of AgNWs were dried out under ambient environment on a pyramidal silica mold, following a spin coating of prepared PDMS precursor at 1000 rpm. The microstructured PDMS films with AgNWs embedded were cured under 60 °C for 4 hours. After the microstructured AgNWs/PDMS films fabrication, the films were laminated on interdigital electrodes Au/PET to measure the current-force and current-pressure curves by a semiconductor parameter (4200A-SCS, Tektronix) using a bias of 0.1V. The precise forces were provided by a motorized vertical test stand (Mark-10 ESM301) combined with a digital force gauge (Mark-10 M5-2).

Assembly of rigid active components on mechanically heterogeneous silk fibroin films: The as-printed Ag circuits on mechanically heterogeneous silk fibroin films were treated with methanol at the back of predesigned locations for rigid components. After the treatment, a small amount of Ag inks same as the printing ink were dipped at the interconnects. The rigid components including microresistors, rectifiers, RFID (ISO15693) and microcapacitors were mounted on top of the interconnects. After mounting, the circuits were annealed again at 60 °C for 20 minutes.

Mechanical tests of the hybrid system were done by a mechanical tester (C42, MTS Systems Corporation) and at the same time the electromagnetic couplings were measured

using vector network analyzer (TTR503A, Tektronix). The voltage at two ends of the connected resistor was measured by a digital phosphor oscilloscope (DPO 5054B, Tektronix). And the RFID was identified through a commercial RFID reader (ISO15693) using a software of eReader (Yihuoyan Technology Co., Ltd.).

On-skin measurements of pressure signals were realized by mounting the hybrid sensors on the wrist of volunteers. Related experiments on human skin were approved by Institute of Review Board at Nanyang Technological University (approval number: IRB-2017-08-035).

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Chapter 4

Plasticizing Silk Protein for On-skin Stretchable Electrodes

Here, by modifying the mechanical properties of silk and combining with wrinkled metals film, this work has realized a stretchable and biodegradable electrode. The modulus of silk was changed from 700 MPa to 200 kPa, which is as soft as our skins, and shows a high stretchability of 400%. This is realized by the introduction of Ca^{2+} ions and ambient moisture, which is consistent with the Molecular Dynamics simulation. The tunable mechanical properties by moisture renders it ease of operation and sufficient softness and stretchability to form seamless contact with soft human skin. Furthermore, stretchable electrodes were fabricated by thermal evaporation of gold on the stretchable silk. After the metallization, atmospheric moisture induces wrinkled structures in the gold film on silk. As a result, remarkably small resistance change ($R/R_0 \sim 2.45$) was obtained by a strain of 40%, while gold on silicone substrate shows a high resistance change of over 20 times at 20% strain. Finally, high feasibility of the silk/gold conductors are demonstrated in electromyography (EMG) signal recording, where the electrodes need to realize conformal contact and low impedance. Based on these, a reliable silk-based bioelectronics application is achieved.

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4.1 Introduction

The emergence of stretchable electronic devices is particularly suitable for the application of wearable and implantable devices. Unlike conventional bulky electronic systems, soft and stretchable electronic devices have similar mechanical properties with human organs including brain or skin. Stretchable on-skin electronic devices can realize conformable contacts to our arbitrarily shaped and moving skin. For example, on-skin healthcare sensors for pressure, temperature, and electrophysiology based on soft and stretchable materials have been demonstrated with reduced discomfort of wearing and improved signal integrity. Biomaterials are unique options of materials for such soft electronics, which include silk, cellulose, chitin and lignin. They can provide nature biocompatibility, degradability, and sustainability. Especially, silk has been proved to have the versatility into desired formats, such as nanofibers, sponges and thin films with high flexibility, robustness, and optical refractive index. These attractive features have been fully utilized in a field of electronics, optics, and biology. In terms of electronics, silk has been employed as the flexible substrates of transient electronics, sacrificial layers for ultra-flexible electronics, dielectrics for transistors, and active layer for memristors.^[1]

Beyond these flexible yet non-stretchable electronics, realization of soft and stretchable biomaterial electronics is important for on-skin wearable electronics. Stretchable conducting fibers have been realized by coating spider silk fibers with carbon nanotube and gold.^[2] The large resistance change against strain was utilized for strain sensor applications. However, the application was limited by the fiber shape, low conductivity, and mechanical mismatch with human skin.

Silk fibroin has been reported to have a very high Young's modulus and low elastic stretchability, as mentioned in Table 3.1. Research on silk fibroin has proved its uniqueness in mechanical property, electronic property, structural adjustability and good biocompatibility. A typical process to purify silk fibroin is as followed: after degumming, dissolution in LiBr solution and dialysis, a fibroin water-based solution can be obtained, so called the regenerated silk fibroin (RSF) solution. Based on this, various formats of silk

fibroin can be realized including films, gels, electrospun fiber mats and porous scaffold, showing various mechanical characteristics based on silk morphologies (Table 4.1).^[3]

Table 4.1 Mechanical properties of silk with various morphologies.

Materials formats	Young's modulus (GPa)	Strength (MPa)	Extensibility (%)	Pore size (μm)/porosity (%)
Films (as cast)	3.9	47.2	1.9	-
Films (methanol-treated)	3.5	58.8	2.1	-
Films (water-annealed)	1.9	67.7	5.5	-
Films (ultrathin)	6-8	100	0.5-3	-
Hydrogels (8% w/v)	-	0.0252	-	-
Electrospun mat (3%)	-	15	40	-
Electrospun mat (15%)	0.515	7.25	3.2	-
Methanol-treated sponges (concentration 6 wt%)	0.00043	0.02	-	151 ± 40 (87.6%)
HFIP-derived sponges (concentration 17 w/v%)	0.00045-0.0001	0.175-0.25	-	15-202
Aqueous-derived sponges (concentration 4 w/v%)	0.00007	0.011	-	940 ± 50 (95%)

In order to achieve a soft and stretchable electronics based on silk protein, a reliable method to obtain soft and stretchable protein film is necessary. Blending with soft materials to obtain soft composite films have been reported to be effective, such as polyacrylamide, sodium alginate,^[4] cellulose, chitosan, poly (vinyl alcohol), poly(ϵ -caprolactone-co-d,l-lactide,^[5] etc. Solution of regenerated silk fibroin (RSF) can be easily modified getting desired chemical reactions with specific side groups in silk fibroin or mixed well to form uniform composites. For example, blending RSF with glycerol can achieve a more flexible silk-based films that the composite silk/50% glycerol has an improved strain to failure of 130% and a reduced stiffness of 0.023 GPa.^[6] Therefore, a proper selection of plasticizer for silk fibroin is extremely important to achieve a soft and stretchable silk fibroin film. Especially, the films should be able to stabilize the mechanical property under ambient environment.

Among the soft materials, hydrogels which contain a lot water have shown extraordinary advantages with crosslinking network tuning the mechanical property, which is promising for applications such as drug delivery for cells and tissues. A method to covalently crosslink the tyrosine residue of silk fibroin via horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) was reported to obtain an elastomeric, fully degradable and biocompatible silk fibroin-based hydrogel (Figure 4.1).^[7] This hydrogel showed a stiffness from 200 to 10000 Pa and a compressive strain >70%, showing great mechanical compliance with biological soft tissues which is significant for extracellular matrix application. Aqueous processing offers possibility for desired functionalization, which makes silk-based hydrogels promising for a lot of applications in regenerative medicine.

Figure 4.1 Silk-based elastomeric hydrogels. a) Schematic illustration of the crosslinking between the tyrosine residue under HRP and H_2O_2 . b) Circular dichroism spectra of silk gel and silk solution. c) The optical images of silk-based elastomeric hydrogels under compressive strains. Reprinted with permission from reference.^[7]

However, hydrogels have the issue of dehydration under ambient environment and they are also difficult to incorporate with active materials due to pore interfacial bonding and embodiment of water. A method to print stretchable silk fibroin was reported by mixing $CaCl_2$ and silk fibroin to obtain stretchable silk ink. This kind of silk fibroin film has shown remarkable stretchability.^[8] Therefore, how this addition of $CaCl_2$ would influence the mechanical properties of silk fibroin and how to integrate with soft and stretchable electronics is our target to investigate in this chapter.

Figure 4.2 Printing of stretchable silk fibroin for strain gauge. Reprinted with permission from reference.^[8]

To achieve soft and stretchable silk electronics, there have been three issues to be solved. First, the mechanical stretchability and softness of silk protein should be lower than skin whose Young's Modulus is around 1 MPa and the maximum stretchability is at the range of 60% -75%.^[9] Soft silk-based hydrogels can be considered as promising candidate, but they need to be prevented from drying.^[10] Thus, the second issue is to control the water content inside silk so that it can remain stable and soft under ambient environment. Thirdly, the processes to integrate highly conductive materials with high stretchability needs to be established.^[11]

Here we show high-conductivity, soft, stretchable silk-electrodes for on-skin application, enabled by controlled plasticization of silk by ambient humidity, and spontaneous wrinkle formation of deposited metal films. The plasticization was obtained through adding Ca^{2+} ions to render the moisture into silk fibroin film from ambient environment. The Young's Modulus, and stretchability were tuned from 700 MPa to 0.4 MPa, and from <10% to >300%, respectively. This plasticization was further understood by the Molecular Dynamics Simulation to analyze the crystallite strength and secondary structure ratio inside

silk protein. Moreover, highly stretchable ($>120\%$) electrode was realized on this plasticized silk via simply depositing a gold layer. The relative resistance change (R/R_0) is as small as ~ 2.45 at 40% strain while gold on silicone substrate shows a high resistance change of over 20 times at 20% strain. Such high performance was obtained by the wrinkles' formation during the plasticization process by ambient humidity. Furthermore, the high conformability to skin microstructures was confirmed by scanning electron microscopy (SEM). Finally, the high feasibility of our electrodes for on-skin bioelectronics was demonstrated in electromyography (EMG) signal recording comparable with commercial gel electrodes.

