Thermogel for surface contact plant electrophysiology

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**THERMOGEL FOR SURFACE CONTACT PLANT
ELECTROPHYSIOLOGY**

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2020

THERMOGEL FOR SURFACE CONTACT PLANT ELECTROPHYSIOLOGY

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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

2020

Statement of Originality

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Abstract

Plant electrophysiology studies the endogenous electrical signals that regulate plant behaviors towards environmental changes, laying the foundation for smart plant monitoring and plant-electronics integration. To read electrical signals from plants, surface contact electrodes are desirable for ad-hoc and on-demand application, but high-quality recording is hampered by the complex surface topography on plants, as well as mechanical disturbance during operation and testing. The ionic interface connecting metal electrodes and plant surfaces play a crucial role in tackling these problems. However, conventional ionic interfaces made of agar gel and solid hydrogels lack either adhesiveness or conformability, thus not competent in overcoming the surface and mechanical challenges. Thermogel, which undergoes sol-gel transition on temperature increase and possesses viscoelasticity, is proposed an adhesive and conformable ionic interface to tackle these challenges.

The current thesis discusses thermogel formulation optimization, material property characterization, electrode preparation, performance comparison, and mechanistic investigation, using hairy plants as a topographically complex model, and electrophysiological recording of wound-induced potential changes as an application demonstration. The thermogel exhibited one order of magnitude reduction in electrical impedance on hairy plants over solid hydrogels and more than four times increment in shear adhesive strength than agar gels, being electrically and mechanically advantageous over conventional hydrogels. It was formed in situ on hairy plants, locking into the abrupt surface irregularities and establishing an adhesive and conformal ionic interface between plants and electrodes. Facile and high-fidelity electrical recording on hairy plants was demonstrated, where conventional gels failed mechanically or electrically. The failure of solid hydrogels is due to lack of deformability or fluidity to conform to hairy surfaces, and the failure of agar gel is attributed to low gelling concentration and lack of viscoelasticity. In contrast, the sol-gel transition of thermogel, as well as amphiphilicity and mobility of the gelling polymers synergistically promotes conformability and interfacial adhesiveness, and additionally gives rise to self-healing property, advantageous for practical application.

Based on experimental results, this thesis verifies the hypothesis that thermogel can form an adhesive and conformal ionic interface between plants and electrodes, which delivers improved signal recording performance than conventional hydrogels. With simplified electrode setup and potential accessibility to other textured biological surfaces, the work discussed in this thesis will promote the development of plant-electronic hybrids towards smart sensing and modulating functions and inspire future exploration of unique properties of soft materials for bio-electronic integration.

Lay Summary

Electrical signals are an important inter-cell communication channel in animals, such as electrocardiogram (ECG) and electroencephalogram (EEG). With the advancement of electronic technology, inferring human health conditions or treating diseases through electrical interrogation and intervention is becoming popular. Analogously, plants also rely on electrical signals in sensing and responding to environmental changes and threats. Utilizing electronics to monitor plant health and to modify plant physiology might be a future solution for sustainable food supply and environmental protection, as the global food scarcity and climate change are escalating at an unprecedented speed.

To read signals from plant surfaces, an ionic interface bridging plant and metal electrode is required. This interface serves two roles: to electrically transmit signals and to mechanically bond plants and electrodes. Conventionally, agar gel is used to ensure good electrical contact, but it lacks adhesiveness and thus requires complex setup and great operational care to prevent electrode detachment. Another possible material is preformed solid hydrogels that exhibit good adhesiveness and mechanical robustness; however, they cannot conform to the roughness on plant surfaces, for instance, hairy plants, deteriorating signal quality. These issues render surface contact electrodes not ideal for plant-electronic hybridization.

To tackle the shortcomings of conventional hydrogels, in this thesis, thermogel is proposed and proved an effective ionic interface. Thermogel is a liquid at lower temperature ($\sim 4^\circ\text{C}$) yet turn into a sticky gel at higher temperature ($\sim 25^\circ\text{C}$). After being applied on hairy plants in a liquid phase, it gradually gels resulting in a gel that locks into the abrupt surface irregularities. Because of its stickiness and robust mechanical integrity after gelation, it can adhere electrodes reliably on plants, which greatly simplifies electrode setup while ensuring high-quality signal recording.

The work discussed in the current thesis provides an effective solution to bridging plants presenting highly textured surfaces with non-invasive electrodes. It paves the way for plug-

and-play integration between plants and electronics for advanced functions such as environmental sensing, plant part actuation, and plant physiology modulation. Furthermore, the demonstration on hairy surfaces provides inspiration for material and device design targeting other textured biological surfaces such as brain, which will enable wider integration between biology and electronics for applications in robotics, prosthetics, and so on.

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Figure 5.6 Analysis of signal intensity read using EPC thermogel and PAAm hydrogel on sunflower plants. **a)** Peak amplitude ratio of EPC thermogel to PAAm hydrogel in a pair, as a function of peak amplitude read from EPC thermogel (open circles). Data points are divided in two groups (orange shading and green shading, mean $\pm$ s.e.), which are statistically different. $***p < 0.001$; two-tailed, unpaired t-tests. **b)** Peak amplitude comparison between PAAm hydrogel and EPC thermogel according to the grouping in (a) (mean $\pm$ s.e., $n = 4$ for large peaks (green), $n = 9$ for small peaks (orange)). $*p < 0.05$; paired t-tests. ns, not significant.

Figure 5.7 Results of flame wounding on tobacco plants. **a-b)** Photographs showing a surface electrode adhered on a tobacco stem through EPC thermogel (a) and a Ag wire pierced in a tobacco stem (b). During testing, the two electrodes are positioned opposite (as E1 and E2 in Figure 5.2b). Scale bars, 5 mm. **c)** Potential signals read from Ag wire and EPC-interfaced Ag plate in a flame-wounding test. Inset photographs show the application procedure of EPC-based electrodes, where white arrows point to EPC droplet and Ag plate, respectively. Results are representative of measurements on three individual plants.

Figure 5.8 Monitoring propagation of flame-induced potential changes in tobacco stems. **a)** Scheme showing the setup for flame-wounding a tobacco plant at leaf tip and recording on its stem at positions E1 and E2 in this study. **b)** Scheme of a single leaf with connected petiole and stem showing the exact location of electrodes (E1 and E2). **c)** Signals read from E1 and E2 using EPC thermogel-based electrodes. Inset, enlarged view to visualize the signal transmission from E1 to E2. Signals are representative of measurements on four individual plants.

Figure 5.9 Signal characteristics of flame-induced potential changes on tobacco stems. **a)** Scatter plot of transmission speed of potential signals as a function of distance from point of wounding. **b)** Scatter plot of peak amplitude as a function of distance from point of wounding. Lines connect two points collected in a single wounding event from two electrodes positioned at E1 and E2, indicated in Figure 5.8a and b.

Figure 5.10 Electric potential recording of tobacco plant (*Nicotiana benthamiana*) using inserted glass electrodes in a previous study. **a)** Image of the setup. Letters b and c represent reading glass electrode and reference glass electrode respectively. **b)** Three electric potential signals propagated after burning injury was inflicted on first (left side signal), second (middle signal) and third leaf (right side signal) of the plant. Arrows indicate potential spikes and dashed circles indicate observed slowing of signal repolarization rate. Adapted with permission.^[7] Copyright 2018, Taylor & Francis Group.

Figure 6.1 Schematics of procedure for tensile adhesion tests of agar gel. Distance between the two Ag/AgCl plates is 1 mm.

Figure 6.2 Surface tension of agar and EPC solutions. **a)** Surface tension of 0.9% NaCl droplets in air ($n \geq 3$) with addition of agar and EPC polymers at increasing concentration. Error bars, s.d. **b)** Enlarged view of surface tension of agar solutions (mean $\pm$ s.e.) with statistical analysis. *** $p < 0.001$; unpaired two-tailed t-test. ns, not significant.

Figure 6.3 Histogram showing contact angles of saline, agar and EPC solution droplets on solid surfaces (mean $\pm$ s.e.). * $p < 0.05$, **** $p < 0.0001$; unpaired, two-tailed t-test. ns, not significant. PDMS = polydimethylsiloxane, LDPE = low density polyethylene.

Figure 6.4 Histogram showing mean contact angles of saline and EPC solution (1%) at 0.9% NaCl ($n \geq 3$) on various polymers and corresponding reduction of contact angles by addition of EPC. Error bars, s.d. PDMS = polydimethylsiloxane, LDPE = low density polyethylene, PET = polyethylene terephthalate, and PI = polyimide.

Figure 6.5 Molecular structures of polymers used in contact angle measurement. a) PDMS, b) LDPE, c) PET, and d) PI.

Figure 6.6 SEM images of polymers used in contact angle measurement at two magnifications. Cracks visible in high resolution images are from sputtered Au coating (20 nm thickness) to enhance contrast. Impurities are for ease of focusing.

Figure 6.7 UV-Vis absorption spectra of DPH solution (6 μM in 0.9% saline) with and without 11% EPC at $\sim 0^\circ\text{C}$.

Figure 6.8 Setup and images for confocal microscopy of EPC sol/gel (with 10 μM DPH) and agar gel (with 0.1 wt% fluorescent beads) at room temperature. **a)** Setup and viewing angle. **b)** Top view and side view of droplets composed of EPC saline solution at varying EPC concentrations. **c)** Top view and side view of droplets composed of saline and 0.5% agar-saline. **d)** Side view of saline and agar droplets with outline highlighted (orange dashed lines). The green shading in agar images denotes blank area where no fluorescent signal was observed even when adjusting image brightness and contrast. Scale bars, 50 μm .

Figure 6.9 Mechanical properties of viscous polymer solutions and pastes, compared to 11% EPC thermogelling solution. **a)** Viscosities and $\tan\delta$ of 20% PVA solution, 43% PVA paste, and 30% PAA paste. The number suffixes indicate wt% of polymers. **b)** Images within 10 s after applying 20% PVA solution between two Ag/AgCl plates with glass backing in a lap-shear configuration. **c)** Results from amplitude sweeps of 43% PVA paste and 11% EPC thermogel at room temperature around 25°C . **d)** Shear adhesive strengths of 43% PVA paste and 11% EPC thermogel on Ag/AgCl electrodes and *Gloxinia* hairy leaves, measured by lap-shear tests. **e)** Photographs of 43% PVA pastes on *Gloxinia* leaves (left, side view) and Ag/AgCl plates (middle and right, side and front view respectively) after shear tests. Scale bars in (b) and (e), 5 mm.

Figure 6.10 Rheological characterization of agar gelling solutions at 0.5% and 3% concentrations in the temperature range of $0 - 50^\circ\text{C}$, as compared to 11% EPC solution. **a)** Viscosity and storage modulus comparison. **b)** $\tan\delta$ comparison.

Figure 6.11 Rheological characterization for gelation time estimation. **a)** EPC thermogelling solution was stabilized at 4°C before quickly heating up to 25°C , whereas agar solutions were stabilized at 50°C before quickly cooling down to 25°C . The different

slope is due to varying heating/cooling rate of equipment (faster heating than cooling). **b)** Storage and loss moduli of 11% EPC, 0.5% agar, and 3% agar solutions under the temperature conditions in (a).

Figure 6.12 Tensile adhesive strengths of agar gels at varying concentrations, Ag/AgCl as the adherends, and the corresponding differences between in-situ and ex-situ adhesive strengths. One-way ANOVA with Tukey correction. Error bars, s.d. ns, not significant.

Figure 6.13 In-situ shear adhesive strengths of 0.5% and 3% agar gels and 11% EPC thermogel on Ag/AgCl electrode and hairy leaf surfaces. Error bars, s.d.

Figure 6.14 Tensile and shear adhesive strengths of EPC thermogel and agar gels on Ag/AgCl plates. **a)** Tensile adhesive strengths of 11% EPC thermogel. Paired t-test. Error bars, s.e. ns, not significant. **b)** Shear adhesive strengths of 3% agar gel and 11% EPC thermogel. $**p < 0.01$; unpaired, two-tailed t test. Error bars, s.e.

Figure 7.1 Rheological, mechanical, and electrical properties of 10% EPC thermogel added with 10 mM KCl (labeled as EPC10-KCl0.01), in comparison with optimized formulation used in this study, 11% EPC-0.9% NaCl (labeled as EPC11-NaCl0.9). **a)** Storage and loss moduli as a function of temperature. **b)** Gelation time comparison when thermogelling solutions are heated from 4 °C to 25 °C. **c)** Yield behavior comparison in amplitude sweep. **d)** Histogram of conductivity of 10 mM KCl without and with addition of 10% EPC polymers (mean $\pm$ s.e.). $*p < 0.05$; unpaired, two-tailed t test.

Figure 7.2 Dehydration of thermogel (11% EPC, 1% NaCl). **a)** Setup for measuring the conductivity of thermogel. Sample was placed in fume hood for air drying. **b)** Percent of water loss (by mass) with time. **c)** Conductivity drops with water loss and reaches a threshold value for effective electrophysiology recording (according to conductivity of 10 mM KCl used in previous reports^[1-2]) at around 70 wt% water loss. **d)** Conductivity change with time on a logarithm scale. At the current setup and drying conditions, it takes around 3 hours for thermogel to reach the threshold conductivity. In real plant measurement, this time varies.

Figure 7.3 Holistic encapsulation to improve temperature tolerance and mitigate dehydration of thermogel-based electrode.

Figure 7.4 Surface height profile of Ag/AgCl (a) and Pt (b) plate electrodes.

Abbreviations

3D	Three-Dimensional
AC	Alternating Current
AFM	Atomic Force Microscopy
ANOVA	Analysis of Variance
AP	Action Potential
APS	Ammonium Persulfate
CA	Contact Angle
CMC	Critical Micellar Concentration
DC	Direct Current
DI	Deionized
DPH	1,6-Diphenyl-1,3,5-Hexatriene
ECG	Electrocardiogram
EEG	Electroencephalogram
EIS	Electrochemical Impedance Spectrometry
EPC	Poly(PEG/PPG/PCL Urethane)
HMDI	Hexamethylene Diisocyanate
IPA	Isopropyl Alcohol
LCST	Lower Critical Solubility Temperature
LDPE	Low Density Polyethylene
LM	Low Magnification
LVR	Linear Viscoelastic Range
MIFE	Microelectrode Ion Fluxes Estimation
mRNA	Messenger Ribonucleic Acid
PAA	Polyacrylic Acid
PAAm	Polyacrylamide
PCL	polycaprolactone
PDI	Polydispersity Index
PDMS	polydimethylsiloxane
PEG	Polyethylene Glycol

PEO	Polyethylene Oxide
PET	Polyethylene Terephthalate
PHB	Poly[(R)-3-Hydroxybutyrate]
PI	Polyimide
PPG	Polypropylene Glycol
PPO	Polypropylene Oxide
PVA	Polyvinyl Alcohol
SEI	Secondary Electron Imaging
SEM	Scanning Electron Microscopy
UV-Vis	Ultraviolet-Visible
VGIC	Voltage-Gated Ion Channel
VP	Variation Potential

Chapter 1

Introduction

This chapter contextualizes the thesis by providing a brief background of plant electrophysiology and describing the research gap in electrode design. The hypothesis on utilizing thermogel in addressing the research gap is then stated and specific objectives are identified. This chapter also gives an overview of the thesis, including experimental and analytical scope and thesis structure. Finally, major novel outcomes generated from this study are summarized.