4.2 Results and discussion

4.2.1 Plasticization of silk fibroin by CaCl_2

The original hard and tough silk fibers into the soft and stretchable films was plasticized by introducing CaCl_2 to silk. Silk fibroin was obtained by degumming *Bombyx mori* silkworm silk and dissolved into formic acid with certain CaCl_2 weight ratio, with experimental details in Experimental Methods.^[8] By introducing up to 30 wt% of CaCl_2 , Young's modulus of silk protein dropped from 10 GPa^[12] to 1.66 MPa under relative humidity of 50% (Figure 4.3a). Increasing the weight ratio of CaCl_2 , the water content increased from 6% into 15% under relative humidity of 50%. To further clarify the role of CaCl_2 and water in this plasticization process, the relative humidity was controlled while fixing the CaCl_2 concentration at 15%. By incubating the silk protein at various relative humidity from 43% to 85%, the Young's modulus further decreased from 233.67 MPa to 0.149 MPa (Figure 4.3b). It's clearly observed that there were increase of water content from 12% to 31% when exposed to various relative humidity from 43% to 85%. And the microscopic images of the fabricated silk films with 15 wt% of CaCl_2 are shown in Figure 3c and d. Under same CaCl_2 concentration, higher water content is in accordance with the lower Young's modulus. In this way, mechanical property of silk can be tuned by introducing proper amount of CaCl_2 depending on the relative humidity.

Figure 4. 3 Young's modulus and water contents of silk fibroin films with various weight ratios of CaCl₂ under 50% relative humidity. b) Young's modulus and water contents of silk fibroin films with 15 wt% CaCl₂ under various relative humidity. Scanning electron microscopic images of silk film 15 wt% CaCl₂ at magnification of c) 3k and d) 40k.

In our lab in Singapore, the relative humidity is at the range of 60% - 70%. The stretchability improved from 6-10% to >400%, which is higher than those of human skin (Figure 4.4). Therefore, we performed the following experiments using silk protein with the concentration of 15% CaCl₂, resulting in the Young's Modulus of 0.4 ~ 2 MPa, which is comparable with that of human skin.

Figure 4.4 Mechanical stress-strain curve of silk film with 15 wt% CaCl_2 in lab whose relative humidity is 68%, showing a stretchability more than 400%.

4.2.2 Molecular dynamics simulation results

To better understand the mechanism of this plasticization process in silk, molecular dynamics simulations were performed. The mechanical characteristics of silk are predominantly determined by highly crystalline domain (crystallites), and amorphous domain as described in Figure 4.5.^[13] Therefore, simulations on the representative crystallites (Figure 4.6 & 4.7), and amorphous domain (Figure 4.9) were performed. Methods for constructing the models and simulations can be found in experimental methods (Chapter 3.2.4).

Figure 4.5 Schematic illustration of semi-amorphous structures of silk.

The weakened mechanical strength of crystallites by water was successfully analyzed through the investigation on hydrogen bond and interchain energy. Crystallites in silk consist of closely packed hexapeptides of Glycine (G), Alanine (A), Tyrosine (Y), and Serine (S). Typical patterns of hexapeptides inside silk include GAGAGS, GAGAGA and GAGYGA.^[14, 15] Therefore, crystallites models for these three patterns were built up in vacuum and water in order to analyze the hydrogen bonds and interchain energy differences. In this study, only two extreme conditions (in vacuum (fully de-hydrated), and in water (fully hydrated)) were investigated because these two models have shown the reliability in simulation.^[13, 16, 17] The hydrogen bonds in the outmost layer of GAGAGA crystallite at equilibrium in vacuum (Figure 4.6a) and water (Figure 4.6b) are shown. In water, the hydrogen bond number is 6 /layer while it's 10 /layer in vacuum.

Crystallite domain

Figure 4.6 a,b) Representative snapshots of GAGAGA crystallites in vacuum, and water, respectively.

Figure 4. 7 a,b) Representative snapshots of GAGYGA crystallites in vacuum, and water,

respectively. c,d) Representative snapshots of GAGAGS crystallites in vacuum, and water, respectively.

Similar decrease in hydrogen bond was also obtained in other structures (GAGAGS, GAGYGA) (Figure 4.7). Furthermore, the interchain energy of three crystallites increased in water compared with in vacuum, while higher interchain energy means less energy is necessary to break the bond (Figure 4.8). The interchain energy of one representative GAGAGA crystallite in water is -176.18 kJ/mol, which shows an increase compared with in vacuum of -180.6 kJ/mol. This change is comparable to the previous simulation results explaining the effect of water in mechanical properties of silk.^[17, 18] Crystallite domain serves as the building block of the silk fibroin, thus the small increase in the interchain energy can strongly weaken the mechanical properties of the whole molecules.^[13]

Figure 4.8 The interchain energy of three typical crystallite structures (GAGAGS, GAGAGA, GAGYGA) in vacuum and water.

The statistical data of hydrogen bonds and interchain energy change in all crystallites can be found in the Table 4.2. These simulations on the crystallites show that the addition of water decreases the crystallites strength, which is consistent with the experimental data showing decreased Young's Modulus. In addition, the effect of water with Ca^{2+} ions was also investigated, and their influence on crystallite was negligibly small compared to water without Ca^{2+} ions (Table 4.3). This simulation reveals that water plays a dominant role in the plasticization process of crystallites.

Table 4.2. Statistical hydrogen bond number of whole crystallites. (The data were collected during the last 50 ns after equilibrium)

Crystallite	In vacuum	In water
GAGAGA	83.52 ± 1.4	73.93 ± 2.7
GAGYGA	83.59 ± 1.4	78.05 ± 3.0
GAGAGS	86.32 ± 1.4	71.91 ± 2.7

Table 4.3. Statistical hydrogen bond number between the two chains on the outmost layer of each crystallites in water with and without Ca^{2+} ions. (The data were collected during the last 50 ns after equilibrium)

Crystallite	In water without Ca^{2+} ions	In water with Ca^{2+} ions
GAGAGA	6.10 ± 0.9	6.20 ± 0.8
GAGYGA	6.21 ± 0.8	6.16 ± 0.8
GAGAGS	6.16 ± 0.8	6.21 ± 0.8

Furthermore, simulations in the amorphous domain explain that water and Ca^{2+} ions introduce the stretchability by influencing the secondary structures.^[19] Secondary structures are dimensional structures of protein, including beta-sheets, random coils, helices and turns, etc. While beta-sheets have relatively strong mechanical properties and are not very extensible, the other secondary structures, such as helices and random coils, possess higher extensibility.^[3] The simulation is performed to reveal which secondary structures are dominant in vacuum and in water with Ca^{2+} ions. In water, the simulation without Ca^{2+} is difficult because they are necessary for balancing the charges in the system. Figure 4.9 a, b show the representative snapshots of the silk protein in vacuum and in water with Ca^{2+} ions, respectively. In water with Ca^{2+} ions, there are less stiff beta-sheets and more extensible secondary structures than in vacuum.

Figure 4.9 a,b) Representative snapshots of amorphous domain in vacuum, and water with Ca^{2+} ions, respectively. In each of the environments, the silk forms different secondary structures (β -sheets, helices, random coils, and etc.), determining the extensibility of the molecules. c) Secondary structure ratio in the amorphous domain in vacuum and water with Ca^{2+} ions.

Figure 4.10 Time evolution of secondary structures (Timeline analysis) of 12 peptide chains in amorphous domain in vacuum (a), and in water with Ca^{2+} ions (b). Each of the chains contains 43 residues.

This trend was supported by the dynamic analysis of structure during the simulation (timeline analysis, Figure 4.10). Figure 4.9c shows the quantitative secondary structure ratio in vacuum and in water with Ca^{2+} ions. Ratio of beta-sheets in vacuum, and in water

with Ca^{2+} ions were 24%, and 16%, respectively. On the other hand, the content of random coils in vacuum, and in water with Ca^{2+} ions were 44.67%, and 68%, respectively. Additional information about the root-mean-square-deviation (RMSD), radius of gyrate, statistical hydrogen number are listed (Figure 4.11 and Table 4.4) demonstrating the stability of the protein structure.

Figure 4.11 Structural stability of the amorphous domain. a) Radius of gyration of amorphous domain in vacuum and in water with Ca^{2+} ions. The stable values after 50 ns showed the structural equilibration. b) Root-mean-square-deviation (RMSD) of amorphous domain of silk protein in vacuum and in water with Ca^{2+} ions. The plateaus of RMSD which were less than 1 nm showed the protein structures were at equilibrium.

Table 4.4 Statistical hydrogen bond number of amorphous domains in vacuum and in water with Ca^{2+} ions at equilibrium. (The data were collected during the last 50 ns after equilibrium)

	In vacuum	In water with Ca^{2+} ions
Hydrogen bond number	452.53 ± 9.1	251.86 ± 10.4

The simulation results on amorphous domain explain water and CaCl_2 introduce extensible structures in silk to improve stretchability. This is consistent with the Fourier-transformed infrared spectroscopy (FTIR) of plasticized silk films, which shows less β -sheets than pure silk films (Figure 4.12). Based on the experimental and simulation results on the mechanical properties and underlying explanation inside silk protein, we have achieved the

plasticization of original tough silk protein. This fulfils the materials requirement for the stretchable on-skin electronic devices.

Figure 4.12 a) The FTIR absorbance spectra of pure silk film, silk film with 15 wt% CaCl_2 , 20% CaCl_2 and 30% CaCl_2 at the range from 4000 cm^{-1} to 1000 cm^{-1} . b) The FTIR absorbance spectra of pure silk film, silk film with 15 wt% CaCl_2 , 20% CaCl_2 and 30% CaCl_2 at the range from 1800 cm^{-1} to 1000 cm^{-1} .

This work demonstrates that silk with CaCl_2 can possess skin-like mechanical properties in ambient, although previous works demonstrate very soft silk gels incorporating much water and CaCl_2 ,^[8] or stiff film by removing CaCl_2 ,^[20] as summarized in Table 4.5.^[21-24] Furthermore, this work explains the plasticization mechanism by molecular dynamics simulation both in crystallite and amorphous domain, while previous simulation studies concentrate on how to increase the crystallite strength and there are few discussions on ions influence on silk protein from simulation. Our study shows that water influences the crystallite strength, while ion does not. More importantly, the significant effect of ion together with water on amorphous domain was demonstrated, while further studies on ions interaction with chain can be explored.^[25] Future development of more powerful simulation tools would bridge these correlations between microscale molecular dynamics simulation and macroscale experimental results, which may realize principle guidance on experimental designs. In addition, experimental studies have demonstrated the effect of plasticization can be observed by T_g shift after post-removal of CaCl_2 . These indicate CaCl_2 and water within silk would have significant influence on the mechanical properties.^[26, 27]

Table 4.5 Comparison of Young's modulus of silk film from various methods.

Methods	Young's modulus	Ref
Raw silk fibers	5~12 GPa	22
Ultrathin film	6~8 GPa	23
As cast silk fibroin film	3.9 GPa	24
CaCl ₂ -FA dissolved silk, CaCl ₂ removed (Wet)	206 MPa	20
Water annealing film	22.0 MPa	21
Methanol-treated	18.7 MPa	21
CaCl ₂ -FA dissolved silk, with CaCl ₂	0.102 MPa (together with pig skin)	8
CaCl ₂ -FA dissolved silk, with CaCl ₂ (Under ambient)	0.15 ~ 412.67 MPa	This work

4.2.3 Electrical properties under strain

The issue to integrate stretchable electronic devices on the plasticized silk was solved by the simple vacuum deposition of metals, and the ambient hydration, realizing 100% stretchable conductors.