1.1 Background

With the advancement of electronic technology, health monitoring and disease treatment using electronic devices is becoming a trend for future healthcare.^[1] However, despite the equally important role of electrical signals in plants as in animals,^[2-3] electronic interrogation and intervention of plant physiology and pathology have yet to receive much attention. Much related work is focused on deciphering the signals, and little work is done to improve the tools used for plant electrophysiological study, i.e., the electrodes that interface with plants for electrical communication.

Conventional surface contact electrodes, composed of an ionic interface connecting metal electrodes and plants, face challenges in tackling the rough and irregular surface topography of plants, as well as accommodating the mechanical disturbance during operation and testing. For example, early plant electrophysiological measurements often used saline borne on felt-tipped pens or cotton threads to bridge plants with electrodes,^[4-7] which require complex mechanical support for holding plants and electrodes in place to maintain contact. Later, agar gel was introduced to improve the mechanical property of the ionic interface,^[8-10] but it lacks adhesiveness thereby still requiring additional fixation and great operational care to avoid electrode detachment.^[9-10] Furthermore, pre-formed adhesive hydrogels are also possible choices, given that they have been widely used at the interface between animal tissues and electronics.^[11-12] However, they lack the deformability to fully conform to abrupt surface irregularities on plants, such as hairs, which are widely present on crops and ornamental plants. This poor contact will reduce adhesion force and deteriorate signal transmission stability and fidelity.^[13-14]

Therefore, to enable facile, effective, wider adoption of electrical interrogation and intervention of plants, much improvement of the ionic interface between plants and electrodes is needed. Specifically, conformability and adhesiveness are two issues pivotal to the quality of measurement yet not fully addressed by conventional hydrogel-based ionic interfaces. Inspired by the sol-gel transition of agar gel and the adhesiveness of chemically crosslinked hydrogel, this thesis aims to utilize thermogel, a material that combines these

two key features in one material system, to address the issue.

1.2 Hypotheses

This thesis tests the following hypotheses:

Hypothesis 1: Thermogel can establish a conformal and adhesive ionic interface between plants and electrodes.

Hypothesis 2: Thermogel ionic interface can deliver improved signal quality and greater operational convenience than conventional hydrogels in surface contact electrophysiology on hairy plants.

1.3 Objectives and Scope

In testing the hypotheses stated above, the following objectives are to be accomplished by the end of the study:

1. To design and fabricate a thermogel ionic interface that is conformable and adhesive to hairy plants
2. To design and fabricate a thermogel-based surface contact electrode that delivers improved electrical recording performance on hairy plants, including higher signal quality and simplified electrode setup, over conventional surface recording materials and methods
3. To explain the mechanisms of conformability and adhesiveness as well as other properties that are related to electrophysiological recording

To achieve these objectives, the following experiments and analyses were conducted:

1. Thermogel-based surface contact electrode design with thermogel material properties specified

2. Thermogel preparation and formulation optimization to meet the application requirements
3. Microscopic observation of gel-plant interfaces and adhesive strength measurement
4. Characterization of other properties central to implementation of thermogel ionic interface, including rheological, mechanical and electrical properties, in comparison to agar gel and chemically crosslinked hydrogel
5. Performance assessment through recording of wound-induced electrical signals on hairy plants, in comparison to agar gel and chemically crosslinked hydrogel
6. Correlation of signal recording performance to material properties
7. Characterization of surface activity, cohesive mechanical property, and interfacial adhesion of solutions and gels of thermogelling polymers, agar polymers, and adhesive polymers
8. Explanation of the observed properties in relation to molecular structures, and analysis of practical considerations for plant electrophysiology application

1.4 Dissertation Overview

This thesis consists of the following 7 chapters:

Chapter 1 provides a rationale for the research, outlines the goals and scope, and summarizes major outcomes.

Chapter 2 reviews the literature concerning plant electrophysiology and common methods for signal recording. Limitations of current materials used in surface contact recording are discussed, which lead to the gap that the thesis is addressing. Prior evidence for the feasibility of the proposed hypothesis is provided.

Chapter 3 describes the materials and methods employed and discusses the principles underlying the synthesis and characterization techniques. The rationale for selection of each material and method is included. Methods of data analysis and minimizing errors are

also briefly introduced.

Chapter 4 elaborates the design, optimization, and characterization of thermogel ionic interface. Conformability and adhesiveness are examined, together with other properties that are central to the implementation of thermogel-interfaced surface electrodes, including rheological, mechanical, and electrical properties.

Chapter 5 describes the signal recording performance of thermogel-interfaced surface electrodes on non-hairy and hairy plants, in comparison with conventional hydrogels and benchmarked against invasive recording.

Chapter 6 details the process of unraveling the mechanisms of conformability and adhesiveness of thermogel, and the molecular origins of disparate properties of EPC thermogelling solutions, agar gelling solutions, and adhesive polymer solutions.

Chapter 7 overviews the thesis with an assessment of the extent to which the hypothesis is proven/disproven and a summary of key findings. Implications to the relevant scientific and engineering fields as well as proposals for future work are also discussed.

1.5 Findings and Outcomes

This research leads to several novel outcomes by:

1. Constructing an ionic interface that enables effective and facile electrophysiological measurement on hairy plants
2. Demonstrating a new application of thermogelling polymers
3. Unravelling the mechanisms for conformability and adhesiveness of thermogel
4. Discovering the limitations of agar gel and understanding the underlying mechanisms
5. Proposing design guidelines for conformal contact of bioelectrodes on rough biological surfaces

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Chapter 2

Literature Review

This chapter provides historical and contemporary background related to this study, identifies the research gap, and justifies the proposed hypotheses. Plant electrophysiology studies the endogenous electric signals in plants, which are critical to their survival and adaptation to the environment. Non-invasive signal recording is preferred to invasive recording due to signal genuineness and avoidance of tissue damage. Surface contact measurement is especially suitable for ad-hoc applications in plant-electronic integration, but faces challenges in insulating, hydrophobic, and complexly structured plant surfaces, as well as mechanical disturbance during testing. Conventional hydrogel-based ionic interfaces, including physical gels and chemical gels, cannot fully address these issues due to lack of adhesiveness or conformability. Combining the merits of these two material systems, thermogel may be an effective solution to the adhesiveness and conformability problems.

2.1 Plant Electrophysiology

It is well known that animal cells communicate through electrical signals (e.g., ECG and EEG). Less widely known, electrical signals are also used by plant cells for communication, and the discovery and research of electrical signaling in plants has equally long history (more than 200 years) to that in animals.^[1] Electrical signals were first studied in plants that produce visible, rapid response to touch, such as insect-trapping plants, but later it was recognized electrical signals generally exist in most (if not all) plants, including those that appear slow and static. The transmission of electrical signals within plants was found to trigger a series of biochemical events that eventually lead to gene expression alterations, which could further result in macroscopic modulation of plant behaviors such as growth and fertilization.^[2-4]

One unique feature of electrical signaling compared to other forms of intercellular communication (e.g., chemical signals) is the rapid and wide spread, the importance of which was not realized until the observation of rapid systemic changes in fundamental processes such as transcription and translation (manifested in increased/reduced protein synthesis, mRNA accumulation, etc.) associated with electrical signals in 1980s. This turns out to be imperative for systemic defensive response to environmental threats, such as viruses, insects, heat, etc. Since then, a major goal of plant electrophysiology, the branch of study on electrical signals in plants,^[5] is to decipher the steps in signaling and modulation,^[1] but till now, there is no complete picture of the mechanisms and processes of electrical signal generation, transmission, and role in physiological modulation.

In the past ten years, with the rapid advancement of electronics technology and the emergence of cyber-physical interface,^[6] there is growing interest in wiring up plants to achieve non-conventional functions, such as environmental sensing, augmented human-plant interaction, crop health monitoring, and plant physiology modulation.^[7-13] In these applications, either electrical signals generated within plants are collected and analyzed by external devices, or electrical stimulation is delivered to plants for behavioral intervention. Accordingly, plant electrophysiology is gaining more practical significance beyond

biological understanding, and this trend is expected to intensify given the continuing expansion of digitalization in the physical world. Moreover, the integration between plants and electronics might provide novel solutions for sustainable food supply and environmental protection.

2.2 Recording Electrodes

To collect electrical signals from plants, there must be a certain electrode that connects the plant with a data acquisition device. Conventionally, electrodes are classified into intracellular and extracellular electrodes, which are placed inside and outside of cells, respectively. Both types of electrodes can be used for organism-level electrophysiology study, and some intracellular techniques such as patch-clamp, AP-clamp and Self-clamp techniques, are powerful tools for single-cell study,^[5, 14] which are beyond the scope of this thesis. In order to read the potential, a complete electric circuit must be formed, and hence a reference electrode, or ground electrode is required (**Figure 2.1**). For a reference electrode placed on plant, the potential difference between the measuring electrode and the reference electrode is recorded, and the reference potential is likely to change given the dynamic behavior of plants. On the contrary, potential of ground electrode inserted in soil is generally assumed stable. Special attention is given to intracellular measurement of membrane potential, where the reference electrode should be placed in the bathing medium surrounding the cell(s) being measured.^[14]

Figure 2.1 Schematic diagram (left) and photo (right) of the digital acquisition system for recording voltage differences between the base of the trunk and the canopy. Reproduced with permission.^[15] Copyright 2012, The Authors, licensee IntechOpen.

2.2.1 Intracellular Electrodes

Figure 2.2 Structure of a glass microelectrode. Reproduced with permission.^[16] Copyright 2012, Elsevier.

Intracellular electrodes are inserted into the interior of a living cell (i.e., cytoplasm), thereby able to accurately measure the absolute value of membrane potential including resting potential. In organism-level plant electrophysiological study, intracellular electrodes are commonly inserted into phloem sieve tubes, i.e., cytoplasm of sieve tube cells.

Figure 2.3 Phloem potential measurement using aphid stylet-attached microelectrode. **a)** An aphid in feeding position with its stylet inserted into a sieve element on the upper side of a leaf. **b)** After the aphid is separated from its stylet by a laser pulse, the stylet stump exuded sieve tube sap

to which the tip of a microelectrode was attached. Cooling the shoot evoked an AP transmitted acropetally within the phloem, while flaming of a leaf generated a VP with different form and of long duration. Reproduced with permission.^[17] Copyright 2006, The Authors, published by Blackwell Publishing.

The major type of intracellular electrodes is microelectrode, composed of a glass capillary (a few micrometers in diameter) containing electrolyte and a thin Ag/AgCl wire, and the electrolyte will be in contact with cells and the Ag/AgCl wire will be connected to the external circuit (**Figure 2.2**). The small diameter of the electrode tip allows insertion into cell cytoplasm without causing large-area tissue damage, but insertion must be carried out with extreme care and requires some experience to ensure the correct insertion position. An effective way to ensure insertion accuracy is to exploit the probing capability of aphids. They attack plants by piecing their stylets into the phloem sieve tubes and suck the nutrients in there. Thus, once an aphid successfully locates the phloem using its stylet, the stylet is cut from the insect and microelectrodes can be connected with the stylet (again with great care), directly accessing to the phloem sieve tube cells (**Figure 2.3**). Obviously, this method relies on some luck in identifying a preying aphid, is limited to those plants that are attacked by aphids, and imposes stringent requirements in electrode positioning, fixation, and operator skill.

2.2.2 Extracellular Electrodes

Extracellular electrodes probe the ion concentration from outside of cell membrane, e.g., apoplastic space between cell membrane and cell wall, and should be placed near the cells that produce potential fluctuations for maximal signal intensity that can be recorded. They are generally easier to handle than intracellular electrodes and allow for long-term measurement. Moreover, non-invasive extracellular electrodes offer additional benefits due to avoidance of tissue damage.

Figure 2.4 Experimental set-up for detecting electrical signals in plants through electrolyte in containers and cotton threads. Reproduced with permission.^[14] Copyright 2009, National Natural Science Foundation of China and Chinese Academy of Sciences, published by Elsevier and Science in China Press.

Invasive extracellular electrodes are predominantly piercing metal wires (silver, platinum), although Ag/AgCl microelectrodes were also used on woody plants^[15]. They are easy to prepare and apply on plants but induce physical damage to plants, which should be avoided in wound-induced electrical signal monitoring. Nevertheless, this damage effect will disappear after plants are allowed to recover for hours or days. Moreover, metal wires are very stable, thus suitable for measurement over days, weeks, and even months.^[1]

Figure 2.5 Schemes of surface contact electrodes using brush (a) or pen (b). 1 – calomel electrode, 2 – container containing KCl electrolyte, 3 – camel hair brush (a) or felt-tipped pen (b), 4 – plant shoot or stem. Adapted with permission.^[18] Copyright 1973, Oxford University Press. Adapted with permission.^[19] Copyright 2006, Scandinavian Plant Physiology Society.

A frequently used non-invasive extracellular measurement is through surface contact electrodes.^[1] They are normally a metal electrode (Ag/AgCl, calomel electrode) making contact with dry plant surfaces (leaves, petioles, stems) through an electrolyte. The electrolyte can be borne in containers,^[14] on cotton threads (**Figure 2.4**),^[14, 20-21] on felt-tipped pens/brushes (**Figure 2.5**),^[18-19, 21] and in agar gel.^[20, 22] An obvious advantage of this method is the avoidance of tissue damage, enabling on-demand measurement without much preparation time, but a shortcoming is drying of electrolyte, making this method only applicable for short-term measurement within a few hours.^[1]

Figure 2.6 Setup for vibrating probe method, or MIFE (Microelectrode Ion Fluxes Estimation). **a)** Scheme of electrode structure and measuring principle. LIX, liquid ion exchanger. **b)** Photograph of a microelectrode in the proximity of a plant root and measuring principle. The ion-selective microelectrode is moved in a square-wave manner between two positions near the root surface. A voltage gradient (dV) is measured by the electrometer between two positions over the travel range dX . Reproduced with permission.^[23] Copyright 2006, Springer-Verlag Berlin Heidelberg. **c)** Photograph of an MIFE measuring system (dual channel test). (a) and (c): Reproduced with permission.^[14] Copyright 2009, National Natural Science Foundation of China and Chinese Academy of Sciences, published by Elsevier and Science in China Press.

Another non-invasive extracellular technique is the vibrating probe method,^[1] or MIFE (Microelectrode Ion Fluxes Estimation) in more advanced term.^[5] It is a technique where

a vibrating microelectrode tip is brought near the cell(s) of interest, and the ion-selective electrode measures local ionic flux through the difference in voltage when it vibrates (**Figure 2.6**). This method is rather limited in application scenario (plant parts soaked in a bathing medium) because it only works in liquid environment. Nevertheless, the ion selectivity and high spatiotemporal resolution make it useful in identifying ionic species responsible for observed membrane potential fluctuations.^[14]

2.3 Challenges in Surface Contact Measurements

As mentioned in Section 2.2.2, the non-invasiveness of surface contact electrodes makes them suitable for on-demand measurement without much preparation time in short-term electrophysiology study. Therefore, surface contact electrodes should be the most promising type of electrodes for plug-and-play integration between plants and electronics in applications like environmental sensing, crop health assessment, and interactive human-plant interface, enabling ad-hoc deployment without affecting normal plant physiology. However, current surface electrodes face several challenges in establishing reliable plant-electronic interfaces.

2.3.1 Challenges from Plant Surface Properties

Figure 2.7 Diagram of a mature plant cuticle indicating the different cuticular components.

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The biggest barrier for surface contact measurement is the insulating, waxy, and rough cuticle, the outmost layer covering the entire plant except roots. Plant cuticle (Figure 2.7) is made of primarily long carbon chain fatty acids (waxes and cutin), with good mechanical strength, barrier property, and water repellence to protect plants from environmental threats, such as insects, predators, toxins, etc.^[24-25] However, just like stratum corneum in human skin,^[26] cuticle contributes substantially to the interfacial impedance between plants and surface electrodes. This is firstly because of the chemical composition, which are all insulating polymers, and secondly because of the rough surface topography,^[11] detrimental to intimate interfacial contact.

Figure 2.8 Diverse surface topography of plants including micro/nano textures for superhydrophobicity (a-d) and hairs for self-protection and preying (e-g). **a)** Optical image of the *B. oleracea* L. leaf. **b-c)** Environmental scanning electron microscope (SEM) images of the leaf surface with a microstructured/nanostructured morphology. **d)** Water contact angle reveals the superhydrophobic property of the leaf surface. Reproduced with permission.^[27] Copyright 2017, The Authors, exclusive licensee American Association for the Advancement of Science. **e)** Seedlings of *Rudbeckia hirta* showing hairs as long as half of the width of the lamina. The stiff hairs on *Rudbeckia hirta* make them difficult to eat for some herbivores. Adapted with permission.^[28] **f)** Hairs on *Salvia glutinosa* can secrete a highly sticky glue to trap insects. Adapted

with permission.^[29] Copyright 2010, Springer-Verlag/Wien. **g)** Flower bud of a *Capsicum pubescens* plant, with many hairs. Adapted with permission.^[30] Copyright 2007, The Authors, under the Creative Commons ShareAlike 2.5 license.

Plant surface topography represents one of the most diverse morphology in the biological world. Intricate hierarchical nanostructures render plants superhydrophobicity (**Figure 2.8a-d**), lotus leaf being a classical example. Presence of trichomes, either sharp or dense, on crops like tomato, sunflower, and tobacco, as well as ornamental plants like Russian sage, catmint, and mullein, makes some plants appear inaccessible (Figure 2.8e-g). For superhydrophobic plant surfaces, contact through saline may be challenging because air pockets exist between water droplets and nanostructured surfaces. Moreover, strong water repellence may prevent adhesion of saline at all, so a mechanical support to hold the liquid saline in place is required. On the other hand, hairy surfaces may be accessible by liquid electrolyte, but completely incompatible with solid-state electrodes, which will be discussed in more detail when introducing current surface electrodes.

2.3.2 Challenges from Mechanical Disturbance

Relative movements between plant parts and electrodes in direct contact can be triggered by various intentional or unintentional mechanical stimuli, and these movements, if not accommodated by the plant-electrode interface, can affect signal recording fidelity and stability, manifested in signal artifacts, noises, and even signal disappearance. Therefore, ensuring stable contact between plants and electrodes even under mechanical disturbance is essential.

The first source of mechanical disturbance comes from thigmonastic movements of plants while electrodes are attached to the moving organs. Plants such as Venus flytrap can actuate fast and drastic motion of the trap organ, generating enough force and displacement to cause electrode detachment.^[31] Hence a certain level of adhesiveness and freedom to dislocate from the electrode fixation setup are necessary in surface electrodes to maintain their stable connection with thigmonastic plants. Relative movements also come from

mechanical wounding operation. In the study of wound-induced electrical signals, severe damage to plant parts needs to be practiced, such as scission and pressing.^[32-33] These damaging operations involve forceful contact with plants, which are likely to cause minor shaking as well as large-scale movements of plant parts, largely depending on the dexterity of operators. Sometimes mechanical structures constraining plants can help alleviate the issue,^[22] but not totally negate the problem. Additional fixture also makes the experiment complex and tedious to conduct, not realistic for plug-and-play deployment. Furthermore, for testing or application conducted in a more natural environment, wind, accidental touch, and many other unpredicted factors prevent plants from standing still. An adhesive and freely moving surface electrode should be designed to accommodate abovementioned mechanical disturbances.

2.4 Limitations of Hydrogel-Interfaced Surface Electrodes

Surface contact electrodes used in plant electrophysiology study differ in the form of plant-contacting electrolyte, whereas the metal electrode has not many variations, mainly Ag/AgCl and calomel electrodes to maintain a stable potential. The traditional ways of using cotton thread^[20-21] and felt-tipped pen/brush^[18-19, 21] are rarely used today because of the complexity and cumbersomeness of electrode setup. Instead, hydrogels including agar gel and chemically crosslinked hydrogels, are often used as the ionic interface between plants and electrodes.

2.4.1 Agar Gel Ionic Interface

Figure 2.9 Structure and morphology of agar molecules and gels. **a)** Molecular structure of the repeating unit of agarose, agarobiose. D-G = β -D-galactopyranose; L-AG = 3,6-anhydro- α -L-galactopyranose; AB = agarobiose. Agar is a mixture of agarose and agaropectin. The latter represents a group of polysaccharides that maintain the agarobiose sugar backbone but contain sulphuric acid, D-glucuronic and pyruvic acid substituents. Reproduced with permission.^[34] Copyright 1990, Springer Netherlands. **b)** Scheme of agarose gel network. Cross-links involve both double helix formation and substantial association of double helices to form microcrystalline junction zones. Reproduced with permission.^[35] Copyright 1987, Springer-Verlag Berlin Heidelberg. **c-d)** Confocal micrographs of 3% agar when cooled to 10 °C (at 1 °C min⁻¹) while shearing at (c) 40 s⁻¹ and (d) 750 s⁻¹. Reproduced with permission.^[36] Copyright 1999, Elsevier Science.

Agar powder is a mixture of polysaccharides derived from algae (**Figure 2.9a**). It dissolves in hot water and physically gels once cooled to room temperature. Bundles of double helices are formed during gelation (Figure 2.9b), giving rise to good strength and stiffness of agar gel. Meanwhile, agar can also be prepared as a viscous fluid with mechanical stirring during cooling.^[22] This mechanical disturbance results in a dispersion of gel particles (of micrometer sizes) in water (Figure 2.9c-d),^[36] which retains largely the fluidic character. Therefore, agar gelling solution or viscous agar solution can conform to most

plant surface textures and provide certain level of mechanical robustness to the contained electrolyte.^[20, 22] The wide availability and ease of preparation further make agar a recommended material in a recently published protocol for plant electrophysiological study.^[22]

Figure 2.10 Agar gel-based ionic interface. **a)** Schematics of agar gel bridging hairy plants with metal electrode with enlarged view of gel-solid interface showing weak adhesion. **b)** Photograph showing the setup of agar gel-based surface measurement on *Arabidopsis* plant. The four thin Ag/AgCl wires are positioned precisely by micromanipulators. **c)** Schematic top view of fixation of an *Arabidopsis* leaf for wounding experiment. The leaf to be wounded is drawn with solid lines and the neighboring leaves that would cover part of the support are drawn with dashed lines. (b) and (c): Adapted with permission.^[22] Copyright 2014, Nature America.

Nevertheless, agar gel is brittle and non-adhesive, originating from the rigid sugar backbone of agar polysaccharides^[35, 37] and high tendency of syneresis (i.e., extruding water on standing, which forms a lubricating liquid layer between gels and interfacing solids^[35-36, 38-39]). Therefore, it is still prone to detachment under mechanical disturbance

(Figure 2.10a). Furthermore, viscous agar solution is even weaker than quiescently set agar gel.^[36] Consequently, in previous studies, complex fixation setup is employed for both electrodes and plants (Figure 2.10b-c).^[22, 33] Obviously, such stringent requirement in plant and electrode positioning complicates testing procedure and does not allow measurement in natural settings.

2.4.2 Chemical Hydrogel Ionic Interface

The recent adhesive and tough hydrogels developed for bio-electronic interfacing^[40-42] may overcome the adhesive and mechanical weakness of agar gels/solutions. The interfacial adhesiveness between these hydrogels and solid surfaces mainly comes from two contributions: specific hydrogel-adherend interactions and maximized interfacial contact/entanglement. As examples of the first aspect, surface treatment^[43-44] and inclusion of certain functional groups in hydrogel polymers^[45-46] enable formation of strong bonds (e.g., covalent bonds, hydrogen bonds) between hydrogels and adherends, thus establishing robust interfacial bonding. For the second aspect, reducing crosslinking density is often found to enhance adhesive performance due to enhanced chain mobility on hydrogel surface to physically/chemically interact with adherends.^[47-48] In addition, infiltration of small molecules^[49] or uncross-linked polymers^[50] on permeable surfaces (polymers, tissues, hydrogels) followed by gelation brings about drastic improvement in bonding strength through topological entanglement.

Figure 2.11 Schematics of solid hydrogel bridging hairy plants with metal electrode with enlarged view of gel-plant interface showing non-conformability.

Most of these hydrogels contain chemical crosslinks to enhance mechanical and adhesive

properties, and so gelation is often done physically away from the adherends for crosslinking to take place. The resultant freestanding hydrogels largely retain solid character, which prevents conformal contact with macroscopically rough surfaces, such as hairy surfaces (**Figure 2.11**). The difficulty in achieving conformal contact is twofold. First, the small radius of curvature of hair tips and the large aspect ratio of hairs make the real contact area between plants and electrodes much smaller than the nominal contact area (**Figure 2.12**), which results in a drastic reduction in adhesion force.^[51] Secondly, as some plant hairs serve important physiological functions and are easily damaged by external forces,^[25] minimal pressure (often used to improve electrode-tissue contact and adhesion^[46, 52]) can be applied to facilitate electrode attachment. As a result of such poor contact, not only adhesion force will be dramatically reduced, but also signal fidelity will be severely deteriorated.^[6]

Figure 2.12 Non-conformal interface between hairy plants and thin-film electrodes. **a)** Schematic illustration of much reduced real contact area (pink) compared to nominal contact area (black). **b)** Photograph of a thin-film electrode (Au nanofilm on $\sim 70\ \mu\text{m}$ thick PDMS (polydimethylsiloxane) substrate) commonly employed in bio-interfacing electronics^[53-55] standing on a hairy leaf, as a realistic illustration of (a).

On the other hand, it is possible to chemically crosslink pre-gel solutions on adherend surfaces in situ, so that conformability can be greatly enhanced.^[49] However, these processes often involve use of toxic chemicals, UV irradiation,^[50] and other factors that are noxious to plants. In this regard, physical hydrogels that can gelate within short time periods without harmful stimuli are most desirable, just like agar gels.

2.5 Thermogel as A Promising Solution

Thermogel, short for thermo-responsive hydrogel, is a water-rich polymer network that undergoes reversible sol-gel transition with temperature change.^[56-57] Normally, a thermogel exists as a liquid at low temperature (e.g., room temperature, 25 °C) and a solid gel at higher temperature (e.g., body temperature, 37 °C). Due to the unique gelling behavior at body temperature, thermogel is commonly used in minimally invasive biomedical applications, such as injectable drug carrier,^[58-59] antibacterial wound dressing,^[60] and vitreous substitute.^[61-62]

Figure 2.13 Mechanical properties in shear of agarose gels and thermogel. **a)** Phase angles obtained from frequency sweeps of agarose gels at varying concentration, showing minor viscoelasticity. Agarose is the main gelling component of agar gel. Phase angle is calculated by $\tan^{-1}(G''/G')$. Reproduced with permission.^[63] Copyright 2005, Elsevier. **b)** Storage and loss moduli obtained from frequency sweeps of 12% EPC thermogel (a representative thermogel). Phase angle at 1 Hz is $\sim 25^\circ$, showing much larger viscoelasticity than agarose gels. Adapted with permission.^[62] Copyright 2019, The Authors, under exclusive licence to Springer Nature.

Thermogel may be a promising material as the ionic interface between hairy plants and metal electrodes, overcoming the shortfalls in adhesiveness and conformability of agar gel and solid hydrogels, respectively, based on the following considerations:

1. The sol-gel transition permits liquid-phase application of pre-gel solution on plants, thereby achieving conformal contact on hairy plants. This sol-gel transition

resembles the gelling behavior of agar solution, but the direction of transition with respect to temperature is reverse. In other words, application of thermogelling solutions at a temperature lower than the ambient temperature will permit in situ gelation on plants.

2. Thermogelling polymers normally incorporate highly flexible chains/chain segments for reversible crosslinking and micelle formation,^[56] and once gelated thermogels possess substantial viscoelasticity^[62, 64] (**Figure 2.13**). This implies their bonding with solid surfaces will be enhanced, as in the case of reducing crosslinking density of chemical hydrogels.
3. As thermogelling polymers normally gelate at much higher concentration (3% – 20%)^[56] than agar (0.2% – 2%)^[65], their interfacial bonding with adherends should be stronger than agar gels, given only physical interactions at interface are involved for both systems.
4. Thermogel contains largely water, so its ionic conductivity should be sufficient for electrical coupling when salt species are added.

2.6 Gap Statement

In summary, hydrogel is the dominant plant-interfacing material in surface contact electrodes for non-invasive plant electrophysiology, due to its ionic conductivity, shape adaptability, and biocompatibility. However, current physical hydrogels, predominantly agar gels, and chemical hydrogels cannot fully address the challenges from plant surface properties and mechanical disturbance during testing. Specifically, agar gels/solutions lack the adhesiveness for mechanical tolerance, and chemical hydrogels lack the conformability for signal fidelity as well as robust adhesion. Therefore, there is a need to develop a conformable and adhesive ionic interface to effectively bridge plants with surface electrodes.

To address the above research gap, this thesis proposes and examines the efficacy of using thermogel as the ionic interface between plants and surface electrodes. Hairy plants are chosen as a representative topologically complex surface. Improved adhesiveness over agar

gels is expected to permit simplified electrode setup, while improved conformability is expected to deliver higher signal recording quality than chemical hydrogels. Experiments are conducted to testify the above hypotheses.

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Chapter 3

Experimental Methodology

This chapter introduces and rationalizes the materials and techniques used in this study. General background, working principles, as well as rationale for selection of each material and technique are given without details on the experimental procedures. In particular, EPC thermogelling polymer was chosen as the demonstrative polymer due to favorable properties for plant application. Two forms of agar gels previously used in plant electrophysiological study and a representative chemical hydrogel PAAm served as controls. Microscopic observation and adhesive tests were used to verify the conformability and adhesiveness of different hydrogels on hairy plants. Rheological and electrochemical impedance characterizations were carried out to study the mechanical and electrical properties. Other techniques including surface tension and contact angle measurement, UV-Vis spectroscopy, and confocal microscopy were employed in an attempt to explain the molecular origins of observed properties. Lastly, plant electrophysiological measurements were conducted on several types of plants to test the performance of hydrogels.

3.1 Overview of Rationale

The current study involves the following experimental segments: preparation of hydrogel materials, characterization of material properties, assembly of electrodes, and electrophysiological measurements on plants. Selection of thermogel material is based on application requirements; selection and preparation of control gels is to be representative of conventional approaches and at the same time ensure the best possible performance for the intended usage; characterization is conducted only on properties that affect performance during electrophysiological measurement; design of electrode configuration is to keep application and operation simplistic; plant electrophysiology experiments follow standard protocols in previous studies to ensure comparability of current results and provide stronger reference support.

Overall, the selection of materials, procedures, and techniques serves to testify the hypothesis and achieve the objectives of the current study. Detailed rationale for each material and technique will be provided in the following sections.

3.2 Synthesis

Three types of hydrogels were prepared: thermogel, agar gel, and chemical hydrogel. Agar gel and chemical hydrogel serve as control samples to compare with thermogel. All gels were prepared in saline solution to render conductivity for electrophysiological measurement.