Figure 4.13 A scheme of on-skin stretchable electrodes based on plasticized silk protein.

A 40 nm Au on stretchable silk showed a low initial sheet resistance of 7 Ohm/sq., and relative resistance (R/R_0) of 2.45 at 40% strain, while control Au on PDMS showed 20 Ohm/sq., and 31, respectively (Figure 4.14a). The lower initial sheet resistance is due to

the uniform Au film formation on silk instead of microcracked Au on PDMS.^[28, 29] Furthermore, lower sheet resistance was obtained by increased thickness of Au (80 and 150 nm (Figure 4.15)) while maintaining the high stretchability of 100%. It's noteworthy that, in the microcracks based stretchable conductors such as Au on PDMS, increased Au thickness degrades the stretchability.^[30] Furthermore, the high electrical performance was maintained in the cyclic strains (100 cycles of 20% strain). The cycling durability was measured by laminating silk-based electrodes on elastomer substrates in order to support the elastic recovery and mimic the situation in on-skin usage. During the cycles, the resistance varied from 10 Ω to 20 Ω , which is smaller than that of Au on PDMS (Figure 4.14b).

Figure 4.14 Electrical resistance change under strain of the plasticized silk electrodes. a) Resistance-strain characteristics of Au (40 and 80 nm) on plasticized silk, and Au (40 nm) on PDMS. b) Resistance change of silk electrodes and PDMS electrodes under cyclic 20% strain.

Figure 4.15 Resistance-strain characteristic of 150 nm Au on plasticized silk.

Besides Au, other stable metals including Ag and Pt become also stretchable on our plasticized silk (Figure 3c). When Pt was sputtered on stretchable silk and PDMS, a stretchability of 60% and 30% were obtained, respectively. Similarly, Ag on stretchable silk has achieved a high stretchability of 50%, while the stretchability of Ag on PDMS is less than 10%. The hydrated phenomena can lead to metal oxidation so that only noble metals were adopted to form the conductive layers.

Figure 4.16 Resistance-strain characteristics of Ag and Pt on plasticized silk and PDMS.

Optical microscope images of silk electrodes showed these stretchability is originated from the wrinkle formation of metal films by the ambient hydration of stretchable silk substrates

(Figure 4.17). As-deposited electrodes showed a relatively flat surfaces (Figure 4.17a), yet, after the plasticization via ambient humidity, the wrinkles of Au were formed (Figure 4.17b). This hydration-induced wrinkle formation has been reported in different material systems such as hydrogels,^[31] but this is the first report for realizing stretchable conductors by substrates hydration. Until a strain of 40%, existed wrinkles were extended, and microcracks started being formed (Figure 4.17c). This observation has a nice correlation with the small resistance change shown in Figure 3a. When the strain was 60%, no wrinkle was observed, and Au on silk started to form microcrack networks (Figure 4.17d). Further increase of strain increased the length and width of the cracks (Figure 4.17e), resulting in larger resistance change than wrinkle unfolding. It is noteworthy that the relationships between electrical performance and surface structures have a nice agreement with previous reports on wrinkles-based stretchable conductors.^[32] And the wrinkling phenomenon of the gold films have been observed with various thickness up to 150 nm. They showed a stable electrical performance until wrinkles are unfolded and large resistance change when cracks are formed.

Figure 4.17 a, b) Wrinkles formation of deposited Au on plasticized silk under ambient environment. Wrinkles unfolding process under strain (b-c) and microcracks formation under strain (d-e).

This wrinkle-assisted stretchability was also confirmed in Ag and Pt on silk substrates under ambient environment as shown in Figure 4.18. Utilizing the wrinkle formation, our stretchable silk realizes a simple fabrication of stretchable conductors with high conductivity and stretchability.

Figure 4.18 Optical images of as-deposited Ag (a), Pt (b) on plasticized silk and Ag (c), Pt (d) after ambient hydration.

4.2.4 On-skin electrodes for electromyography recordings

The soft and stretchable silk electrodes enabled on-skin electronics with high conformability. Figure 4.19 shows that our electrodes were conformably laminated on skin while showing the high conductivity to operate a light emitting diode (LED) at a low voltage of 2.7 V.

Figure 4.19 On-skin electrodes for light emitting diodes based on plasticized silk protein.

Our silk electrodes maintain the skin-like softness as the silk without Au layer (Figure 4.20). Moreover, the electrodes can be easy to deal with because they are rather stiff when it's kept under relatively dry environment. By moisturizing skin prior to the lamination, the electrodes can be immediately plasticized on skin, realizing conformal contacts to the skin. The high conformability was confirmed by cross-sectional scanning electron microscope (SEM) images of the silk electrodes laminated on skin replica (Figure 4.21b). No gap was observed between silk electrodes and skin microstructures. Furthermore, even with a smaller microstructured surface, our electrodes could maintain the conformability (Figure 4.21a). The adhesion strength with human skin was quantified by the 90° peeling tests. The adhesion strength was as high as ~ 80 N/m, although that of commercial adhesive tape (RS polyimide tape) was ~ 50 N/m (Figure 4.22). Furthermore, the adhesion of Au and silk is strong that a quantification process can't peel Au off the plasticized silk substrates rather than break the silk substrates (Figure 4.23), which indicates the good adhesion and anti-abscission of the active layer of our plasticized silk electrodes.

Figure 4.20 Mechanical tensile curve of pure plasticized silk protein (w/o Au) and silk electrodes (w/ 80 nm Au) under relative humidity of 60%, showing the comparable softness.

Figure 4.21 a, b) Cross-sectional SEM image of the plasticized silk electrodes attached on skin replica, and microstructured substrates, respectively.

Figure 4.22 90° peeling test of a plasticized silk electrode and polyimide tape from skin, showing their adhesion strength with skin.

Figure 4.23 a) The setup to quantify the adhesion strength between gold and our plasticized silk fibroin; b) An image showing the gold can't be peeled off from the plasticized silk fibroin.

Finally, the advantage of our stretchable silk electrodes with biocompatibility and skin adhesiveness is demonstrated by the on-skin electrophysiological signals recording. Figure 4.25b shows the on-skin recording of electromyography (EMG) obtained by our silk electrodes on forearm skin. Our electrodes exhibited as low interfacial impedance with skin as commercial gel electrodes, which is crucial for the signal integrity (Figure 4.24). This

result can be explained by the above-discussed high conformability. The importance of conformal contact to skin was confirmed by attaching the Au side of silk electrodes to the skin, increasing the interfacial impedance in one order of magnitude. This result can be explained by the above-discussed high conformability to skins. In addition, the plasticized silk substrates contain water and Ca^{2+} ions which contribute to ionic conductivity between Au and skin. In this way, our plasticized silk serves the same function as the hydrogels in commercial EMG electrodes. Previous studies on long-term on-skin electrodes show higher interfacial impedance than commercial gel electrodes because they are normally dry.^[33-35] Low impedance was achieved by the use of ionic liquid,^[36] while our silk electrodes can provide low impedance by utilizing moisture in skin or ambient. The detected EMG signals, compared to the signals collected by commercial gel electrodes, achieved comparable amplitudes (Figure 4.25). The signal-to-noise ratios obtained by silk electrodes, and commercial gel electrodes were 17.17 dB, and 17.95 dB, respectively, which was calculated by the open source code to calculate EMG signal-to-noise ratio in Supplementary information. Furthermore, the accuracy of signals obtained by our silk electrodes was confirmed by comparable amplitude-frequency spectrum, and energy spectrum analysis (Figure 4.26). In addition, the silk electrodes maintained good interfacial impedance with skin even after 4 hours of wearing (Figure 4.27) and led to no remarkable skin irritation while commercial gel electrodes caused discomfort among 5 volunteers tested (Figure 4.28).

Figure 4.24 An optical image of our plasticized silk electrodes on skin for electromyography signals recording.

Figure 4.25 a) On-skin interfacial impedance of the plasticized silk electrodes in comparison with commercial gel electrodes. The results were obtained when the silk sides were laminated (Skin/Silk/Au) and Au side were laminated (Skin/Au/Silk). b) EMG signals obtained by the plasticized silk electrodes and commercial gel electrodes. Two-direction arrows describe when motions happened.

Figure 4.26 Comparison between the EMG signals from our plasticized silk electrodes and commercial gel electrodes. a) Signal-to-noise ratio (SNR) of the EMG signal detected by plasticized silk electrodes and commercial gel electrodes. b) Amplitude spectrum of the EMG signals recorded by plasticized silk electrodes and commercial gel electrodes. c,d) Energy spectrum analysis of the EMG signals recorded by plasticized silk electrodes (c), and commercial gel electrodes (d).

Figure 4.27 On-skin interfacial impedance of plasticized silk electrodes after 4-hour wearing in comparison with initial interfacial impedance.

Figure 4.28 a) Skin inflammation was observed after wearing commercial gel electrodes for 4 hours. b) No skin inflammation was observed after wearing our plasticized silk electrodes for 4 hours.

4.3 Conclusion

In conclusion, we have demonstrated the plasticization of silk protein for highly stretchable substrates in on-skin electronics. The plasticization of silk into skin-like softness was achieved by the addition of Ca^{2+} ions and the subsequent hydration via ambient humidity. The mechanisms of the plasticization were analyzed by molecular dynamics simulation, which are weakened strength of crystallite and more extensible secondary structures of amorphous domain. Furthermore, metal thin films on the silk form wrinkled structures during its plasticization by ambient humidity, resulting in highly conductive and stretchable electrodes. These features were fully utilized in on-skin electrophysiological electrodes. The softness of the silk substrates provides the conformal contact with skin, which enables low interfacial impedance with skin, and high-quality on-skin EMG signals recording. The plasticized silk will open up more progress in biomaterials-based wearable and implantable electronics with green features. For example, the silk electrodes will accelerate future research on the integration of other active elements like sensors and transistors. We anticipate that the incorporation of biomaterials into wearable and implantable electronics will advance the harmonized integration between electronics and human.