3.2.1 Thermogel Preparation

A multi-block copolymer synthesized from polyethylene glycol (PEG), polypropylene glycol (PPG), and polycaprolactone (PCL), denoted as EPC (short for poly(PEG/PPG/PCL urethane)) (**Figure 3.1**)^[1-2] was used in this study as a demonstrative thermogelling polymer. Thermogelling solutions made of EPC were previously shown to possess drastic rheological change upon sol-gel transition^[1] with a low critical gelation concentration (3%)

and transition temperatures (20 – 40 °C) near ambient temperatures.^[2] A low critical gelation concentration will facilitate free movement of ions, thus promoting conductivity; a transition temperature near ambient temperature will be suitable for plant application. Therefore, EPC thermogelling polymer was selected as the precursor of thermogel used in this study. It should be noted that other thermogelling polymers may also be applicable,^[3] and many concepts introduced in this thesis can be applied to other thermogelling systems. To prepare EPC thermogel, EPC polymers are dissolved in water at 4 °C. The solution will solidify into a gel within a minute once its temperature is brought to higher than its sol-gel transition temperature.

Figure 3.1 Molecular structure of EPC copolymer.^[1]

The sol-gel transition behavior of EPC solution originates from dehydration of PPG segments upon temperature increase, causing hydrophobic associations between segments of a single polymer and between different polymer chains, thereby forming a continuous crosslinked polymer network.^[4] Both PPG and PEG exhibit lower critical solubility temperature (LCST) in water, which means when the temperature increases to a threshold value, the polymer will no longer be soluble regardless of concentration. PPG has a LCST in water in the range of 10 – 30 °C and PEG has a LCST in water in the range of 100 – 150 °C.^[3] Therefore, for applications around room temperature and body temperature, PEG will stay soluble, or hydrophilic in solution. However, PPG will become insoluble, or hydrophobic once temperature increases to higher than its LCST.

Such reduced solubility with increased temperature is somewhat counter-intuitive in that insoluble substances normally become soluble upon heating. This is a result of battling between entropy and enthalpy, summarized in the following expression for Gibbs free energy change:

$$\Delta G = \Delta H - T\Delta S \quad \text{Equation 3.1}$$

where ΔH denotes enthalpy change, ΔS denotes entropy change, and T is temperature. Gelation of block copolymers consisting of PPG, PEG, with or without small proportions of a more hydrophobic polymer (e.g., poly[(R)-3-hydroxybutyrate], PHB, and PCL) was previously found to be entropy-driven.^[5-7] Enthalpy change due to the breakage of polymer-water interactions and formation of polymer-polymer interactions is positive ($\Delta H > 0$), meaning less energetically favorable. Nevertheless, the strong polymer-water interaction through hydrogel bonding in solvated state results in largely restricted molecular motion for both polymer and water. Once temperature increases, ordered water molecules are released from bonding with polymer chains and so polymer chains become more flexible like in polymer melts, resulting in entropy gain ($\Delta S > 0$) that surpass the effect of positive enthalpy change. The net consequence is a decrease in free energy ($\Delta G < 0$), favoring water expulsion and gelation, which dictates the direction of spontaneous reaction.

3.2.2 Agar Gel Preparation

Agar is a common gelling polymer used for biological studies. It dissolves in hot water at low concentrations (normally $>90^\circ\text{C}$ and $<2\text{ wt\%}$) and gelates into a solid gel when temperature reduces to $\sim 40^\circ\text{C}$.^[8] Agar fluidic gel (also known as viscous agar solution) was recently reported for plant surface electrophysiological recording,^[9] where the solution gelates while being mechanically stirred and a gel particle-water mixture is obtained and stored for long-term usage (more than a month). However, the protocol was not followed entirely in this study, because such fluid gel easily flows and does not adhere to any surface. Moreover, agar gel formed from quiescent setting of warm solution (i.e., without mechanical interference such as shaking and stirring) possesses the highest possible mechanical stiffness and strength.^[10] In testing such type of agar gel, EPC thermogel would be compared with a stronger control gel.

Therefore, freshly dissolved agar solution stored at $50 - 60^\circ\text{C}$ (to prevent gelation before test commencement) was used in the current study. Prolonged heating may cause polymer degradation, so agar gelling solution was stored at room temperature (in monolithic gel

state) after experiment, and re-liquified by heating every time before testing. Meanwhile, viscous agar solution was also tested for rheological and mechanical properties to compare with agar gelling solution.

3.2.3 Chemical Hydrogel Preparation

Polyacrylamide (PAAm) hydrogel was selected as the control gel for chemically crosslinked solid hydrogel because it is one of the most common hydrogels used for biological research, easy to prepare, and safe for plant tissue contact. Thermally induced radical polymerization was employed to synthesize PAAm hydrogel from precursor solution composed of monomer, crosslinker, initiator and accelerator in water. The crosslinker concentration was adjusted to a relatively low level to maximize adhesive strength. According to previous studies,^[11-12] the interfacial adhesion of chemically crosslinked hydrogel on rough surfaces increases with decreasing crosslinking density, as a result of enhanced chain mobility to follow the surface roughness. In testing such soft and sticky PAAm hydrogel, EPC thermogel will be compared with a strongly adhesive chemical hydrogel.

3.2.4 Electrode Preparation

Ag/AgCl is the gold standard electrode for electrophysiological testing due to its ability to maintain a stable potential despite external potential fluctuations in tested objects. For the same reason, it is a common type of reference electrode. Electrolytic chlorination was conducted on bare Ag plates or Ag wires to prepare Ag/AgCl electrodes used in this study. Ag plates or wires were immersed in HCl electrolyte together with Pt counter electrode. Reaction proceeded according to the following half-cell reactions under DC voltage bias:

3.3 Characterization

3.3.1 Rheological Characterization

Rheological characterization is an important technique to study the mechanical/rheological properties of viscoelastic solids/liquids in shear. For solid materials like hydrogels, large shear deformation is not possible. Hence oscillation shear tests are commonly practiced, where small oscillated shear strains are applied without damaging the samples. In this study, two modes of operation were employed: amplitude sweep, and temperature ramp.

3.3.1.1 Amplitude Sweep

Figure 3.2 Illustration of determining linear viscoelastic range from amplitude sweep tests.

In amplitude sweep, samples experience oscillations at constant frequency and temperature but varying amplitude, usually from small to large values across several orders of magnitude. This test is usually the first to conduct on an unknown material to find out the linear viscoelastic range (LVR). As shown in **Figure 3.2** Illustration of determining linear viscoelastic range from amplitude sweep tests. , LVR is determined as the oscillation strain range where G' (storage modulus) and G'' (loss modulus) are almost invariant. Within LVR, the strain is small enough to preserve the internal structure of tested materials such that no mechanical property alterations are induced by the application of oscillation strains. Subsequent testing in other modes, like temperature ramp and frequency sweep, is normally conducted within LVR. On the other hand, LVR reflects a material's ability to

resist irreversible structural destruction. A larger upper limit of LVR indicates stronger resistance to shear-induced structural rearrangement. Such structural changes result from molecular conformations and interactions within material systems, such as polymer chain sliding, physical crosslink disruption, etc.

Figure 3.3 Determination of yield strength using dynamic stress sweep method.

This critical strain value on the onset of irreversible deformation is also termed yield strain, and the corresponding stress is the yield strength. Analogous to static tensile tests, yield strain defines the elastic region, and yield strength is the upper bound of stress without causing plastic deformation. Using amplitude sweep for yield analysis is called dynamic stress/strain sweep method (stress or strain depending on the rheometer setting, i.e., stress-controlled or strain-controlled), and it is especially useful for high-viscosity materials in the efficacy of preventing wall slippage. A practical guide in determining yield point from a graph of G' vs. oscillation stress is given in **Figure 3.3**.

3.3.1.2 Temperature Ramp

In temperature ramp, samples are exposed to constant oscillation amplitude (strain) and oscillation frequency within LVR, while sample temperature is changed in a continuous manner according to programmed procedures. This test gives information of mechanical properties under varying temperature, which is a fundamental characterization of

temperature-responsive hydrogels. By recording shear strain and shear stress as a function of temperature, critical parameters can be obtained: storage modulus (G'), loss modulus (G''), $\tan\delta$ (G''/G'), complex viscosity (η^*), complex modulus (G^*), etc. Among these parameters, $\tan\delta$ is especially important in gauging the sol-gel transition of thermogelling solutions. A $\tan\delta$ larger than 1 indicates a higher tendency of viscous flow (irreversible deformation), whereas a $\tan\delta$ smaller than 1 indicates a higher tendency of elastic deformation (reversible deformation). The former signals a viscous sol, and the later signals an elastic gel. While there is normally no sharp change of mechanical properties when $\tan\delta$ passes 1 as temperature varies, a sol-gel transition temperature is defined as the temperature where $\tan\delta$ equals 1.

3.3.2 Scanning Electron Microscopy

To visualize the contact between hydrogels with hairy leaves, microscopy has to be used. Scanning Electron Microscopy (SEM) was used because of higher resolution and depth of field than optical microscopy. Secondary Electron Imaging (SEI) was conducted on cross-sectioned samples for topographical information at the gel-leaf interface.

Figure 3.4 Signals generated when an electron beam bombards a sample surface. Image reproduced from <https://www.nanoscience.com/techniques/scanning-electron-microscopy/components/>. Copyright 2020, Nanoscience Instruments.

When a beam of electrons is shot on the sample surface, many types of signals are generated simultaneously (**Figure 3.6**Error! Reference source not found.). In SEI, secondary electrons generated from inelastic interaction of primary electronics with sample atoms are collected and counted to produce an image. Because SEs only originate from the top surface of a sample, it gives information on surface topography, i.e., how the sample looks like in shape. For biological samples and other non-conductive samples, a thin layer of conductive coating is necessary to improve contrast and resolution, as well as to prevent burning damage to samples due to accumulation of excess electrons within the area of scanning.

3.3.3 Adhesive Strength Measurement

Figure 3.5 Lap-shear test (a) and tensile test (b) for adhesive strength measurement. Image adapted with permission.^[13] Copyright 2019, The Authors, under exclusive licence to Springer Nature.

Commonly used procedures for measuring adhesive strength include lap-shear tests and tensile tests (**Figure 3.5**Error! Reference source not found.). They respectively quantify the shear and tensile strengths of an adhesive on a certain adherend. During testing, force as a function of displacement is recorded using a mechanical tester, and the largest force until fracture is extracted for computation of strength according to the following equation:

$$\text{Adhesive strength} = \frac{F_{max}}{WL} \quad \text{Equation 3.4}$$

This equation applies for both shear and tensile strength calculation. Moreover, integrating the area under stress-strain curve gives adhesive energy, which is another important parameter to evaluate the adhesion performance of an adhesive.

For plant electrophysiology, shear is a more common type of stress than tension experienced by surface electrodes attached on stems (which is the plant electrophysiology setup demonstrated in this study). Therefore, adhesion performance characterization of hydrogels was conducted in lap-shear tests. On the other hand, to investigate the adhesion property of agar gel in more depth and more quantitatively, tensile adhesion tests were conducted on agar gels of varying concentrations. The choice of tensile tests instead of shear tests is based on the following considerations. First, interfacial friction contributes more significantly to shear adhesion than tensile adhesion, so tensile adhesion tests can reflect the interfacial bonding more authentically. Secondly, injection of pre-gel solution in a tensile configuration (horizontal) is easier to implement than in a shear configuration (vertical) so that more repeatable and accurate results can be obtained.

3.3.4 Electrochemical Impedance Spectrometry

Electrochemical Impedance Spectrometry (EIS)^[14] is a powerful approach to characterize the electrical conduction property of a material or material combinations (electrochemical systems). Resistance, R , describes the behavior of an ideal resistor, which follows the Ohm's law,

$$R = \frac{E}{I} \quad \text{Equation 3.5}$$

where E is voltage and I is current, under a handful of assumptions, including independency of current and voltage levels, independency of frequency, and AC signals being in phase. However, in many (in fact most) electrochemical systems, the current-to-voltage response does not fit into these assumptions and cannot be described simply by Ohm's law. Therefore, impedance, Z , is introduced to give a more comprehensive, realistic description of the resistance to electric current flow.

In EIS, a sinusoidal AC voltage at a preset small amplitude is applied across a sample (often an electrochemical cell) through working electrode and counter electrode (a reference electrode can be included for more accurate potential control), scanning through a frequency range. The applied voltage, E , is a function of time, t , angular frequency, ω , and amplitude, E_0 , in the following form,

$$E_t = E_0 \sin(\omega t) \text{ and } \omega = 2\pi f \quad \text{Equation 3.6}$$

where f is frequency in hertz. The response current I also has a sinusoidal waveform, where a phase shift of φ exists between I and E .

$$I_t = I_0 \sin(\omega t + \varphi) \quad \text{Equation 3.7}$$

Then the impedance Z is defined as

$$Z = \frac{E_t}{I_t} = \frac{E_0 \sin(\omega t)}{I_0 \sin(\omega t + \varphi)} = Z_0 \frac{\sin(\omega t)}{\sin(\omega t + \varphi)} \quad \text{Equation 3.8}$$

Mathematical transformation of the above equations gives expression of Z as a complex number:

$$Z(\omega) = Z_0(\cos\varphi + j\sin\varphi) \quad \text{Equation 3.9}$$

Common data representation of impedance includes Nyquist Plot and Bode Plot. In the former, the real part and the imaginary part of Z at each frequency is plotted with x axis denoting real part and y axis denoting negative imaginary part, respectively. This plot does not tell information about frequency. In contrast, Bode Plot displays the absolute value of impedance, $|Z| = Z_0$, and phase shift φ against frequency.

Table 3.1 Electrical circuit elements used for EIS analysis and their characteristics.

Component	Parameter	Current vs. voltage	Impedance	Phase shift	Frequency dependency
Resistor	R	$I = \frac{E}{R}$	$Z = R$	0°	None
Capacitor	C	$I = C \frac{dE}{dt}$	$Z = \frac{1}{j\omega C}$	90°	Negative correlation

Electrical circuit models are often fitted to experimental results for detailed analysis of electrical properties within an electrochemical system. The most frequently used circuit elements for modelling are resistors and capacitors. Their characteristics are summarized

in **Table 3.1**. Electrical circuit elements have their physical meanings to the electrochemical system under testing. Resistor is often used to model solution/electrolyte resistance, and capacitor is often used to describe electrical double layer established on the surface of an electrode inserted in electrolyte. While model fitting gives more accurate estimation of electrical properties within a system, the characteristics listed in Table 3.1 provide a handy rule of thumb for gaining a rough understanding of the system being tested. For example in a system where an electrode is inserted in an electrolyte, since impedance of bulk solution is independent of frequency (modelled by resistor) and impedance of electrode-electrolyte interface decreases with increasing frequency (modelled by capacitor), the total impedance at low frequency might be dominated by interfacial impedance and at high frequency by bulk impedance, respectively.

3.3.5 Surface Tension and Contact Angle Measurements

Dilute solutions of EPC and agar polymers were used to investigate their surface activity through surface tension and contact angle measurements. Surface activity is the tendency of molecules to adsorb at an interface between two or more phases in contact.^[15] Such spontaneous adsorption lowers the interfacial energy, and can profoundly affect the wetting behavior of a liquid on solids. In this study, the liquid of interest is aqueous saline, as the solvent in hydrogels, and the molecules in interest are EPC copolymers and agar polymers, as both of them form gelling solutions.

Figure 3.6 Setup of pendant drop method for surface tension measurement. Image reproduced from <https://www.dataphysics-instruments.com/knowledge/>. Copyright, DataPhysics Instruments GmbH.

Pure saline was added with EPC and agar polymers respectively in a range of

concentrations. Surface tension of the resultant solutions were measured in air using pendant drop method (**Figure 3.6**). Briefly, a liquid droplet was dispensed from a needle and suspended in air. A photo is captured from side and the shape of the pendant drop is analyzed using computer software. Lastly, Young-Laplace fitting of the droplet geometry gives its surface tension. Young-Laplace equation describes the relationship between pressure across an interface and surface curvature, as following:

$$\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad \text{Equation 3.10}$$

where γ is surface tension, R_1 and R_2 are principal radii of curvature. The shape of a pendant droplet is determined by the balance between surface tension, which tends to minimize surface area thus forming a spherical droplet, and gravitation, which drags the droplet to become more elongated. Surface tension causes Laplace pressure, while gravitation causes hydrostatic pressure. Thus, the total pressure difference across an interface is the sum of the two components, as reflected in the following equation (at a distance z from an arbitrary reference plane):

$$\Delta P(z) = \Delta P_0 \pm \Delta \rho g z \quad \text{Equation 3.11}$$

On the right side of the equation, the first term is Laplace pressure, which depends on surface tension and droplet shape, and the second term is hydrostatic pressure, which depends on density of liquid, gravitational constant, and shape of droplet. The computer software performs numerical approximation of differential equations derived from $\Delta P = \gamma \left(\frac{1}{R_1} + \frac{1}{R_2} \right)$ Equation 3.10 and $\Delta P(z) = \Delta P_0 \pm \Delta \rho g z$

Equation 3.11 with input variables (liquid density, local gravitational constant) and geometrical parameters from image analysis, for the calculation of surface tension.

Figure 3.7 Setup of sessile drop method for contact angle measurement. Image reproduced from <https://www.dataphysics-instruments.com/knowledge/>. Copyright, DataPhysics Instruments GmbH.

Figure 3.8 Illustration of contact angle at a solid-liquid-gas contact line. Reproduced with permission.^[16] Copyright 2016, Springer International Publishing Switzerland.

Contact angle of dilute solutions of EPC and agar polymers on various solids were measured in air using sessile drop method (**Figure 3.7**). The procedure is similar to pendant drop method. The difference is that a liquid droplet is now brought in contact with a solid surface with needle withdrawn. At equilibrium, the solid-liquid-gas three-phase contact line should be like that illustrated in **Figure 3.8**. The computer software will analyze the captured image to get the value of contact angle, θ . θ is reflective of the relative magnitude of surface and interfacial tensions between the three phases. The simplest model to describe their interrelation is Young equation:

$$\gamma_L \cos \theta = \gamma_S - \gamma_{SL} \quad \text{Equation 3.12}$$

where γ_L is the surface tension of liquid, γ_S is the surface tension of solid, and γ_{SL} is the interfacial tension between liquid and solid. In the present study, contact angles of different aqueous solutions (pure saline, added with agar, or added with EPC) are compared on a certain solid, where γ_S is a constant. Hence changes in γ_{SL} can be inferred from measured θ and making assumptions on γ_L gotten from surface tension measurements. Importantly, γ_{SL} provides insights into the physical interactions between polymers in solution and solid surfaces.

3.3.6 Dye Solubilization for Micelle Detection

Dye solubilization is a standard technique used for determination of critical micellar concentration (CMC). By measuring the light absorption of a dye, whose absorption behavior is highly sensitive to local chemical environment, in the solution of a micelle-forming polymer, formation of micelles can be detected.

1,6-diphenyl-1,3,5-hexatriene (DPH) was used as the hydrophobic dye in this study, which is non-fluorescent in water but exhibits strong fluorescence when exposed to hydrophobic environment. In a polymer solution added with DPH, when there is no micelle formation, DPH is directly exposed to water and does not show obvious UV-Vis light absorption. When the polymer concentration is sufficiently high for micelle formation, DPH is enclosed within the hydrophobic core of micelles, where water is expelled from the close proximity of DPH. In this case, strong absorption of UV-Vis light can be observed. Therefore, by comparing the UV-Vis absorption spectra of solutions at different polymer concentration, CMC can be determined. In this study, dye solubilization was not used for CMC determination. Instead, it was used as a qualitative examination of micelle formation of a certain solution composition at different temperatures.

The absorption of light by organic molecules originates from electron excitation from a lower energy level to a higher energy level. The molecular electronic state distribution is highly sensitive to the chemical environment in direct contact with the molecule, such as solvents used to dissolve the molecule, or intercalation into lipid membranes (often used for cell membrane imaging). In the case of DPH in aqueous solution, as water is a highly polar solvent, it is able to effectively distort the electronic clouds around DPH and so alter the absorption spectrum.

Figure 3.9 Simplified configuration inside a UV-Vis spectrometer. Image reproduced from <https://www.ssi.shimadzu.com/products/uv-vis-spectrophotometers/faqs/instrument-design.html>.

Copyright, Shimadzu Scientific Instruments.

Absorption spectrum is collected using UV-Vis spectrometer, where a beam of monochromatic light is shine upon a solution contained in a cuvette, and the passed light is detected at the other end of the light path (**Figure 3.9**). Absorbance is defined as

$$A = \log_{10} \frac{P_0}{P} \quad \text{Equation 3.13}$$

where P_0 and P refer to light power before and after passing through the sample, respectively. A is governed by the Beer-Lambert law:

$$A = \epsilon bc \quad \text{Equation 3.14}$$

ϵ is the molar absorptivity (material specific), b is the path length, and c is the concentration of compound in solution. Since the path length is fixed by using the same type of cuvette, concentration of light-absorbing compounds must be kept constant when comparing other features, such as in dye solubilization tests.

3.3.7 Confocal Microscopy

Confocal microscopy is a powerful technique for visualizing internal structure of biological samples in 3D, commonly used for cell imaging. Samples are usually stained with fluorescent dyes. A laser of wavelength corresponding to the excitation wavelength of the dye shines upon the sample, and the emitted photons are collected and counted. The difference between confocal microscopy and normal optical (widefield) microscopy lies in its ability to collect light generated only on a single plane, rather than across a range of depth (**Figure 3.10**). This ability enables distinction of features in vertical direction (z axis) with resolution down to sub-micrometer. A stack of images at different z height allows reconstruction of a 3D model of the sample. Moreover, multiple dyes can be used simultaneously to label different molecules within a sample, so a comprehensive spatial distribution of structures of interest can be produced.

Figure 3.10 Comparison of widefield microscope (a) and confocal microscope (b). Image reproduced from <https://www.ptglab.com/news/blog/if-imaging-widefield-versus-confocal-microscopy/>. Copyright 2019, Proteintech Group.

In this study, molecular distribution of gelling polymers (EPC and agar) within an aqueous droplet was investigated using confocal microscopy. Hydrophilic fluorescent microbeads were modified on agar polymers, and hydrophobic DPH was mixed with EPC to probe the internal hydrophilicity/hydrophobicity distribution.

3.4 Plant Electrophysiology

Plants generate potential fluctuations in response to many environmental stimulations. In this study, mechanical wounding and heat were selected as the stimuli to induce endogenous electrical signals, because they are commonly studied in plant biology, easy to control, and reproducible.

Figure 3.11 Photographs of sunflower (a) and tobacco (b) plants used in this study, showing hairs on stems.

Arabidopsis thaliana was used as a control plant to test the usability of thermogel-based surface electrodes. It has minimal hairs on leaves and is frequently studied in plant biology as a model plant. A recent study reports on its electrical signaling behavior and genetic origin.^[17] Furthermore, sunflower and tobacco plants were selected to demonstrate usage of different hydrogels in capturing electrical signals on hairy plants, because they have noticeable hairs on stems (**Figure 3.11**) and are previously studied^[18-24] so that reference to previous reports can be drawn to validate the results obtained in the current study. Moreover, both sunflower and tobacco plants are economic crops, study of which has practical significance.

3.5 Data Analysis

For all measurements conducted in this study, wherever possible, multiple replications (more than three) of tests were done to improve the accuracy and reliability of data acquired. For plant electrophysiology, more data points were collected due to the natural variation among individual plants. Statistical analyses were conducted to compare the difference between groups of samples, including unpaired or paired t-tests, and ANOVA (analysis of variance).

3.6 Overview of Methodologies

This study encompasses the synthesis, processing, and application of a material, as well as relevant characterization and mechanism study, which requires a wide range of experimental techniques and analytical methodology. The selection rationale and working principles are discussed and introduced in this chapter, which provides strong technical and knowledge background for discussions in the following chapters.

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Chapter 4

Design and Properties of Thermogel Ionic Interface

This chapter details the design, optimization, and characterization of thermogel ionic interface. The target properties of thermogel material were firstly set according to the design of surface contact electrode. Formulation optimization of thermogelling polymer solution was then conducted in terms of polymer and salt concentrations. Interfacial conformability to hairy plants and adhesive properties were verified. Moreover, rheological, mechanical, and electrical characterizations of thermogel all showed comparable or superior performance to conventional hydrogels. These results prove that thermogel can establish an adhesive and conformal ionic interface, and also point to the promise for surface contact electrophysiology on hairy plants.

4.1 Introduction

To achieve the first two objectives of the current study, i.e., 1) to design and fabricate a thermogel ionic interface that is conformable and adhesive to hairy plants, and 2) to design and fabricate a thermogel-based surface contact electrode, the first few tasks include proposal of electrode design, specification of target thermogel properties, selection of thermogel material and optimization, and property characterization with a focus on conformability and adhesiveness. Only if the thermogel material developed satisfies application requirements and outperforms conventional hydrogels, can it be promising for electrophysiological study on hairy plants. Therefore, in this chapter, design, fabrication, optimization, and characterization of thermogel material in comparison with conventional hydrogels are discussed in detail.

4.2 Experimental Methods

4.2.1 Hydrogel Preparation

Synthesis of EPC polymer: The synthesis protocol follows a previous work.^[1] Poly(PEG/PPG/PCL urethane) (denoted as EPC) is synthesized from polyethylene glycol (PEG), polypropylene glycol (PPG), and poly(ϵ -caprolactone)-diol (PCL-diol) using hexamethylene diisocyanate (HMDI) as a coupling reagent. HMDI is added in equal molar amount as the molar sum of PEG, PPG, and PCL-diol. Typically, 12 g of PEG ($M_n = 2050 \text{ g mol}^{-1}$, $5.85 \times 10^{-3} \text{ mol}$), 3 g of PPG ($M_n = 2000 \text{ g mol}^{-1}$, $1.50 \times 10^{-3} \text{ mol}$), and 0.15 g of PCL-diol ($M_n = 2000 \text{ g mol}^{-1}$, $7.50 \times 10^{-5} \text{ mol}$) were weighed out into a 250 mL round bottom flask. The reactants were then dissolved in 40 mL anhydrous toluene at 60 °C for 10 min before undergoing azeotropic distillation to remove moisture from the reactants. Azeotropic distillation was carried out until the reactants become a slurry. The slurry was dissolved in another 40 mL of anhydrous toluene at 60 °C for 10 min before doing a second round of azeotropic distillation. The slurry obtained was further dried under high vacuum for 3 h at 110 °C to ensure complete removal of water. After the high vacuum step was completed, the slurry was dissolved in 10 mL of anhydrous toluene and two drops of

dibutyltin dilaurate (~8 mg) were added sequentially. 1.23 mL (7.09 mmol, 103%) of HMDI was then injected into the round bottom flask to start polymerization. The reaction was done at 110 °C for 24 h. During the first 4 h, 10 mL of anhydrous toluene was added each time whenever the reaction becomes too viscous to be stirred. After 24 h, the reaction solution was precipitated in 2 L of diethyl ether. The crude polymer product was blown dry of the ether and re-dissolved in 150 mL isopropyl alcohol (IPA) at 60 °C for 30 min.

The polymer in IPA solution was transferred into dialysis tube of 3.5 kDa cut-off molecular weight and dialyzed against 2 L of deionized (DI) water. The tub of DI water was changed twice each day and the dialysis was done for a total duration of 3 days. After dialysis was completed, the polymer solution in the dialysis tube was frozen in 50 mL centrifuge tubes and freeze dried for 5 days to obtain the pure EPC polymer. The yield was 85% after isolation and purification. The synthesized polymer was stored at below -20 °C to minimize hydrolysis degradation. Weight ratio (%) of EG/PG/CL is around 81.3:17.4:1.3 and molar ratio of EG/PG/CL is around 165.8:26.9:1.0. $M_n \approx 25000 \text{ g mol}^{-1}$, $M_w \approx 38000 \text{ g mol}^{-1}$, and PDI ≈ 1.52 .^[1]

Preparation of EPC thermogelling solutions: EPC thermogelling solutions were prepared at different polymer concentrations and salt concentrations. First, NaCl (Sigma-Aldrich, 746398) was added to DI water at desired concentrations to prepare saline stocks. EPC polymer (equilibrated at ambient temperature if taken from fridge) was weighted and added in a saline stock at a desired concentration. The mixtures were allowed to equilibrate at 4 °C for 3 days to ensure complete solvation.

Preparation of agar solutions: Agar solutions are prepared in two forms: viscous agar solution and agar gelling solution. The preparation of viscous agar solution followed the protocol by Mousavi et al.^[2] Briefly, agar powder (Sigma-Aldrich, A1296) was weighted and added in physiological saline (0.9% NaCl) at 0.5 wt%. The mixture was heated to boil and kept boiling for 10 min before cooling to room temperature with magnetic stirring at 300 rpm. The resultant mixture is a viscous translucent liquid, stable at room temperature for months. To prepare agar gelling solution, the same ingredients were mixed and heated

to boil, but only cooled to 50 °C without gelation. As-prepared solution can be directly used for testing. If not used immediately, the solution was naturally cooled at ambient temperature, when it became a solid gel. Next time before usage, the gel was reheated to boil for 5 min and cooled to 50 °C.

Preparation of PAAm hydrogel: To prepare PAAm hydrogel with different shapes and sizes, plastic (polylactic acid) molds printed by a Ultimaker 2 desktop 3D printer (Ultimaker BV) were used. Precursor solution for synthesizing ~11 wt% PAAm was prepared as following. First, 0.11 g acrylamide (Sigma-Aldrich, A8887) and 5.5 μL 10 mg mL^{-1} N,N'-methylenebis(acrylamide) (Sigma-Aldrich, 146072) as crosslinker were added in 1 mL physiological saline. The mixture was bubbled with blowing N_2 for 8 min. In a separate container, 100 mg ammonium persulfate (APS) (Sigma-Aldrich, 215589) was dissolved in 1 mL DI water and N_2 -bubbled for 8 min. Next, 5 μL 100 mg mL^{-1} APS as initiator and 1 μL N,N,N',N'-tetramethylethylenediamine (Bio-Rad, 1610800) as accelerator were added in the monomer-crosslinker solution and mixed well. The precursor solution was immediately charged into a 3D mold and capped by a glass slide leaving no air inside the mold. A 5 g weight was placed on top of the glass to ensure airtightness. Lastly, overnight polymerization at room temperature gave a sticky and stretchy hydrogel.

Physiological saline (0.9% NaCl) was used for preparation of all solutions/gels throughout the study unless otherwise stated. This is to avoid discrepancy in test results caused by ion effects and to replicate the conditions during surface recording on plants as much as possible. Although lower concentrations were used in previous reports (1 – 10 mM),^[3-5] plant surfaces of gel contact showed no visible change over the lifetime of plants after testing, suggesting the general safety of the salt content used in this study.