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Chapter 5

Glycerol Plasticized Silk Fiber Mats for Highly Conformal, Highly Gas-permeable and Lowly Evaporative-Resistant Sensors under Sweaty Conditions

Electrodes bridged the skin and machine are crucial for signal acquisition of wearable electronic devices. However, the development of skin-friendly electrodes is still challenging due to the high sensitivity of skin and gas-impermeability of electrode materials. Here, we demonstrated a conformal, highly breathable, metal-free, and stretchable electrode directly attachable to human skin, which is extremely suitable for sweaty exercise use. This is realized by incorporating glycerol plasticized porous silk protein with conducting polymers poly (3,4-ethylenedioxythiophene): polystyrene sulfonate (PEDOT: PSS). The silk electrode is highly stretchable with good breathability (low thermal insulation, low evaporative resistance, and high water-vapor transmission rate) compared with commercial gel and polymer electrodes. In sweaty status after exercise, our silk electrodes show better electrocardiography (ECG) signals quality than commercial gel electrodes, and they did not disturb the heat dissipation during sweat evaporation.

5.1 Introduction

The development of wearable on-skin electronics has benefited the intimate, long-term health monitoring as they are as stretchable and soft as skin, which greatly reduce the discomfort during wearing.^[1-3] They are emerging as a trend in the medical diagnostics and continuous health monitoring such as body temperature monitoring, pulse detection and electrocardiography recording.^[4-6] However, these patchable devices (electrodes and sensors) on top of skin have always interfered the functions of skin which regulates the body temperature and fluid balance via sweat and breathe.^[7] Moreover, some may even cause chronic skin rash and skin damage.^[8, 9] Especially, for the disabled who have prosthetic limbs in long-term daily use or athletes whose electromyography signals need to be recorded by on-skin electrodes during training in sweaty conditions, the thermal-wet comfort of the electrodes is of great importance. Therefore, the design of electronic electrodes can not only be used for monitoring of the biological signals, but also minimally suppresses the functions of the skin is highly desirable.

To transcend these obstacles, gas permeable devices have raised a lot of attention as they are competent for the long-term physiological tracking without blocking the metabolism of skin. Some efforts have been made by developing such kind of breathable electrodes based on metal nanomesh, air permeable, or porous substrates (Figure 5.1).^[10-14] These electrodes truly possess high gas permeability, but to some extent, many of them suffered from either the limited stretchability, high interfacial impedance (in the case of metal), or reduced skin conformability (in the case of thick porous substrates). More importantly, besides the gas permeability, the indexes of breathability including thermal resistance and evaporative resistance are more crucial for electrodes eliminating the discomfort in sweaty conditions.

Figure 5.1 Gas-permeable, and stretchable on-skin electrodes based on porous structures. Reprinted with permission from reference. Scale bar, b) 1 mm; c) 5 μm.^[10]

Optionally, conducting polymers incorporated with stretchable, porous, biocompatible, and solution processable biomaterials may deal with this issue. Several biomaterials like cellulose, chitin, and lignin have attracted great attention for building biological *in vivo* and *in vitro* sensing devices.^[15-17] However, there are still no such biomaterials-based skin electrodes which can possess excellent skin conformability, high stretchability, good gas permeability, and low evaporative resistance (also known as breathability). The main challenges lie in the lack of combined capability of these materials with versatile processability, easy handleability, modulated stability, and tunable mechanical properties. Luckily, silk is an ideal candidate which can meet the requirements and has been extensively applied into research of biological application.^[18]

Here, a silk based breathable electrodes was reported by utilizing glycerol plasticized silk fiber mats and incorporating conducting polymers PEDOT: PSS. The doped glycerol in PEDOT: PSS aqueous solution have significantly increased the conductivity of silk electrodes up to ~24 S/cm, and the stretchability as high as ~250%. The high stretchability of silk electrodes was originated from the hydration of glycerol and the random mesh

structures of the fiber mats. In addition, the silk electrodes possess a very low evaporative resistance ($\sim 23 \text{ Pa} \cdot \text{m}^2 \cdot \text{W}^{-1}$) compared with commercial gel and SEBS polymer electrodes ($> 300 \text{ Pa} \cdot \text{m}^2 \cdot \text{W}^{-1}$). And the water vapor transmission rates are also higher than transepidermal water loss (TEWL) of the skin, which means the silk electrodes will not block sweat evaporation in normal and exercise use. Electrocardiography signals were not disrupted by sweat and heat dissipation during exercise. The proposed silk electrodes demonstrated good physiological comfortability and will undoubtedly advance the development of wearable skin-friendly electronics.

5.2 Results and discussion

5.2.1 Fabrication and glycerol tuned properties of the silk electrodes

The composite gas-permeable, metal-free, and stretchable electrodes based on silk protein were prepared by electrospinning of silk and subsequent dip-coating of PEDOT: PSS which contained glycerol as additive, as shown in Figure 5.2. The experimental details can be found in Methods part.

Figure 5.2 Fabrication process of stretchable and gas-permeable electrodes based on silk fiber mats.

Here, glycerol plays the roles of plasticizing the silk fiber mats for stretchability as well as enhancing the conductivity of PEDOT: PSS. It is noted that silk fibroin is the mixtures of β -sheet nanocrystals (crystalline region) and noncrystalline regions (amorphous domain) which are thought to determine the stability and mechanical properties of silk (Figure 5.6). After glycerol stabilization, the characteristic amide I absorption band in Fourier Transform Infrared Spectroscopy (FTIR) spectra shifted from $\sim 1648 \text{ cm}^{-1}$ to $\sim 1625 \text{ cm}^{-1}$, indicating that the random coils dominated silk conformation had converted to the stable β -sheet structures (Figure 5.3).

Figure 5.3 FTIR spectra of as-spun silk, glycerol-stabilized silk (labeled as G-silk), and glycerol stabilized silk coated with PEDOT: PSS which contains 15 vol% glycerol (labeled as Silk-P-15G).

This stabilization process renders the silk fiber mats with structural stability and solution processability,^[19, 20] which was stable without structural damage even after lateral dip-coating process of PEDOT: PSS (with glycerol) and thermal anneal treatment (Figure 5.4).

Figure 5.4 SEM images of (a) as spun silk fiber mat, (b) glycerol stabilized silk fiber mat, and (c) PEDOT: PSS (with 15 wt% glycerol) coated silk fiber mat.

Besides, there are strong absorption bands increasing in the range of 3600 - 3000 cm^{-1} (free and bound -NH and -OH groups) as glycerol concentration increased in the PEDOT: PSS aqueous solution (Figure 5.4), which means the increased amounts of residual glycerol and water in the silk electrodes.

Figure 5.5 FTIR spectra of silk electrodes with different glycerol contents, and the spectra of Triton X-100, PEDOT: PSS, and glycerol.

The glycerol added in the coating solution significantly modified the mechanical properties of silk electrodes. Glycerol could be hydrogen bonded to the peptide matrix and lead to less-organized silk II structures than commonly used methanol treated silk materials. Such more-random β -sheet structures leave exposed reactive sites available for water or other small polar molecules to re-interact via hydrogen bonding. The amorphous domain plays a key role in the elasticity of silk. Molecular dynamics simulation on the amorphous domain show that glycerol can bind with silk peptide, which plays a role to tune structural and mechanical properties of silk. The details of the model and simulation process of this molecular dynamics simulation are illustrated in Methods part. There are plenty of hydrophilic groups, like $-\text{COOH}$, $-\text{NH}_2$, and $-\text{OH}$ in silk fibroin, making it hygroscopic with strong affinity to water. The glycerol-absorbed water molecules can disrupt the hydrogen bonds in the amorphous phase and enhance the chains mobility, weakening chain-chain interaction, and thus reducing the Young's modulus.^[21-23] Therefore,

simulation results show an increased ratio of extensible secondary structures (random coils and turns) in the amorphous domain of silk (Figure 5.5).

Figure 5.6 Molecular dynamics simulation showing the stabilization and plasticization mechanisms of silk with glycerol.

No matter for glycerol or methanol/ethanol treated silk fiber mesh, or even raw silk fibers, higher water content in silk fibroin significantly improves their stretchability with sometimes one order of magnitude (Figure 5.7), which is consistent with literatures.^[24-26]

Figure 5.7 (a) FTIR spectra of ethanol treated silk (E-silk), methanol treated silk (M-silk), and glycerol treated silk (G-silk). (b) Stretchability of E-silk, M-silk, and G-silk in dry and wet states, respectively.

Moreover, glycerol can absorb moisture from ambient environment and prevent silk electrode from dehydration (Figure 5.8).

Figure 5.8 Load rate of silk electrodes with different glycerin content after moisture balance in ambient environment for 12 h.

As a result, when the glycerol content in PEDOT: PSS aqueous solution was increased from 0 to 40 vol%, the stretchability of silk electrode in ambient conditions was dramatically increased from <10 % to up to ~340 %, while the Young's modulus was decreased from ~220 MPa to <3 MPa (Figure 5.9). Glycerol also could lead to a structural reorientation of PEDOT: PSS chains that promotes the coating stability and conductivity of the blend (Figure 5.9).^[27, 28] With glycerol addition, the conductivity was efficiently tuned from ~0.18 S/cm (0 vol% glycerol) to ~24 S/cm (15 vol% glycerol). However, higher glycerol content would make the silk electrode too slimy to handle, and the conductivity decreased as the reduced effective loading of PEDOT: PSS. Therefore, considering the conductivity, proper stretchability, and good handleability of the electrodes, 15 vol% of doping rate was adopted (labeled as Silk-P-15G) in the following experiments.

Figure 5.9 The influence of glycerol content in the PEDOT: PSS coating solution on the stretchability, Young's modulus, and conductivity of final silk electrodes.

5.2.2 Electrical performance of the silk electrodes

The conductivity of the silk electrode (Silk-P-15G) can be maintained up to ~250% strain upon stretching, and obtained a relative low resistance change of ~4 at 100 % strain (Figure 5.10a), which is much more than enough for on-skin electronic applications. The resistance changes of the electrodes remained stable after 100 strain cycles at 10%, 20%, and 30% strain (Figure 5.10b), showing high cyclic durability. No broken fibers and delamination were observed (Figure 5.11), exhibiting high mechanical robustness of the porous structures.

Figure 5.10 Electrical performance and morphology evolution under strain of the gas-permeable, stretchable silk-based electrodes. a) Resistance-strain characteristic of silk-based electrode silk-P-

15G and comparison with other commonly used electrodes. b) The resistance changes of silk electrodes silk-P-15G under cyclic 10%, 20%, and 30% strain.

Figure 5.11 SEM image of silk electrode (Silk-P-15G) under cyclic 30% strain for 100 times.