4.2.2 Rheological Characterization

Rheological testing was conducted in modes of temperature ramp and amplitude sweep to characterize the mechanical properties of EPC thermogelling solution, agar gelling solution and PAAm hydrogel. A TA Instruments Discovery Hybrid Rheometer Series (DHR-3,

Research Instruments, Inc.) fitted with a 40 mm diameter flat geometry (40 mm crosshatched geometry for agar gel) and a temperature-controlled Peltier plate was used. EPC thermogelling solutions were applied on the Peltier plate at around 10 °C (agar solution at 60 °C) to fully fill the gap between the parallel plates. Gap distance was fixed at 900 μm .

Temperature ramp was conducted from 0 to 50 °C for EPC solution and PAAm hydrogel, and from 50 °C to 0 °C for agar solution, at an oscillation frequency of 5 rad s^{-1} and an oscillation amplitude of 1%, with ramp rate of 3 °C min^{-1} . Storage modulus (G'), loss modulus (G''), $\tan\delta$, and complex viscosity were extracted and plotted against temperature. Sol-gel transition temperature was determined by the crossover of G' and G'' curves. Gelation time was measured by recording $\tan\delta$ while EPC solutions were heated from 4 °C to 25 °C at the maximum power. Amplitude sweep was conducted at an oscillation frequency of 5 rad s^{-1} at 25 °C, from 0.1% to 3000%. Storage modulus and oscillation stress were plotted against oscillation strain to determine yield strength and yield strain.

4.2.3 SEM

Cross-sectional SEM images of *Gloxinia* leaves applied with EPC thermogel, agar gel, and PAAm hydrogel were taken to compare their degree of conformability. *Gloxinia* leaves were chosen because of high hair density and acceptable deformation during freeze-drying. First, fresh *Gloxinia* leaves were cut in ca. 2 cm $\times$ 1 cm pieces. Warm agar solution (0.5%, 50 °C) and cool EPC solution (11%, 4 °C) were dropped on the entire upper surface of cut leaves, respectively. They were left at ambient environment for 5 min to allow complete gelation. Meanwhile, a PAAm hydrogel sheet ($\sim$ 0.5 mm thick) cut in a similar size was adhered to the upper surface of a leaf by gentle pressure. Next, as-prepared leaf-gel assemblies were immersed in liquid nitrogen until fully frozen and quickly transferred to vacuum in a freeze-drier (ScanVac CoolSafe 110-4, LaboGene). Samples were freeze-dried for 24 hours before sectioned by scissors in liquid nitrogen. Lastly, the sectioned samples were freeze-dried for another 24 hours. For SEM imaging, samples were fixed on a sample holder using carbon tape and sputter-coated with $\sim$ 10 nm of Au. SEI was done by a JSM-

7600F Field Emission Scanning Electron Microscope (JEOL) at LM (low magnification) mode with 10.0 kV accelerating voltage.

4.2.4 Shear Adhesion Tests

Shear adhesion tests were conducted on an MTS Criterion[®] Electromechanical Universal Test System Model 42 (MTS Systems Corporation) fitted with a 50 N load cell and a pair of screw action grips.

Figure 4.1 Setups for shear adhesion characterization in lap-shear configuration (a) and of complete plant-gel-electrode assemblies (b).

Shear adhesive strengths of agar gel (0.5%), PAAm hydrogel (11%), and EPC thermogel (11%) on hairy leaves and metal electrodes were determined by lap-shear tests (**Figure 4.1a**).^[6] *Gloxinia* leaves were used as model plants because of high density of hairs and ease of acquisition. Ag/AgCl plates (1.5 V DC bias across Ag plates (+) and Pt counter electrode (-) in 0.1 M HCl for ~100 s^[2]) were used as model electrodes because they are the standard electrodes for plant electrophysiology. Fresh leaves and electrodes were cut in a size of 1 cm × 1 cm and pasted on glass slides to ensure flatness as well as for easy clamping by the screw action grips. Before testing, metals were cleaned with ethanol and blowing N₂, and leaves were cleaned with blowing N₂ only. To achieve the best possible contact between gels and surfaces, agar solution and EPC solution were first dropped on surfaces of investigation. After brief gelation, the glass slides were clamped on the

mechanical tester with precise alignment and a horizontal distance of ~ 1 mm between the adherend surfaces was kept. Any gap between the upper and lower samples was filled by additional drops of agar or EPC solution, and gelation completed in about 3 min. As for PAAm hydrogel, simple placement and gentle pressure was applied, indicated by a non-zero force reading on the mechanical tester. Shear tests were conducted at an extension rate of 3 mm min^{-1} . Forces were recorded versus displacement and divided by apparent contact area, 1 cm^2 , for calculation of stress. The maximum value in stress up to rupture was taken as the shear adhesive strength.

Shear tests of plant-gel-electrode assemblies (Figure 4.1b) were conducted on tobacco stems which are highly hairy. A tobacco plant cultured in pot was cut from top, leaving only a relatively straight portion of the stem (3 – 6 cm long). The pot was fixed on a stationary platform on the mechanical tester. A piece of Ag/AgCl was cut in size of $1 \text{ cm} \times 2 \text{ cm}$ and pasted on a backing glass slide. Gel was applied using the same method as for lap-shear tests. The upper clamp of the mechanical tester moved upwards at a rate of 8 mm min^{-1} and force was recorded as a function of displacement. Due to the variation of stem geometry between plants and geometry mismatch between stem and metal electrode, it is difficult to accurately calculate adhesion strength. Therefore, force readings were reported as a rough comparison between different gels. To minimize the effect of stem geometry variation, stems of similar diameter were chosen (4 mm) and contact length was fixed at 20 mm. This set of adhesion tests serves as a verification of results from lap-shear tests in complete electrode assemblies.

4.2.5 EIS

Electrical properties of EPC thermogel were studied in terms of ion concentration dependent conductivity, molar conductivity, interfacial impedance with electrodes, and electrode system impedance on hairy leaves. All measurements were carried out using an Electrochemical Workstation Zennium (ZAHNER-elektrik GmbH & Co. KG) in EIS mode scanning from 10^5 Hz to 10^{-1} Hz at an amplitude of 50 mV.

Figure 4.2 Setups for measuring intrinsic conductivity (a), interfacial impedance with electrodes (b), and impedance of surface electrodes on hairy leaves (c).

Conductivities of different gels with 0.9% NaCl were measured in a long-strip configuration shown in **Figure 4.2a**. 3D printed plastic molds were used to confine gels in precise dimensions. Impedance at the minimum absolute value of phase angle was taken for approximation of conductivity. The following equation was used to calculate conductivity σ from measured impedance R :^[7]

$$\sigma = \frac{L}{RWT} \quad \text{Equation 4.1}$$

Here, L , W , and T are length, width, and thickness of gel, indicated in Figure 4.2a. Conductivities of EPC thermogelling solutions at varying NaCl concentration were measured using the same method. Complete gelation was ensured before testing yet without significant dehydration ($\sim 3 \text{ min}$ after application of 4°C solution).

Interfacial impedances between gels and electrodes (Pt and Ag/AgCl) were measured in a thin-plate configuration shown in Figure 4.2b. The dimensions of gels were maintained using square PDMS (polydimethylsiloxane) frames laminated in between two electrodes. Slight difference in thickness ($\pm 0.15 \text{ mm}$) was found to have negligible influence on total impedance.

Impedance of surface electrodes consisting Ag/AgCl and different gels, interfacing with

hairy plants, were measured in a setup shown in Figure 4.2c. A *Gloxinia* leaf was cut from its mother plant and cleaned with strong N_2 airstream. Immediately after this, measurements were done at the tip, middle, and rear regions of the leaf (three pairs of electrodes tested for each region). A single leaf was used for fair comparison between gels by excluding the intrinsic impedance variation between individual leaves. The whole process was completed before observable dehydration occurred to minimize changes of leaf impedance.

4.3 Thermogel Formulation

4.3.1 Design Target of Thermogel Properties

Figure 4.3 Proposal of surface electrode with thermogel as the ionic interface.

The application procedure of thermogel-interfaced surface electrode is summarized in **Figure 4.3**. First, thermogelling solution in sol state at a low temperature (at $\sim 4^\circ\text{C}$ if stored in fridge) is dropped on hairy surface through a plastic pipette (dropper). Next, a plate electrode (instead of wire to maximize contact area for large adhesion force and small contact impedance) is placed on the gelling solution while the solution gradually gelates into a solid gel within a reasonable period of time. The final thermogel interface should be a mechanically robust (able to bear the weight of electrode) and electrically conductive (sufficient for signal transmission) bridge between hairy plant and surface electrode. According to the above application procedure, the following property parameters of thermogel material are required:

1. A sol viscosity at $\sim 4 - 10^\circ\text{C}$ of $< 1\text{ Pa s}$ (for storage in fridge and pipette handling)

2. A transition temperature slightly lower than the ambient temperature in this study (i.e., in the range of 20 – 23 °C)
3. A gelation time within 20 – 40 s (sufficient time for electrode placement)
4. A gel yield strength of >35 Pa (based on areal mass density of Ag/AgCl plate electrode using in this study, 2.8 – 3.6 mg mm⁻²; can be lighter if thin-film electrodes are used)
5. Conductivity of >1 mS cm⁻¹ (based on electrolyte previously used in plant electrophysiology^[5])

4.3.2 Tuning of Thermogel Composition

There are two components (besides water) in EPC thermogelling solutions: EPC polymer and NaCl. The polymer provides thermogelling property while the salt provides ionic conductivity. The composition of each of the components was carefully optimized to fit the requirements of practical application listed in Section 4.3.1, with additional consideration of biosafety.

4.3.2.1 Influence of Polymer Concentration

An increase in polymer concentration is expected to increase viscosity and moduli, yet decrease gelation temperature, due to higher density of polymer interactions and larger entropy gain upon gelation. Rheological characterization of three compositions with varying polymer content at fixed NaCl content (physiological saline, i.e., 0.9%, used as the benchmark) shown in **Figure 4.4** conforms this trend. It was observed during manipulation of these samples that the viscosities of 9% and 11% EPC solutions at 4 °C are sufficiently low for sample withdrawal and dispense through a disposable pipette, but 13% EPC solution is too viscous for pipette handling (also reflected in a viscosity close to 1 Pa s in Figure 4.4c). Moreover, as the ambient temperature in the current study is in the range of 23 – 25 °C, 9% EPC solution is not able to form an elastic gel because its gelation temperature is too close to the ambient temperature, while 11% and 13% EPC solutions can satisfy the gelation temperature requirement. Furthermore, the measured gelation time

from 4 °C to 25 °C is within 30 – 50 s for 11% and 13% EPC solutions, which also encompasses the equipment cooling time. Hence the true gelation time should be smaller than the measured values, and 11% and 13% EPC solutions should approximately satisfy the gelation time requirement. Based on these considerations, 11% EPC was chosen as the optimal concentration.

Figure 4.4 Effect of polymer concentration on rheological properties and gelation behavior of EPC solution. **a-c)** Rheological characterization of 9%, 11%, and 13% EPC in physiological saline (0.9% NaCl). Sol-gel transition temperatures are indicated. **d)** $\tan \delta$ curves when EPC solutions (at the above concentrations) were heated from 4 °C to 25 °C. $\tan \delta$ of 1 signals gelation.

4.3.2.2 Influence of Salt Concentration

Ionic salts can not only directly affect the electrical conductivity of gels, but also influence the gelation behavior of water-soluble polymers. NaCl is a salt-out salt (increase of salt concentration reduces polymer solubility), previously reported to reduce critical gelation

temperature and improve gel strength of some thermogelling polymers.^[8-9] This phenomenon is because Cl^- anions are highly hydrated in water and compete with polymers for water bonding, weakening the interactions between polymers and water molecules, which originally promote solvation; consequently, hydrophobic association between polymers is favored causing gelation.^[9] As demonstrated in **Figure 4.5**, the gelation temperature is reduced by an increment in NaCl concentration, but viscosity and moduli are not significantly affected as by EPC concentration. Judging based on gelation temperature and gelation time, a salt concentration of 1.0 – 1.5% would be appropriate for 11% EPC solution. Additionally, a higher salt concentration may permit the use of lower polymer concentration, further promoting ionic conductivity. However, previous studies on plant electrophysiology usually employed a salt concentration of 1 – 10 mM^[3-5] to minimize disturbance of plant internal ionic environment (1.0% NaCl is equivalent to 171 mM NaCl). Therefore, a high salt concentration should be avoided to ensure biocompatibility of hydrogels in direct contact with plants. 0.9% NaCl is the concentration of physiological saline, safe for human use. Therefore, this concentration was selected as a benchmark for plant measurement in this work. Prolonged contact between hydrogels at this salt concentration on various species of plants did not produce any visible changes in plant appearance or physiology, so the concentration of 0.9% NaCl was considered safe during the course of the current study. Nevertheless, salt species and concentration should be adjusted for optimal plant biocompatibility for future plant study.

Figure 4.5 Effect of NaCl concentration on rheological properties and gelation behavior of EPC solution. **a-c)** Rheological characterization of 11% EPC solution with 0.5%, 1.0%, and 1.5% NaCl. Sol-gel transition temperatures are indicated. **d)** $\tan\delta$ curves when EPC solutions (at the above NaCl concentrations) were heated from 4°C to 25°C . $\tan\delta$ of 1 signals gelation.

Conductivity is another factor to consider when tuning the salt concentration. Conductivity of 11% EPC solution added with varying NaCl wt% is compared with pure saline (measured at ambient temperature), shown in **Figure 4.6a**. A slightly lower conductivity is observed in EPC thermogel than saline, but the conductivity of 0.9% NaCl-11% EPC is still much higher than 10 mM (0.058 wt%) pure saline, indicating the sufficient electric conductivity of thermogel at a composition of 0.9% NaCl-11% EPC.

Figure 4.6 Effect of NaCl concentration on conductivity of 11% EPC solution. **a)** Conductivities of saline and 11% EPC thermogel at varying NaCl concentration ($n = 3$). Lines are linear fitting of experimental data. **b)** Molar conductivity derived from (a). Error bars, s.d.

Molar conductivity is further calculated and plotted (Figure 4.6b) to explain the difference between thermogel and saline. Lines are fitting curves according to the empirical law for ionic mobility:^[10]

$$\Lambda_m = \Lambda_m^0 - K\sqrt{c} \quad \text{Equation 4.2}$$

Here, Λ_m is molar conductivity, Λ_m^0 is limiting molar conductivity, K is a constant, and c is molar concentration. Λ_m^0 reflects the network hindrance to ionic movement; a smaller value indicates stronger hindrance. This is reflected in the graph as the intercept with y-axis. K reflects the interactions between ions, which restrict ionic movement; a smaller K suggests weaker interactions. It is manifested in the slope of a curve. These two features are well demonstrated in Figure 4.6b: a smaller intercept with y-axis indicates stronger hindrance to ionic movement by the EPC gel network, and a smaller slope indicates weaker interactions between ions due to the shielding effect from EPC polymers.

4.4 Rheological Properties

4.4.1 Temperature-Dependent Behavior

Rheological properties of the optimized EPC thermogelling solution (0.9% NaCl-11% EPC)

in response to temperature change was investigated in detail, with 0.5% viscous agar solution, 0.5% agar gelling solution, and 11% PAAm hydrogel^[11-12] as controls. All gels were exposed to constant oscillation frequency (5 rad s^{-1}) and amplitude (1%) in a temperature range of $0 - 40^\circ\text{C}$, and their viscosity and $\tan\delta$ (G''/G') were recorded (**Figure 4.7a**).

Designed to be a sol at low temperatures and a gel at elevated temperatures, EPC solution attains the lowest viscosity and the highest $\tan\delta$ at $\sim 4 - 8^\circ\text{C}$, as expected. This trend is opposite to the behavior of agar, which dissolves in hot water and solidifies at below $\sim 40^\circ\text{C}$ (Figure 4.7a, green dots and lines).^[13] Even though EPC solution is more viscous than agar gelling solution at their respective application temperatures ($\sim 220 \text{ mPa s}$ for EPC at $4 - 8^\circ\text{C}$ vs. $3.9 - 6.9 \text{ mPa s}$ for agar at $36 - 40^\circ\text{C}$), it was found adequately fluidic for conformability on hairy plants. Moreover, its much higher $\tan\delta$ than agar, in sol state ($18 - 19$ for EPC vs. $1.3 - 2.4$ for agar) and even after gelation, indicates a higher tendency of viscous flow by mobile polymer chains, beneficial for filling surface roughness and thus enhancing conformability and interfacial adhesion.^[14] In contrast to physical gels with sol-gel transition, PAAm hydrogel has the highest viscosity ($\sim 400 \text{ Pa s}$) among all gels and a $\tan\delta$ (~ 0.17) less than 1 across the entire temperature range, reflecting its minimal fluidity. Absence of sol-gel transition is also observed in viscous agar solution, which is a dispersion of agar gel particles in water.^[15] A more detailed look at G' and G'' of various hydrogels/solutions around the ambient temperature (Figure 4.7b) reveals that EPC thermogel is more robust than agar gel and viscous agar solution as reflected in higher storage modulus and loss modulus. The much higher loss modulus reaffirms the stronger capability of energy dissipation through viscous flow of EPC polymers.

Figure 4.7 Rheological properties of hydrogels/solutions as a function of temperature. **a)** Viscosity (dots) and $\tan \delta$ (G''/G') (lines) at 0 – 40 °C of 0.5% viscous agar solution, 0.5% agar gelling solution, 11% PAAm hydrogel, and 11% EPC thermogelling solution. **b)** Storage modulus (solid symbols) and loss modulus (open symbols) comparison among EPC thermogelling solutions of different concentrations (9%, 11%, and 13%), viscous agar solution, agar gelling solution, and PAAm hydrogel, from 20 °C to 30 °C.

4.4.2 Yield Behavior

The yield behaviors of different hydrogels were characterized by dynamic strain sweep at 25 °C. Due to the difficulty in adhering agar on testing plates, strain and stress recorded at drop of storage modulus might be caused by wall slippage instead of true network yielding. Nevertheless, the data were still plotted for comparison with other hydrogels (**Figure 4.8**), as a demonstration of weak adhesion.

Figure 4.8 Yield behavior of various hydrogels/solutions. **a)** Storage modulus (dots) and oscillation stress (lines) as a function of oscillation strain under constant oscillation frequency (5 rad s^{-1}). **b-c)** Histograms showing mean yield strength (b) and mean yield strain (c) of different gels. Yield point was determined as the intercept of tangent lines to horizontal and declining segments of storage modulus versus oscillation strain curve (a). Error bars, s.d.

One astonishing feature of EPC thermogel is the high extensibility. Its LVR is exceptionally wide among all hydrogels. This might stem from the reversibility of physical crosslinks (dominantly van der Waals forces if not considering ions in electrolyte) responsible for maintaining gel stiffness. As larger and larger oscillation strain is applied, the polymer chains uncoil, slide over each other, and recoil, constantly forming new hydrophobic associations with adjacent segments, maintaining the overall crosslinking density and gel internal structure (associated micellar structure).^[16] The instant breaking and reforming of hydrophobic associations is a result of interaction non-directionality. In detail, the type of physical interactions holding up hydrophobic associations are primarily London dispersion force (between PPG and PCL segments), which does not depend on the orientation of interacting molecules but only distance between them.^[17] In contrast, other van der Waals forces involving dipoles or induced dipoles are sensitive to dipole orientation,^[17] especially hydrogen bonding, which is believed to contribute significantly to agar gelation.^[18] This bonding non-directionality resulting in dynamic maintenance of hydrophobic association density among EPC polymers might explain the astounding LVR.

For PAAm hydrogel, covalent crosslinks present in the gel network. They restrict free movement of polymer segments and once broken cannot be reformed, resulting in high strength but low extensibility. As expected, viscous agar solution exhibits the lowest modulus and strength due to absence of monolithic gel network. As for agar gel, wall slippage due to poor adhesion is the limiting factor in mechanical robustness of a system incorporating agar gel, which is critical for the intended application in the current study and will be discussed in detail in Section 4.6 and Section 6.4.2.

4.5 Conformability on Hairy Plants

Conformability of thermogel on hairy plants is an important parameter to verify, as stated in the hypothesis of the thesis. To this end, cross-sectional SEM was used to visualize the degree of conformability of EPC thermogel, agar gel, and PAAm hydrogel on *Gloxinia* leaves. **Figure 4.9a** reveals seamless contact between EPC thermogel and *Gloxinia* leaf, and the magnified image in Figure 4.9b depicts a hair wrapped tightly with EPC polymer, suggesting direct contact between EPC polymers and plant surfaces. As for agar gel, tiny slits around 37 μm wide (Figure 4.9c) present at the interface, possibly because the differential thermal expansion/contraction during freeze-drying (for sample preparation) induced interfacial detachment. For PAAm hydrogel, large gaps around 270 μm wide (Figure 4.9c) separate the gel and the leaf, with some hairs damaged and some piercing the hydrogel. From the above results, it can be concluded that EPC thermogel is able to establish a conformal contact on hairy leaves with robust adhesion while preserving the intactness of vulnerable hairs.

Figure 4.9 Contact between hydrogels and hairy leaves. **a)** Cross-sectional SEM images of gels applied on *Gloxinia* leaves. Orange shading outlines the gels, and grey regions are plant tissues. **b)**

Enlarged cross-sectional SEM image of EPC-leaf interface. **c)** Histogram showing gap widths between leaf surfaces and gels (mean $\pm$ s.e., $n = 18$ for PAAm and agar, $n = 9$ for EPC, on ca. 2 cm² samples). ** $p < 0.01$, *** $p < 0.001$; unpaired, two-tailed t-test.

4.6 Adhesive Properties

The other critical parameter in gauging the potential performance of thermogel ionic interface is adhesiveness, as part of the thesis hypothesis. In this section, the adhesive properties of different hydrogels are quantitatively investigated and compared.

4.6.1 Hydrogel as Adhesives

In a typical electrode assembly, two interfaces are involved: electrode-gel interface and plant-gel interface. Hence, the adhesive strengths of EPC thermogel on electrodes (Ag/AgCl plates) and hairy plants (*Gloxinia* leaves) were measured, respectively, in lap-shear tests, agar gel and PAAm hydrogel being controls. Both agar gel and EPC thermogel were applied in sol state to attain maximal adhesive strengths. As summarized in **Figure 4.10**, EPC thermogel fails cohesively (i.e., inside gel bulk) with a strength around 170 – 180 Pa, regardless of adherends. This implies robust interfacial bonding such that the bulk gel strength becomes the limiting factor in shear adhesion. This feature of cohesive failure will help preserve plant tissues during electrode detachment. In addition, the not dissimilar behavior on hairy plants and metal electrodes underscores the versatility of amphiphilic polymers in establishing physical contact with surfaces of distinct chemistry, concurring with previous results on the adsorption of PEO (polyethylene oxide) and PPO (polypropylene oxide)-based block copolymers.^[19-20] Conversely, agar gel and PAAm hydrogel tend to fail adhesively (i.e., at interface). Agar gel adheres very weakly on electrodes (34 Pa), which might originate from low solid content and syneresis (extruding water on standing^[21]), whereas PAAm hydrogel adheres weakly on hairy leaves (48 Pa), indicating the prominent effect of poor contact due to presence of hairs.^[22]

Figure 4.10 Histogram showing shear adhesive strengths of gels of agar, PAAm, and EPC on Ag/AgCl electrodes and hairy leaves of *Gloxinia* (mean $\pm$ s.e.). * $p < 0.05$; unpaired, two-tailed t-test. ns, not significant.

4.6.2 Electrode Assemblies

Following the characterization of individual interfaces, shear tests of plant-gel-electrode assemblies were conducted to verify the results from lap-shear tests. Tobacco stems in pots were fixed while electrodes bridged through a hydrogel layer were pulled vertically upwards. In such assemblies, EPC thermogel should fail cohesively with the largest shear strength whereas agar gel and PAAm hydrogel should fail adhesively, inferred from lap-shear testing results. The load-displacement curve confirms the largest shear strength of EPC-based assemblies (**Figure 4.11a-b**). In addition, benefitting from the exceptional extensibility and energy dissipation through viscous flow, EPC thermogel-based assembly possesses outstanding adhesion energy as compared to other gels (**Figure 4.11c**). Furthermore, photographs of samples during shearing show failure modes as predicted (**Figure 4.11d**). These results prove that EPC thermogel is mechanically reliable, as a bridge between hairy plants and electrodes.

Figure 4.11 Adhesive properties of electrode assemblies. **a)** Representative load-displacement curves from shear tests of complete electrode assemblies on tobacco stems. Force is not converted to stress due to difficulty in accurately measuring contact area (roughly controlled at 1 cm^2). **b-c)** Mean peak load (b) and adhesion energy (c) for each type of gels ($n \geq 2$) calculated from the load-displacement curves. Error bars, s.d. **d)** Left, scheme showing a side view of the experimental setup for shear test on tobacco stems. Right, schemes and photographs illustrating different failure modes of assemblies with agar gel, PAAm hydrogel, and EPC thermogel, respectively (from left to right). A, adhesive failure; C, cohesive failure. Red arrows indicate presence of gels. Scale bar, 5 mm.

4.7 Electrical Properties

Figure 4.12 Overview of electrical conduction in a gel-based surface electrode. The right side displays the equivalent circuit, where the letters in the subscript have the following meanings: c = conductor, g = hydrogel, b = biological tissue.

The non-invasive electrode designed in this study has a general construction shown in **Figure 4.12**. In such configuration, three parts of electrical conduction need quantitative assessment: gel-electrode interface, hydrogel bulk, and gel-plant interface. In the following sections, each of these issues is respectively discussed.

4.7.1 Intrinsic Conductivity

Figure 4.13 Intrinsic conductivity of hydrogels and solutions. **a)** EIS results of saline, agar gel (0.5%), PAAm hydrogel (11%), and EPC thermogel (11%) at 0.9% NaCl, obtained using the setup shown in inset. **b)** Conductivities derived from (a) (mean $\pm$ s.e.). *** $p < 0.001$; unpaired, two-tailed t-tests. ns, not significant.

The geometry of the gel/solution under testing was thoughtfully designed to maximize the resistance to current flow within gel/solution and minimize resistance across gel/solution-electrode interface. Such reasoning is confirmed by the very small phase shift (right y-axis in **Figure 4.13a**) across a wide range of frequencies. As introduced in Chapter 3, a small phase angle (near zero) indicates dominant resistive impedance, corresponding to electrolyte resistance. Hence, impedance values at phase angle around zero were extracted for the calculation of conductivity (Figure 4.13b). Statistical comparison through unpaired two-tailed t-tests shows that the intrinsic conductivity of EPC thermogel is similar to that of PAAm hydrogel at the same polymer content. The higher conductivity of agar gel and saline should be attributed to lower (and no) polymer content resulting in minimal hindrance to ionic movement.

4.7.2 Interfacial Impedance with Electrodes

Figure 4.14 Interfacial impedance with metal electrodes. Mean EIS results of gels and solutions at 0.9% NaCl ($n = 3$) laminated between plate electrodes (Pt in (a) and Ag/AgCl in (b)), in a configuration depicted in the inset of (b).

Again, the geometry design of gel-electrode laminates was carefully drafted to maximize the interfacial contribution to overall impedance, and this was successfully implemented based on the large phase angle (near 90°) across a wide range of frequencies (right y-axis in **Figure 4.14a**). As introduced in Chapter 3, a 90° phase angle indicates dominant capacitive impedance, corresponding to electrolyte-electrode interfacial double layer

resistance. Since Pt is a noble metal, electrical double layer formation should be the main contributor to interfacial impedance, which can well explain the results shown in Figure 4.14a, and proves that the setup indeed captured the interfacial impedance between gels and electrodes. On the other hand, since Ag/AgCl electrodes constantly exchange ions with electrolyte, rates of Faradic reaction and ion diffusion contribute to the interfacial impedance. This results in a significantly different impedance level as compared to Pt electrodes. Overall, for both types of metal electrodes, EPC thermogel is equally capable as control gels of gel-electrode electrical coupling.

4.7.3 Surface Electrodes on Hairy Plants

Figure 4.15 Impedance of surface electrodes on hairy leaves. EIS results of Ag/AgCl electrodes interfacing with hairy leaves through various gels. Shaded areas around solid lines represent mean $\pm$ s.d. from 3 independent tests on a single leaf.

After verifying the competency of gel intrinsic conductivity and gel-electrode coupling, the next step is to compare gel-plant interfacial impedance on hairy leaves. One can conclude, from Figure 4.14b and **Figure 4.15**, that the gel-plant interfacial impedance dominates the overall impedance of a surface electrode. Thus, the comparable impedance of EPC thermogel and agar gel indicates similarly conformal contact, whereas the more than one order of magnitude higher impedance of PAAm hydrogel should be attributed to poor gel-leaf contact. These results prove that the dominant factor in electrical coupling of

a surface electrode lies in the gel-plant interface, where EPC thermogel is an effective electrical bridge due to intrinsic conductivity and excellent conformability. The comparable impedance of EPC thermogel to agar gel proves effective gel-leaf electrical contact, outperforming PAAm hydrogel.

4.8 Electrode Removal

Safe electrode removal after experiment is important to preserving the intactness of plants. Due to the mechanical softness of thermogel, the electrode can be manually detached without hurting the plants (**Figure 4.16**). Note that this is only possible when the strength and modulus of gel is smaller than those of plant tissues. Thermogel satisfies this criterion with a shear strength of 580 Pa and a shear modulus of 147 Pa, smaller than the shear strength (23 kPa) and shear modulus (14 kPa) of tobacco leaf, respectively. (Note: mechanical property of tobacco stem was not successfully tested due to difficulty in fixation. Nevertheless, the normal range of plant tissue mechanical properties reported in literature is still well above the values of thermogel: shear strength around 0.1 – 50 MPa and shear modulus around 50 – 500 kPa.^[23-24]) The remaining thermogel on plant after electrode detachment can be either wiped off using wet tissue or rinsed off by water, because EPC polymer becomes soluble in water when concentration is low. The last bit of residue can self-degrade through hydrolysis of PCL segments, causing no visible harm to plants.

Figure 4.16 Detachment process of thermogel-based electrode from tobacco stem.

4.9 Conclusions

In this chapter, formulation of thermogelling polymer solution was optimized according to realistic application requirements. The rheological, adhesive, and electrical properties, as well as conformability of the optimized EPC thermogel were characterized in comparison with conventional hydrogels. Based on experimental results presented here, it can be concluded that EPC thermogel can establish a conformal and adhesive ionic interface between hairy plants and surface electrodes, outperforming conventional hydrogels. Moreover, EPC thermogel also possesses superior mechanical and electrical properties for effective plant-electrode bridging, promising for improved electrophysiological recording using hydrogel-interfaced surface contact electrodes.