It is hypothesized that the high stretchability and low resistance change under strain are mainly originated from the porous structure and random arrangement of fibers in our silk electrodes. For electrodes without porous mesh structures prepared by spin coating PEDOT: PSS on elastic polymer substrates (such as PDMS and SEBS), the ultimate strain are only ~49% and ~48%, respectively. In addition, a Au mesh electrode by depositing Au on as-spun silk fiber mat (labeled as Silk-Au) was prepared and found that the ultimate strain was only ~24%, which is similar to the reported Au mesh electrode on electrospun PVA fiber mat.

Interestingly, the resistance of Silk-Au electrode dramatically increased up to hundreds of M Ω magnitude after glycerol plasticization due to the hydration-induced fiber expanding (Figure 5.12). These electrodes with non-infiltrated metal or PEDOT: PSS distributed only on the surface, which tended to form disconnected cracks during stretching.

Figure 5.12 a) Conductivity of silk fiber mat coated with Au. b) Conductivity of Au coated silk fiber after glycerol plasticization.

To demonstrate the superiority of the random fiber structures in stretchability of the electrodes, silk electrodes with high-degree fiber alignment (labeled as A-Silk-P-15G, as shown in Figure 5.13a) were tested in the bidirectional stretch conditions. Results showed that the resistance changed much faster and electrical performance no longer maintained at ~160%, and ~70% strain when being stretched along, and perpendicular to the fiber direction, respectively (Figure 5.10).

Figure 5.13 a) SEM image of silk electrode with aligned silk fibers and b) the corresponding FFT analysis.

Scanning Electron Microscope (SEM) images of our silk electrodes showed that during stretching, the fibers tended to slip and realign in the strain direction (Figure 5.14). With further stretch up to 200% strain, most of the fibers were realigned and stretched in the strain direction without obvious breaking down.

Figure 5.14 SEM images of silk electrodes under variable strain.

Quantitative analysis by Fast Fourier Transform (FFT) of the SEM images also confirmed that with higher strain, the fibers showed a higher degree of orientation in the direction of stretch (Figure 5.15). Morphological observations agree well with the electrical performance under strain, which further proves that both random mesh structures and infiltrated compliant conducting polymer contribute to the high stretchability of our silk fiber mats electrodes.

Figure 5.15 Corresponding FFT analysis by radial summation of the pixel intensities of Fast Fourier Transformed SEM images for each degree between 0° and 180° under strain.

5.2.3 Gas-permeability, thermal insulation and evaporative resistance of the electrodes in thermal-wet conditions

Besides the high stretchability, breathability including low thermal insulation, low evaporative resistance, and high water-vapor permeability of electrodes are particularly important for physiological comfort in daily use, especially during exercise. Human body

can exchange heat through several pathways like dry heat exchange (conduction, convection, and radiation) and evaporation, and among them sweating evaporation is an effective way to cool human body in a hot environment or during exercise. The thermal insulation of electrodes will decrease the dry heat transfer between local skin and external ambient environment. Sweat evaporation from skin serves as the primary way in which the human body cools itself and maintains thermal equilibrium in sweaty conditions. And accordingly, the resistance to evaporative heat transfer from skin to the environment can be determined by the evaporative resistance of electrodes. Thermal insulation and evaporative resistance are important indices to determinate thermal-wet comfort and have been widely used for evaluating the physiological comfort of textiles and clothing. Here, we evaluated these properties of our electrodes by referring international standards ISO 15831 and ASTM F2370 using a 34-zone sweating thermal manikin (Figure 5.16). The shape and heat capacity of the manikin are highly similar to those of human body. Therefore, the transient response of the manikin to the wearing textiles and clothing is very close to that of real human. It can effectively and quantitatively determine the thermal-wet comfort properties when wearing electrodes.

Figure 5.16 Optical photo and schematic illustration of the 34-segment thermal “Newton” manikin, the marked gray area (zone 15) is the used test segment.

Figure 5.17 shows that commercial gel electrode provided the highest thermal insulation value of $\sim 0.16 \text{ }^{\circ}\text{C}\cdot\text{m}^2\cdot\text{W}^{-1}$ because of the thick layer of encapsulation layer which is polyethylene terephthalate (PET). For silk electrode with same thickness as the polymer

film electrode, it exhibited a lower thermal insulation value owing to its porous structures, demonstrating the least negative effect on the dry heat exchange at the skin surface.

Figure 5.17 Thermal insulation of silk-based electrodes (Silk-P-15G) compared with commercial gel electrodes and Au electrode on SEBS polymer substrates.

With regarding to the evaporative resistance, our silk electrodes showed an overwhelming performance over the commercial gel and polymer film electrodes (Figure 5.18). Typically, the evaporative resistance values of commercial gel and SEBS polymer electrodes are all higher than $300 \text{ Pa} \cdot \text{m}^2 \cdot \text{W}^{-1}$ (Figure 5.18), which means almost no evaporation of sweat could occur when they were put on skin. However, the evaporation resistance is only $\sim 23 \text{ Pa} \cdot \text{m}^2 \cdot \text{W}^{-1}$ for our silk electrode, which is comparable to that of naked manikin skin ($\sim 11 \text{ Pa} \cdot \text{m}^2 \cdot \text{W}^{-1}$). This is because our silk electrode is highly hydrophilic (as illustrated by the small contact angle in Figure 5.19), and then the porous structures easily allow the transportation of sweat through the electrode mats and away from the skin. On the other hand, high water-vapor permeability is the other parameter to determine the comfort level of an electrode or electronic device on skin.^[29]

Figure 5.18 Evaporative resistance of silk-based electrodes (Silk-P-15G) compared with commercial gel electrodes and Au electrode on SEBS polymer substrates.

Figure 5.19 Contact angle of the silk electrode (Silk-P-15G).

To quantitatively demonstrate the excellent water-vapor permeability of our silk electrodes among all samples, we measured their water vapor transmission rates (WVTRs) at 20 °C and 37 °C as well, which mimic the comfortable and heavy sweating conditions of human bodies, respectively. Under both conditions, the WVTRs of our silk electrodes are even larger than the TEWL of the skin of adults under normal ($TEWL_{skin} = \sim 5-10 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$) and exercise state ($TEWL_{skin} = \sim 6-66 \text{ g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$),^[30, 31] exceeding those of commercial and SEBS polymer electrodes (Figure 5.20). This indicates that water-vapor loss and sweat evaporation by skin would not be blocked by our silk electrodes under calm and even

sweaty situation. Based on the above series of tests, the high gas-permeability, low thermal resistance and evaporative resistance of our silk-based electrodes are undoubtedly demonstrated.

Figure 5.20 WVTRs of silk electrodes under simulated comfortable (20 °C) and sweaty (37 °C) conditions compared with commercial hydrogel electrodes and Au electrode on SEBS. Marked areas are the ranges of TEWL for skin of adults in normal state and during exercise.

5.2.4 On-skin applications for electrophysiological signals recording

The highly gas-permeable, lowly evaporative resistant and stretchable silk electrodes with skin adhesiveness is demonstrated by on-skin electrophysiological signals recording especially doing exercise. The silk electrodes on forearm skin showed high skin conformability under compression and deformation (Figure 5.21), whose microscopic image shows no difference in microstructures formed from naked skin (Figure 5.22).

Figure 5.21 A photographic image silk electrode laminated on a human forearm at normal and wrinkling state (inset).

Figure 5.22 An optical microscope image showing the conformability of our silk electrodes to the irregular structure the human skin.

Furthermore, the silk electrodes exhibited low interfacial impedance with skin, which is comparable to commercial gel electrode and lower than Au mesh-based electrode (Figure 5.23). Also, for silk-based electrodes with various amount of glycerol, they show similar interfacial impedance with skin (Figure 5.24). The low interfacial impedance and high skin conformability are crucial for the signal integrity and sensitivity in electrophysiological measurement.

Figure 5.23 On-skin interfacial impedance of the silk electrodes in comparison with commercial gel electrodes and silk-Au electrodes.

Figure 5.24 On-skin impedance of all silk electrodes with different glycerol contents.

To assess the reliability in practical situations, ECG signals were collected under calm/resting state as well as sweaty state after running. Before exercise (resting state), the detected ECG signals have comparable amplitudes with the signals collected by commercial gel electrodes (Figure 5.25). The P-waves, QRS complexes, and T-waves are all could be explicitly recognized in both cases. However, after wearing the electrodes and running, there are some noise peaks arising in the ECG signals collected by commercial gel electrodes, while the curves acquired by silk electrodes remained clean and unaffected, showing a higher stability with less fluctuation by sweat. This is because the commercial electrodes are unbreathable, and a sweat film is formed between the skin and ionic hydrogel. This sweat film will disable the adhesion of the commercial electrode and thus decrease its conformability, reducing the signals fidelity. On the contrast, our silk electrodes are breathable, which allow the sweat to permeate and evaporate easily, and no detachment was observed on sweaty skin.

Figure 5.25 ECG signals recorded by silk-based electrodes and commercial electrodes before and after exercise.

Simultaneously, the interfacial impedance decreased after the silk electrode was wetted (Figure 5.26). Therefore, the signals were minimally impacted by sweat during exercise. Meanwhile, it should be noted that the silk electrodes were almost imperceptible on skin, which would not cause discomfort and distraction during real-time healthcare monitoring or training for athletes.

Figure 5.26 On-skin impedance of silk electrodes Silk-P-15G as attached and after wetted.

Furthermore, the breathability of our electrodes also prevents thermal insulation of electrodes and sweat accumulation during exercise. There is no obvious temperature variation between the skin where wearing our breathable silk electrode and adjacent naked skin (Figure 5.27), significantly reducing the discomfort caused by poor heat dissipation. But temperature increases as high as $\sim 2.3\text{ }^{\circ}\text{C}$ and $\sim 3.1\text{ }^{\circ}\text{C}$ were observed for the skin areas

with a polymer electrode and a commercial gel electrode, respectively (Figure 5.27). These results demonstrated that our breathable silk electrodes provide superior physiological comfortability compared with commonly used commercial electrodes and polymer electrodes, which is important for healthcare monitoring during sports and sweaty conditions.

Figure 5.27 a) Optical and infrared photos of various electrodes on skin before and after exercise to demonstrate the good thermal transmission of silk-based electrodes. b) Central temperature variation of the skin where wearing electrodes compared with the temperature of surrounded naked skin after exercise.

In addition, the silk electrodes could also obtain stable ECG signals during long-term wearing in normal daily activities (Figure 5.28), which means our electrodes are reliable and stable in continuous and long-term working.

Figure 5.28 Long-term ECG signals recorded by silk electrodes in normal daily activities.

5.3 Conclusion

In conclusion, we have demonstrated stretchable, highly gas-permeable, lowly evaporative-resistant and conformal conducting polymer electrodes through glycerol plasticizing of electrospun silk protein fiber mats. Our silk-based electrodes with randomly orientated fibers showed higher electric stretchability owing to their porous structures and fiber realignment during stretching. More importantly, the silk electrodes possessed rather low thermal insulation, low evaporative resistance, and high water-vapor permeability. These features made our silk electrodes highly reliable for electrophysiological signals detections such as ECG recording in calm and sweaty situations after exercise. Furthermore, the silk electrodes did not block the sweat permeation and evaporation, and thus almost no disturbance was observed for the regulation of local body temperature by skin. These results demonstrated that our silk-based electrodes possess superior physiological comfortability and may open more progress in biomaterials-based wearable and skin-friendly electronics.

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Chapter 6

Mechanically Heterogeneous Silk Fibroin Films for Hybrid-integrated Stretchable Sensors

Except from the development of soft and stretchable elements for wearable electronics, a stretchable system which is fully functional and compatible with data processing and computation is of highly desire for better human-machine interface. Due to the heavy reliance and established standards for electronic system, novel sensors and devices have to be integrated with rigid electronic components. The giant mechanical mismatch and compatibility of manufacturing are two challenges for the system-level implementation. In this chapter, we have demonstrated a mechanically heterogeneous silk film for hybrid-integrated sensors system which is ease of fabrication and greatly improve the systematic stretchability. This heterogeneous film is realized selectively treatment of methanol on a plasticized silk film with CaCl_2 , realizing locally hardening of specific sites. The rigid electronic components were placed on the sites, realizing strain isolation and functionality under strain. The interconnects were screen-printed with Ag inks with stretchability more than 120%. With this heterogeneous silk film, the stretchability of whole devices could achieve 50% strain. At last, a wireless powered sensor system was demonstrated with improved stretchability and functionality. Our proposed mechanically heterogeneous silk film further broadens the horizon of biomaterials in the application of stretchable electronics.

6.1 Introduction

Stretchable electronic system that are capable of detecting biosignals and transmitting into machine greatly promote the continuously health monitoring and big data of healthcare. Besides stretchable elements such as stretchable electrodes,^[1] stretchable sensors,^[2-4] stretchable energy harvesting devices,^[5-7] etc, a stretchable electronic system should contain not only sensors, power supply, but also processing and communication units, which are indispensable for data transmission and human machine interface.^[8] Although recent decades have witnessed the great progress in stretchable sensors, stretchable conductors for better devices-skin interfaces, complicated signals read-out circuits still highly depend on the rigid complementary metal-oxide-semiconductor (CMOS) manufacturing-based integrated circuits (IC). Therefore, a hybrid electronic system is proposed to achieve the same function as conventional rigid electronics.^[9-15]

A flexible printed circuit board (FPCB) assembles the flexible electrodes, sensors and rigid electronic components, which is a milestone for system-level implementation of flexible and stretchable devices, specifically for the application of wearable electronics. It provides a bridge to combine the novel skin-conformal sensors with rigid silicon-based electronic systems to be compatible with existing computation standards.^[16-18] A hybrid flexible electronic device typically includes four components: 1) soft skin-sensors and devices; 2) hard electronics based on silicon for computation; 3) power source and 4) interface in between rigid and soft electronics.^[19]

In particular, the interface of rigid electronics and soft sensors and electrodes is the key challenge for their integration due to the mechanical mismatch (variation of Young's modulus $> 10^3$) between each other. Firstly, advanced soft and stretchable sensors always require special manufacturing techniques, which hinders the integration.^[18, 20] Secondly, the bonding between the rigid electronics and soft interconnects is prone to break and delaminate due to the giant variation of Young's modulus of rigid electronics and soft sensors, which is resulted by the stress distribution between each other.^[21, 22]

To achieve an integration which is ease of manufacturing and possesses higher mechanical flexibility is a research thrust. Especially, stretchable wearable system which is under tensile strain deformation is even more fragile at the interface and is of difficulty with proper substrate selections. For the stretchable hybrid electronics, a strategy called “rigid” islands and “soft” interconnects has come up for systematic integration of hybrid electronics (Figure 2.10).^[1] The soft regions are designed for the stretchable conductors to maintain the electrical conductivity under strain. The rigid islands are intended to place the rigid active components, which are wired out using the stretchable conductors. The rigid islands can isolate the strain when the whole system was stretched, promising the functions of active components. However, the rigid-to-soft interface would suffer from stress concentration, limiting the durability of the devices.^[21] Also, the stretchability of the electrodes must be high enough to maintain the electrical conductivity as most of the applied strain would be concentrated at the stretchable electrodes.^[23]

A method to fabricate elastomeric substrates (PDMS) with embedded stiff platforms (SU8) for stretchable electronics was achieved by Stephanie P. Lacour et. al (Figure 6.1).^[24] With these embedded rigid platforms, the stress concentration at the rigid-to-soft interface has been greatly reduced into a gradual transition of surface strain. Therefore, the systematic strain can be improved to 20% although there is sacrificial stretchability of electrodes (original 50%). This method is proved to be effective to reduce the stress concentration at the interface while the fabrication process requires lithography and the systematic stretchability is still not high enough.

Figure 6.1 a) Schematic illustrations of elastomeric substrates with embedded rigid platforms. b) The surface strain mapping image of the engineered substrates with spacing (S) of 11 mm at 20% strain. c) The strain profiles of the engineering substrates along x-axis from the center of rigid SU8 platforms. Reprinted with permission from reference.^[24]

Also, several works have demonstrated that heterogeneous polyurethane (PU) composites with gradient elastic moduli over several orders of magnitudes show reversibly stretchable up to 350%.^[25] The other work proves a programmable rigidity (rigidity contrast is ~ 50) of polymer substrate through oxidation reaction, isolating the strain of the rigid region.^[26]

However, these methods all need complicated processing relating chemical reactions or lithography. With the complicated fabrication, the compatibility with active materials deposition is poor for later integration of electronic system. Therefore, it is highly desirable to have an easy method to realize heterogamous substrates for stretchable hybrid electronics.

In this chapter, we propose a mechanically heterogeneous silk fibroin film realized by a drop casting of methanol on specific sites on plasticized silk fibroin films. The plasticization of silk film is realized by adding CaCl_2 as reported in chapter 4.^[27] It was reported that methanol treatment on silk fibroin can induce more beta-sheets formation, therefore higher strength.^[28, 29] Methanol treatment can induce an elastic modulus contrast of ~ 600 with various weight ratio of CaCl_2 , which is hardly realized on silicone-based

elastomers. The giant mechanical heterogeneity is due to the large amount of beta-sheets formation inside silk fibroin due to the methanol treatment. Based on this mechanically heterogeneous silk fibroin film, stress concentration at the rigid-to-soft interface was reduced simulated by finite elements analysis (FEA). Further, stretchable conductors obtained by screen printing of Ag inks on soft and stretchable areas can maintain the electrical conductivity under 120% strain, which undertake the systematic strain. The SEM image show that the interconnected microcracked network contributes to the electrical conductivity of Ag traces and there is no cracks formation at the region treated by methanol. Based on these, the integrated active components (resistors, capacitors, rectifiers and RFID) can be integrated on this mechanically heterogeneous silk fibroin film. A radio frequency coil has been designed to power the connected sensors. With the ease of assembly, a hybrid-integrated sensor system can work under 50% strain without mechanical failure, proving the feasibility of our heterogeneous silk fibroin film for the application of stretchable electronics. Herein proposed heterogeneous silk protein film opens up more possibility of biomaterials-based electronics for high-performance, environment-friendliness and biocompatibility.

6.2 Results and discussion

6.2.1 Mechanically heterogeneous silk fibroin film

The plasticized silk fibroin film was obtained based on CaCl_2 addition into silk fibroin as discussed in chapter 4. Following methanol treatment of various volume to control the area of the rigid area can realize mechanically heterogeneous silk films. Figure 6.2 shows the optical images of the heterogeneous silk films under 0% and 10% strain.

Figure 6.2 a) An optical image of mechanically heterogeneous silk fibroin films at 0% and b) 10% strain.

For various weight ratio of CaCl_2 , the Young's modulus before and after treatment would vary over 1 even 2 orders of magnitude. (Figure 6.3a). For pure silk fibroin films, the films with and without methanol treatment are tough and brittle. For silk fibroin films with 15 wt% and 20 wt% CaCl_2 , they exhibit favorable softness (100 kPa $\sim$ 4 MPa) at lab environment whose relative humidity is around 60%. Therefore, silk fibroin films with 15 wt% CaCl_2 showing a value of $E_{\text{rigid}}/E_{\text{soft}} \sim 6$ and with 20 wt% CaCl_2 showing a value of $E_{\text{rigid}}/E_{\text{soft}} \sim 250$ are chose for the following experiments as comparison for better surface strain regulation and ease of manipulation.

Methanol treatment can induce the secondary structures of silk fibroin shifting from less-organized random coils into rigid beta-sheets. FTIR characteristics reflect that amide I absorption band spectra shifted from $\sim 1648 \text{ cm}^{-1}$ to $\sim 1625 \text{ cm}^{-1}$ with methanol treatment, indicating that the random coils dominated silk conformation had converted to the stable β -sheet structures (Figure 6.3b), which is consistent with literature.^[30-32]

Figure 6.3 a) Young's modulus of mechanically heterogeneous silk fibroin film with and without methanol treatment of various weight ratio of CaCl_2 , b) FTIR results of silk fibroin films with and without methanol treatment, indicating secondary structures change. Relative humidity: 60%.

As silk protein is a semi-amorphous biopolymer, the microstructural change would be reflected in macroscale mechanical properties. Methanol treatment here induces crystallization of silk I conformation, forming large amounts of H-bonds (Figure 6.4). Therefore, the area with methanol treatment is rigid and unstretchable while the other part of the film is soft and stretchable, which consist of the mechanically heterogeneous silk fibroin films.

Figure 6.4 Molecular illustration of a) plasticized silk fibroin and b) methanol-treated tough silk fibroin.

Based on these, a mechanically heterogeneous silk fibroin with desired softness and contrast of rigid to soft areas has been obtained. Also, they show high compatibility with thin film-based manufacturing technology for active materials, which is ease of fabrication and shows high tunability. Following integration with stretchable electronics further demonstrate the feasibility of this mechanically heterogeneous silk fibroin film.

6.2.2 Stretchable electrodes enable by printing

Roll-to-roll printing technology has been regarded as the potential technique to develop large-area and low-cost flexible electronics, which can provide a platform for hybrid integration and assembly with high production. Our mechanically heterogeneous silk fibroin films are compatible with the roll-to-roll fabrication process that the printing process can be done first. Methanol treatment can be done after the circuits printing on desired locations. Conductive inks based on Ag nanoparticles have attracted a lot of research efforts due to the high conductivity, low cost, ease of synthesis and modification. For the application of stretchable electrodes, the poor adhesion with elastomeric substrates hinders the high performance under strain and induces dysfunctionality of the electrodes. Here, the heterogeneous silk fibroin film has good adhesion with Ag traces that the sintering of AgNPs show high uniformity and density (Figure 6.5).

Figure 6.5 Scanning electron images of Ag nanoparticles after annealing at 80 °C for 10 min on 15 wt% CaCl₂/silk and 20 wt% CaCl₂/silk substrates.

The printed Ag traces with 1 mm width can maintain its conductivity up to 100% and 120% strain on plasticized silk fibroin with 20 wt% and 15 wt% CaCl_2 , respectively (Figure 6.6a). As glass transition temperature of silk fibroin is around 130 °C, the printed Ag traces can not be annealed at very high temperature, otherwise the films would suffer from severe deformation and torsion. In our cases, annealing at 80 °C for 10 min was chosen. Compared with Ag traces on PDMS substrates (Figure 6.6b), the Ag traces on plasticized silk substrates can form interconnected microcracks network to maintain the electrical conductivity. The printed Ag traces can only show 20% stretchability on PDMS, which is consistent with the technical details of *LS-411AW Silver Paste* whose flexibility is under 10%. Also, the polymer ink is based on acetate that molecularly can bind better with silk fibroin materials, which is hypothesized to be the reason of good adhesion.

Figure 6.6 a) Resistance-strain characteristics of screen-printed Ag electrodes on 15 wt% $\text{CaCl}_2/\text{silk}$, 20 wt% $\text{CaCl}_2/\text{silk}$ and PDMS substrates, annealing at 80 °C for 10 min. b) Scanning electron microscope image of Ag traces on 15 wt% $\text{CaCl}_2/\text{silk}$ under 50% strain. Scale bar: 10 μm .

Figure 6.7 The SEM images of AgNPs on PDMS substrates under a) 0% strain and b) 50% strain.

Therefore, successful integration of our mechanically heterogeneous silk fibroin film with roll-to-roll printing technology, has realized good adhesion, low sheet resistance and high stretchability.

6.2.3 Strain redistribution on heterogeneous silk film for devices integration

After checking the stretchability of electrodes, the next step is to verify whether our mechanically heterogeneous silk films can isolate the stress concentration so that rigid active devices can be placed on top. Referring to the work with embedded rigid platforms inside PDMS, we have conducted the methanol treatment at the front surface of the electrodes and back surfaces at the same time (Figure 6.8a). In both cases, the methanol-treated areas would be placed on the rigid active components (Figure 6.8b).

Figure 6.8 a) Cross-sectional schematics of methanol treatment in front and back, showing the placements of rigid components and soft electrodes. b) An 3D schematic of wiring and placement of rigid active devices on our heterogeneous silk fibroin films.

Firstly, the stretchability of the Ag traces with methanol treatment at front and back were tested, compared with the no methanol-treated Ag traces (Figure 6.9a). With the front treatment, the stretchability of Ag traces on heterogeneous silk fibroin films decay from 100% to 10%, showing the electrical breakdown on the rigid-to-soft interfaces. On contrast, the Ag traces on heterogeneous silk fibroin films with back methanol treatment maintain the electrical conductivity until 50% strain. This stretchability is reflecting the systematic stretchability that the decay contributes to protect the rigid areas. Therefore, the methanol treatment at the back outperforms on stretchability compared with front methanol treatment.

Figure 6.9 a) Resistance-strain characteristics of Ag traces printed on mechanically heterogeneous silk fibroin films with 20 wt% CaCl_2 . b) A SEM image of Ag traces on mechanically heterogeneous silk fibroin film showing various morphologies of cracks under 50% strain.

From the scanning electron microscope image of Ag traces on mechanically heterogeneous silk fibroin films with back methanol treatment, a clear transition zone of microcracks slightly higher than stretchable electrodes can be observed. This is still due to the rigid-to-soft interfaces, but it is undoubtedly better than the front treatment case. At the same time, there's almost no crack formed at the area with back methanol treatment, demonstrating successful stress isolation. From electrical characteristics and morphological observations, our heterogeneous silk fibroin films with back methanol treatment show good stretchability of the stretchable interconnects part and stress isolation of the rigid treated areas, which is extremely suitable for stretchable hybrid-integrated electronics.

Finite-element analysis of the mechanically heterogeneous silk fibroin has proved the fine details of the rigid-to-soft transition region when the heterogeneous silk film is stretched. The main purpose is to exactly know the difference of front and back treatment of methanol of silk fibroin films and predesign the devices location in order to perfectly avoid the stress for them. In our heterogeneous silk fibroin films, we followed the experimental details of mechanical tests and electrical tests setup of Ag traces on heterogeneous silk fibroin with 20 wt% CaCl_2 . The height of the silk fibroin films is measured to be around 200 μm and the height of methanol-treated rigid platform is around 50 μm . The width of the substrate was set to be 2 mm and the length is 2.5 cm. The dimension of methanol treatment is 2 mm $\times$ 2 mm. The Young's modulus of both follow the mechanical tests data in Figure 6.3a whose average values are 0.13 MPa of the soft region and 33.21 MPa of the rigid region, respectively. The surface strain was calculated being applied 50% systematic strain. From Figure 6.10a, the front and back sides correspond to our back treatment and front treatment, respectively. In front side, there is no stress concentration at the rigid-to-soft regions. On the contrast, the back side shows a red region showing the surface strain concentration.

Figure 6.10 a) Finite-element analysis (FEA) of the front side and back side of mechanically heterogeneous silk fibroin film with 20 wt% CaCl_2 under 50% applied strain. b) Surface strain profiles along x-axis of the mechanically heterogeneous silk fibroin film with 20 wt% CaCl_2 under 50% applied strain.

To further verify the strain profiles, surface strain along the x-axis were plotted to see the detailed surface strain of both cases (Figure 6.10b). The surface strain of front side shows no peak at the rigid-to-soft zone but gradual strain reductions while the back side has a peak of strain concentration. And we can see a strain isolation length of more than 1 mm. Therefore, we can pattern the devices at the center. These simulation results are consistent with our experimental results, showing feasibility of our mechanically heterogeneous silk fibroin films for stretchable hybrid electronics.

Lastly, one single unit was tested on our mechanically heterogeneous silk fibroin film using one resistor. By placing the resistor on the center of the methanol-treated area, the devices can be stretched without delamination up to 100% strain (Figure 6.11) although the electrical conductivity can only be maintained at 50%. To conclude, the mechanically heterogeneous silk fibroin films have been proved from experiments and simulation that are ready for hybrid devices integration as the platform.

Figure 6.11 Optical images of a resistor on a heterogeneous silk fibroin film wired out by Ag electrodes under a) 0% strain, b) 50% strain and c) 100% strain.

6.2.4 Stretchable wireless hybrid-integrated sensors

Mechanically heterogeneous silk films are compatible with the printing technique and have multiple freedom of active devices selection for communication and data process purposes. A wireless powered sensor with rigid active components such as rectifier to transform AC signals into DC signals and capacitors for energy storage, etc, were connected to demonstrate the viability of our hybrid-stretchable platforms. Also, a stretchable passive RFID was demonstrated for on-skin stretchable electronic since wireless network is extremely important for wearable body sensor network.^[33]

Figure 6.12 Schematic illustration of a wireless hybrid-integrated sensor system on the mechanically heterogeneous silk fibroin films under 0% and 50% strain.

Figure 6.12 shows a schematic of a wireless stretchable hybrid system based on our mechanically heterogeneous silk fibroin films. The stretchable coils were printed to be stretchable while the rigid areas are predesigned for active devices. Stretchable antenna for wireless data and power transmission is extremely significant for wireless application that the antennas should be equipped with non-sensitive electromagnetic behaviors under mechanical deformation and is operated under proper frequency. Firstly, the stretchability of our printed radio frequency antenna coils was characterized (Figure 6.13). The resonant frequency of the stretchable antennas is in the radio frequency range and shows small variation of various printed antennas (Figure 6.13b). The electromagnetic-mechanical behaviors of our stretchable antenna were characterized through a vector network analyzer (VNA) incorporating with mechanical tester MTS C42. The corresponding S_{11} characteristics are shown in Figure 6.12a, when being stretched from 0% to 50% strain. The resonant frequency is not changed obviously but the amplitude of return loss decayed due to the resistance increase of the antenna (Figure 6.14). Quality factor is an important parameter to evaluate the performance of antennas, which is experimentally defined as resonant frequency versus the bandwidth with -3 dB. Therefore, the Q factors of the coils under strain were summarized in Table 6.1. Based on the equivalent LC circuits, the capacitance value of 6.5 pF and inductance value of 1.17 μH were calculated.

Figure 6.13 a) The return loss (S_{11}) of printed Ag coils on plasticized silk fibroin films with 15 wt% CaCl_2 under 0% strain to 50% strain. b) The return loss (S_{11}) of 4 printed Ag coils on plasticized silk fibroin films with 15 wt% CaCl_2 , showing no obvious variation.

Table 6.1 Q factors of Ag coils under strain.

Strain (%)	0	10	20	30
Q factor	15.28	8.125	4.24	2.034

Figure 6.14 a) Resistance-strain characteristics of stretchable antennas on heterogeneous silk fibroin film. b) Optical images of the Ag coils under 0% strain and c) 50% strain.

The working distance of radio frequency antenna is limited that only when the distance is within 10 cm, the antennas can work to obtain enough power. (Figure 6.15)

Figure 6.15 The radio frequency intensity at 13.56 MHz of printed coils on plasticized silk fibroin films vs. distance from 0 to 10 cm.

After checking the stretchable radio frequency coils, subsequent circuits for a wireless sensor system was designed for active devices integration and on-skin demonstration. Blank circuits printed was firstly treated with methanol at the back on desired locations, showing no damage of the circuits (Figure 6.16).

Figure 6.16 Optical images of printed antennas and circuits with back methanol treatment from the a) front side and b) back side.

After the methanol treatment, the rigid active devices were laminated into desired locations to achieve various functionalities (Figure 6.17). Firstly, the AC signals can be transformed into DC voltage through the rectifier and capacitor, providing power supply for wireless pressure sensors (Figure 6.19).

Figure 6.17 a) Circuits scheme of AC to DC power transmission with a $1\mu\text{F}$ capacitor, a rectifier, a resistor and a pressure sensor. b) An optical image of printed hybrid-integrated sensor system on mechanically heterogeneous silk fibroin film.

Here, a provided 5 V AC signal with 10 MHz frequency can be transformed into around a DC signal with 4.2 V amplitude (Figure 6.17a). A resistive pressure sensor with a resistor with 1 k Ω resistance were connected. The resistive pressure sensor was fabricated based on pyramidal microstructures with AgNWs as the active layer.^[34] The characterization of the pressure sensors is unveiled in Figure 6.17. This wireless-powered pressure sensor system can distinguish the gentle touch through voltage, showing the successful integration on our mechanically heterogeneous silk fibroin films. Figure 18b is the detected voltage at the two ends of connected 1 k Ω resistors when there is a gently touch on the pressure sensor, maintaining the high sensitivity.

Figure 6.18 a) Current-force characteristics of pressure sensor based on pyramidal structures and AgNWs. b) Current-pressure characteristics of pressure sensor based on pyramidal structures and AgNWs.

Figure 6.19 a) The received AC signals and DC signals after circuits. Input signals: 5 V amplitude and 10 MHz. b) The detected voltage signals at the two ends of 1 k Ω resistor upon gentle touch.

Following the electrical tests of the hybrid-integrated sensor on our mechanically heterogeneous silk fibroin film, we conducted the mechanical tests of the whole system to verify the advancement of our system. Firstly, the plasticized silk film has a good adhesion with our skin and any curved surface (Figure 6.19), which benefits the real application of our hybrid sensors for on-skin health monitoring with high fidelity and signal-to-noise ratios. Secondly, the mechanically heterogeneous silk fibroin films can isolate the strain of the rigid active devices, ensuring their working under mechanical deformation. Considering the skin deformation under motion would not exceed 35%,^[35] the hybrid-integrated sensor on our mechanically heterogeneous silk film can function well on the area besides joints, which is promising for on-skin electronics.

Figure 6.20 An image of the wireless hybrid-integrated RFID system on hand.

6.3 Conclusion

In conclusion, a mechanically heterogeneous silk fibroin films was prepared by simply solution treatment of certain locations on plasticized silk fibroin films. The contrast of Young's moduli of rigid to soft areas can be as high as 250 by varying the ratio of CaCl_2 . The soft areas with printed Ag traces can maintain high electrical conductivity under 120% strain, which compasses that on PDMS substrates. At the same time, the rigid areas can be placed with rigid active devices, which prevent them from strain concentration. Based on this mechanically heterogeneous silk fibroin film, a hybrid-integrated sensor system has been demonstrated with wireless power and good skin conformability. A printed radio

frequency coil was prepared to transmit the wireless power under strain of 50%. Benefited from the mechanically heterogeneous silk fibroin films, the whole system can sustain 50% principle strain, which get rids of complicated lithography process and chemical process. Therefore, our proposed mechanically heterogeneous silk fibroin films have further broadened the scope of biomaterials-based electronics for better sustainability and environmental friendliness.

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Chapter 7

Conclusions and Future Recommendations

This chapter summarizes the whole thesis with main three conclusions of the application of silk fibroin into the field of stretchable electronics. Not only the plasticization of silk materials into skin-like softness has been realized, detailed mechanisms have been discussed. Furthermore, multiple electronic functions from stretchable electrodes, sensors to hybrid-integrated systems have been realized. From the point of view of materials science and engineering, future perspectives and recommendations exploring more feasible formats and functions are proposed.

7.1 Conclusions

In this thesis, silk fibroin, as an FDA-approved biomaterial, has been utilized in flexible and stretchable electronic devices. The main drawbacks of pure silk fibroin include the limited stretchability and high mechanical modulus, which hinders its application in bioelectronics. Also, the rational to integrate electronic functions should be carefully considered. In respect of the microstructures of silk, two methods have been utilized to realize the plasticization of silk fibroin by tuning the secondary structures of protein. Metal films and conductive polymers have been incorporated with electrical conductivity. Besides homogeneous thin films of silk fibroin, nanomesh structures and mechanically heterogeneous silk films were deliberated with good gas permeability and ease of hybrid integration. Therefore, not only single devices, a systematic integration with desired electronic functions has been realized based on silk.

The homogeneous silk fibroin films with skin-like softness are obtained through modification with various ratios of CaCl_2 and ambient hydration. The Young's modulus and stretchability of silk fibroin were tuned into skin-like range at ambient environment, which is $0.4 \sim 2$ MPa and $>75\%$, respectively. Molecular dynamics simulation further proves the feasibility of our method to plasticize silk fibroin that CaCl_2 and absorbed water would decrease the interlayer energy of crystallites domain and increase the ratio of extensible secondary structures of amorphous domain. Thermal-evaporated Au films can form wrinkles after ambient hydration, which contribute the electrical stretchability together with the microcracks network. The good adhesion and conformability with skin is promising for on-skin electrophysiological signals recordings.

For on-skin electrodes, apart from softness and stretchability, good gas permeability and low thermal and evaporative resistance are also important which allows normal metabolism activities of skin, especially under sweaty conditions. Therefore, silk fiber mats by electrospinning were prepared which allow sweat to evaporate and heat to dissipate. The regenerated silk fibroin solution can be electrospun and following glycerol stabilization and plasticization renders the silk fiber mats solution processability. Thus, PEDOT: PSS

was incorporated on silk fiber mats through a simple drop casting method, which has high conductivity up to 24 S/cm and stretchability up to 250%. This silk fiber mats-based electrodes have lower thermal insulation and evaporative resistance, and higher water vapor-transmission rate. During electrophysiological signals recording, the silk fiber mats-based electrodes show better signal-to-noise ratio under sweaty situation compared with commercial gel electrodes. Also, no heat is blocked by silk fiber mats-based electrodes, which provide better skin comfortability. This kind of biomaterials-based mesh-structured electrodes pave the way for imperceptible and skin-conformal electronics.

Apart from stretchable electrodes based on plasticized silk fibroin, a complete electronic function has to be accomplished with rigid processing and communication devices. Therefore, hybrid integration is a significant road for a multifunctional electronic system. Further, homogeneous silk fibroin films were treated into mechanically heterogeneous silk fibroin films, which can isolate the mechanical strain for the rigid active devices. The patterned areas were treated with methanol which can induce rapid conformation change from random coils into beta-sheets. Therefore, a high moduli contrast of ~ 250 can be achieved. The stretchable electrodes are prepared by screen printing of Ag inks and a high stretchability of 120% can be obtained. Based on these, a hybrid-integrated sensor system with wireless power has been demonstrated to work under 50% principle strain. Our mechanically heterogeneous silk fibroin films have expanded the application of silk fibroin from single devices into electronic systems. This method is ease of operation while can obtain good electrical performance, which further pave the way for silk fibroin-based electronic devices.

7.2 Future recommendations

7.2.1 Materials exploration of silk fibroin

Silk fibroin has attracted a lot of research efforts due to the nature biocompatibility and solution processability. From the perspective of materials society, silk fibroin is an attractive material to be tuned with desired mechanical properties. Specifically, for

bioelectronics, wearable applications and implantable applications require different materials properties. For example, the implantable devices have developed one specific area-transient electronics. This kind of electronic devices can degrade with controllable manners after its functioning *in vivo*. As declared in Chapter 1, silk fibroin is a kind of semi-amorphous materials that its degradability can be regulated through adjusting the microscale secondary structures. Therefore, the fundamental of materials science and engineering requires further attempts to understand the mechanical properties, degradability and intramolecular interactions with additives of silk fibroin from experiments perspective and theoretical perspective.

Further, not only silk fibroin thin film is of great importance of wearable applications, other formats such as tubes, sponges and gels are also meaningful for implantable applications including drug delivery, extracellular matrix and biointerface electronics.^[1] Biological tissues always have 3D structures that fibers, and tubes show superiority in biomedical applications realizing a better silk/tissue interface. For example, silk fibroin-based nerve grafts have been reported to guide the regeneration of nerves with oriented silk fibroin filaments.^[2] The morphology, mechanical property, biodegradability should all fulfill to the nerve regeneration application. Further, how to make the various formats of silk fibroin be compatible with active materials is critical to obtain biosignals into machine-readable signals. The form factor of 3D structures would increase the complicity of the integration with thin film-based active materials such as metals.

Lastly, the molecular mechanism of silk fibroin should be deliberated. Although there are a lot of research works discussed how molecular structures of silk fibroin affect the macroscale properties, in the case of *in vivo* and *in vitro* applications, new research filed about the interaction between molecules of silk fibroin and biological environments should be further investigated.

Figure 7.1 Various materials formats fabricated from silk fibroin based on solution process. Reprinted with permission from reference.^[1]

7.2.2 More functional devices based on silk fibroin

Silk fibroin-based electronic devices show the uniqueness in developing sustainable electronics and biocompatible electronics. Unlike previous applications,^[3] our research outcomes have demonstrated skin-conformal, stretchable electronics based on silk fibroin beyond being just flexible. Although we have proved successful integration of plasticized silk fibroin with thermal-evaporated Au films, solution-processed conducting polymer (PEDOT: PSS) and hybrid devices, there are still a lot of challenges to tackle in order to achieve a silk-based electronic skin as human skin possesses a lot of mechano-receptors, chemo-receptors and neural coding system (Figure 7.2).^[4]

Figure 7.2 Schematics of a) cold receptors and b) mechanoreceptors in human skin. Reprinted with permission from reference.^[4]

Various sensor units can be fabricated to detect strain signals, chemical signals, optical signals and electrophysiological signals as well. Recent advancements in biosensors have revealed that chemical signals are of great significance to evaluate the health status.^[5] Protein displays a better molecular affinity with biomolecules that how silk fibroin can interact with sweat and ions, should be considered. Furthermore, how to utilize this kind of interactions to enhance biosensing capability is of great interests to develop silk-based electronic skin. For mechanical sensors, rationale to select sensitive active materials to integrate with silk fibroin is vital.

Figure 7.3 A schematic of silk fibroin-based electronics for better human machine interface with

sustainability and environmental friendliness.

Not only sensors based on silk fibroin can be developed. Due to the great dielectricity of silk fibroin,^[6, 7] memory devices and transistors which are regarded as processing units can be fabricated on plasticized silk fibroin films. A fully stretchable and all silk fibroin-based system can be realized patterning stretchable silk fibroin with stretchable interconnects and fabricated the silk fibroin-gated transistors or resistive switching memory devices for data processing. And energy harvesting devices can also be realized based on silk fibroin as well. These proposed electronic devices based on silk fibroin can open up more opportunity for biocompatible, green and high-performance electronics (Figure 7.3).^[8]

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