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Chapter 5

Plant Electrophysiology Using Thermogel

This chapter elaborates plant electrophysiological studies using thermogel-interfaced surface contact electrodes to examine the practical usefulness of thermogel ionic interface. Wounding experiments were conducted on hairy and non-hairy plants and induced electrical signals were collected using surface electrodes bridged by various hydrogels and invasive metal electrodes. Statistical analyses of recorded signals reveal the superiority of thermogel in terms of signal fidelity, and electrode application was greatly simplified from previous studies. These results prove the efficacy of thermogel ionic interface for surface contact electrophysiology on hairy plants.

5.1 Introduction

The previous chapter has illustrated the potential of EPC thermogel for non-invasively bridging hairy plants with metal electrodes. In this chapter, EPC thermogel is deployed as an ionic interface in a series of plant electrophysiology studies. First, a surface electrode is dedicatedly designed for simplistic operation. Next, mechanical and heat wounding-induced electrical signals in plants will be collected from EPC thermogel-interfaced electrodes in comparison with control hydrogels and invasive electrodes. Operational considerations and signal quality will be the criteria for assessing the efficacy of EPC thermogel as an ionic interface for electrophysiological study on hairy plants.

5.2 Experimental Methods

5.2.1 Electrode Preparation

Two types of electrodes were used for plant electrophysiological study: plate-shaped surface electrodes and wire-shaped invasive electrodes, both of which utilized Ag/AgCl as the metallic portion. The structure of surface electrodes is illustrated in **Figure 5.1**, consisting of Ag/AgCl plate, Cu wiring, and Ecoflex encapsulation. First, a Ag plate was cut in size of 2 mm × 2 mm and connected with an enameled copper wire through a conductive tape. Next, Ecoflex (00-35, Smooth-On) part A and part B were mixed in a weight ratio of 1:1 and immediately dropped on the conductive tape side of the Ag plate. After curing for at least 5 min, the exposed side of Ag plate was chlorinated in a 0.1 M HCl solution under 1.5 V DC bias across Ag plates (+) and Pt counter electrode (-).^[1] Normally 60 – 100 s chlorination resulted in a dull grey AgCl coating, ready for use. The same chlorination procedure was conducted on polished Ag wires (~1 mm diameter) to produce invasive wire-shaped electrodes.

Figure 5.1 Scheme of surface electrodes used in this study for plant electrophysiology. Gel is either agar gel, PAAm hydrogel, or EPC thermogel.

It should be noted that unlike previous studies on sunflowers where complex surface recording are used,^[2-3] the plate electrodes constructed here allows for facile and versatile placement on plants with great operational simplification.^[1, 4-5]

5.2.2 Plants Preparation and Setup

Arabidopsis thaliana and tobacco plants (*Nicotiana benthamiana*) were obtained from Temasek Life Sciences Laboratory, and sunflowers were purchased from a local market. On the day before test, plants were watered and left undisturbed in an air-conditioned room maintained at 24 – 25 °C and 50 – 60 % humidity. All tests were carried out in daytime. During tests, plants were placed in a Faraday cage, supplied with additional white light from a desktop lamp.

To apply as-prepared electrodes on plants, different approaches were used due to the disparate material properties and electrode configuration. For testing using agar gel and EPC thermogel-based surface electrodes, the corresponding solutions were dropped on plants followed by placement of Ag/AgCl electrodes on top of gels. Gentle pressure was applied to facilitate adhesion for ~10 s. For testing using PAAm hydrogel, a piece of hydrogel sheet (<1 mm thickness, ~2 mm × 2 mm area) was first adhered to a Ag/AgCl plate and the assembly was pressed against a plant gently without causing damage. For invasive measurement, Ag/AgCl wires were inserted in tobacco stems at ~2 mm depth, and plants were left undisturbed for at least three hours before testing. A Ag/AgCl reference

electrode was inserted in soil setting the reference potential.

5.2.3 Wounding and Data Acquisition

Mechanical wounding on *Arabidopsis thaliana* followed the protocol by Mousavi et al. (2014) using forceps.^[1] First, serrated forceps were prepared by pasting rectangle pieces of plastic bottle cap on the inner side of tips of a pair of metal forceps. Before wounding, the leaf to be wounded was stabilized by a self-made stage with adhesive tapes (orange parts in **Figure 5.2a**) to avoid signal artefacts due to leaf movements. 3-s pressing of the leaf tip using the serrated forceps generate visible damages. To compare the efficacy of EPC thermogel with control gels (agar gel and PAAm hydrogel), thermogel and a control gel were placed at opposite sides of the midrib at equal distance from the midrib (Figure 5.2a). Synchronized signals were recorded from the two electrodes.

Figure 5.2 Setups for wounding experiments on plants and electrical signal measurement. **a)** Photograph showing a mechanically wounded *Arabidopsis thaliana* leaf and placement of surface electrodes. **b)** Scheme showing the flame wounding method and position of electrodes (E1 and E2) for recording on hairy stems. **c)** Scheme showing the exact location of electrodes on stems for comparison within a pair. The two electrodes in a pair are positioned opposite at equal distance (pink lines) from the extrapolation (dashed black line) of the midrib of the leaf.

Flame wounding on sunflowers and tobacco plants followed methods reported previously.^[2-3, 6-7] In brief, flame from a household lighter was placed underneath a leaf tip for 3 s. Approximately 2 cm² of leaf was injured from visual inspection. Flame wounding

induced a series of potential fluctuations in the stem,^[3] and two electrodes to compare were adhered side by side to capture synchronized signals (Figure 5.2b). To minimize possible influence on signal intensity of different electrode positioning on stems, electrodes in a pair were placed at equal distance from a line extrapolated from the midrib and petiole of the wounded leaf (Figure 5.2c).

Signals were collected at a sampling frequency of 10 Hz by a data acquisition system (USB-2610 Series DAQ, Smacq Technologies). The ground terminal of the data acquisition system was connected to the Faraday cage to minimize electrical noise.

5.2.4 Data Representation and Analysis

Raw data were plotted in potential vs. time without processing. Peak amplitudes were extracted for comparison of signal quality from different types of electrodes. Data were collected on at least three individual plants for each set of experiments. Unpaired or paired t-tests were used for statistical analysis depending on the nature of data.

5.3 Mechanical Wounding on Control Plants

Figure 5.3 Signals from mechanical wounding of *Arabidopsis thaliana*. **a)** Representative signals recorded with pairs of gels. **b)** Peak amplitudes of signals read from EPC thermogel, agar gel, and PAAm hydrogel (mean $\pm$ s.e.). Paired t-tests. ns, not significant.

Arabidopsis thaliana is considered as a control plant because its hair density and length

have negligible influence on gel-leaf contact. The effectiveness of signal recording of EPC thermogel was compared to control gels, i.e., agar gel and PAAm hydrogel, on such non-hairy plants to verify the quality of electrode-plant coupling. The waveform of potential fluctuations are generally identical among each pair of electrodes (**Figure 5.3a**). Furthermore, statistical analysis on multiple plants indicates that the peak amplitude read from EPC thermogel does not deviate from those of agar gel and PAAm hydrogel (**Figure 5.3b**). This suggests the quality of electrical coupling between EPC thermogel and plants is comparable to control gels, if not considering surface topography.

5.4 Flame Wounding on Sunflower Plants

Figure 5.4 Results of flame wounding on sunflowers. **a)** Photograph showing surface electrodes adhered on a sunflower stem at positions E1 and E2 (indicated in Figure 5.2c) through EPC thermogel and PAAm hydrogel, respectively. Scale bar, 5 mm. **b)** Typical signals induced by 3-s flame-wounding showing a large and broad potential drop followed by small peaks. The blue and red lines represent signals from a single wounding event, read using PAAm hydrogel and EPC thermogel, respectively. Dotted lines show the boundaries for determining peak amplitudes of strong and weak signals. **c)** Box plot of peak amplitudes read from EPC thermogel and PAAm hydrogel on sunflower stems (median, 25th and 75th percentiles, minimum and maximum, $n = 13$ from 5 plants). $*p < 0.05$; paired t-tests.

To demonstrate the superiority of EPC thermogel for non-invasive electrophysiology on hairy plants, flame-induced potential fluctuations on hairy stems of sunflower were recorded using EPC-based electrodes in comparison to agar and PAAm-based electrodes.

Due to the poor adhesion between agar gel and electrodes, measurement using agar gel was not successful. PAAm hydrogel could barely adhere on hairs, whereas EPC thermogel firmly anchored plate electrodes on stems (**Figure 5.4a**). Synchronized signals in a single flame wounding event were captured by EPC thermogel and PAAm hydrogel-based electrodes adhered side by side (Figure 5.4b). The recorded potentials display depolarization amplitudes in a range of 41 – 73 mV and signal transmission speed within 7.8 – 23 cm min⁻¹, consistent with previous reports.^[2-3] Analysis of peak amplitude (including large depolarizations and small fluctuations, indicated in Figure 5.4b) shows that EPC thermogel collects stronger signals than PAAm hydrogel in a pair (Figure 5.4c).

Figure 5.5 Equivalent circuit of surface contact plant electrophysiology setup used in this study. The dashed line encloses the elements inside an amplifier, whose gain is denoted as A .

To explain the origin of the different recorded signal intensities by different electrodes, it is essential to understand the equivalent circuit of plant, electrode, and data acquisition system during signal recording (**Figure 5.5**). When a plant generates potential fluctuations that deviate from the resting potential through transmembrane ionic current flow, a potential drop of V_p is developed across the recording electrode and reference electrode, both of which have a certain impedance (Z_e and Z_{ref} , respectively) that causes signal attenuation when reaching the input end of an amplifier. The amplifier then amplifies the input potential by a factor of A , and output a potential of V_a . The recorded signal V_r is then a portion of V_a depending on equipment internal impedance. The relation between plant

signal and recorded signal is

$$V_r = \left(\frac{Z_r}{Z_o + Z_r} \right) V_a = \left(\frac{Z_r}{Z_o + Z_r} \right) A \left(\frac{Z_i}{Z_e + Z_{ref} + Z_i} \right) V_p \quad \text{Equation 5.1}$$

Since Z_r , Z_o , Z_i , and A are kept constant by using a fixed equipment setting, and Z_{ref} is the same for tests on a single plant, the main factor affecting the intensity of recorded signals in a pair of electrodes is Z_e , impedance of surface electrode. The larger the impedance, the weaker the recorded signals. Moreover, as summarized in Figure 4.12, impedance of gel-interfaced electrode comes from three aspects: 1) interfacial impedance between metal and gel, 2) intrinsic resistance of gel, and 3) interfacial impedance between gel and plant. In Section 4.7, it has been proved that the third aspect predominantly determines the overall impedance. Therefore, the weaker signal recorded from PAAm hydrogel should be attributed to the higher impedance between gel and hairy plant, consistent with impedance measurement on hairy leaves (Figure 4.15).

Figure 5.6 Analysis of signal intensity read using EPC thermogel and PAAm hydrogel on sunflower plants. **a)** Peak amplitude ratio of EPC thermogel to PAAm hydrogel in a pair, as a function of peak amplitude read from EPC thermogel (open circles). Data points are divided in two groups (orange shading and green shading, mean $\pm$ s.e.), which are statistically different. *** $p < 0.001$; two-tailed, unpaired t-tests. **b)** Peak amplitude comparison between PAAm hydrogel and EPC thermogel according to the grouping in (a) (mean $\pm$ s.e., $n = 4$ for large peaks (green), $n = 9$ for small peaks (orange)). * $p < 0.05$; paired t-tests. ns, not significant.

Notice that the data spread widely for both EPC thermogel and PAAm hydrogel in Figure 5.4c. This is because both large depolarizations and small fluctuations are counted in peak amplitude comparison. To investigate any possible difference between large and small peaks, the ratio between peak amplitude read from EPC thermogel to that read from PAAm hydrogel (i.e., a value of 1 indicates similar performance) is plotted against amplitude read from EPC thermogel (**Figure 5.6a**). There seems to be a threshold around 40 mV, above which PAAm hydrogel records strong peaks equally well as EPC thermogel, but below which it becomes less effective than EPC thermogel in capturing weak signals. Indeed, when grouping all data points into “strong peaks” and “weak peaks” (green and orange in Figure 5.6 respectively) and comparing the performance of EPC thermogel and PAAm hydrogel for these two groups, respectively, the superiority of EPC thermogel is more obvious for weak peaks, where the signal intensity is more than twice that of PAAm hydrogel (Figure 5.6b).

This voltage-dependent signal quality is not reflected in the previous circuit analysis (Equation 5.1). If Z_e , the impedance of surface electrode, is assumed a material property and remains unchanged when recording different magnitudes of potential drops, then V_r should be proportional to V_p for any magnitude of V_p . Consequently, for a pair of EPC thermogel and PAAm hydrogel, the ratio of V_r between them should be a constant for any magnitude of V_p . The only possible explanation for the observed phenomenon is Z_e changes with V_p , which may be due to different transmission pathways of strong and weak signals within plants such that EPC thermogel touching the stem base could capture the weak signals but PAAm hydrogel adhering only on hair tip could not. Moreover, in most cases observed in this study, the strong peaks are the starting VPs (variation potentials) and the weak peaks are possibly APs (action potentials),^[3] which might imply a stronger transmission of VPs in cells within or near trichomes (hairs). This hypothesis agrees with the fact that APs in general are more restricted in transmission pathways than VPs.^[8] Nevertheless, more detailed study is necessary to unravel the underlying mechanism.

5.5 Flame Wounding on Tobacco Plants

Figure 5.7 Results of flame wounding on tobacco plants. **a-b)** Photographs showing a surface electrode adhered on a tobacco stem through EPC thermogel (a) and a Ag wire pierced in a tobacco stem (b). During testing, the two electrodes are positioned opposite (as E1 and E2 in Figure 5.2b). Scale bars, 5 mm. **c)** Potential signals read from Ag wire and EPC-interfaced Ag plate in a flame-wounding test. Inset photographs show the application procedure of EPC-based electrodes, where white arrows point to EPC droplet and Ag plate, respectively. Results are representative of measurements on three individual plants.

To further challenge EPC thermogel, tobacco plants were chosen to conduct the flaming experiment, whose stem hair density is much higher than that of sunflowers. Agar gel still failed to anchor electrodes on plants and the straight thin hairs on tobacco stems even prevented adhesion of PAAm hydrogel. Only did EPC thermogel adhered reliably (**Figure 5.7a**). Therefore, the signal quality of EPC surface electrodes was benchmarked against inserted Ag/AgCl wires (Figure 5.7b), a standard invasive method.^[1, 3] In a typical test shown in Figure 5.7c, potential was recorded using the Ag wire during the application of an EPC surface electrode (blue shading), and the small transient peaks indicate minimal interference to plant electrophysiology induced by solution coolness and gentle pressure. Following flame wounding an upper leaf, depolarization up to 35 mV was recorded from the EPC surface electrode, approaching 37 mV from the inserted Ag wire.

Figure 5.8 Monitoring propagation of flame-induced potential changes in tobacco stems. **a)** Scheme showing the setup for flame-wounding a tobacco plant at leaf tip and recording on its stem at positions E1 and E2 in this study. **b)** Scheme of a single leaf with connected petiole and stem showing the exact location of electrodes (E1 and E2). **c)** Signals read from E1 and E2 using EPC thermogel-based electrodes. Inset, enlarged view to visualize the signal transmission from E1 to E2. Signals are representative of measurements on four individual plants.

Figure 5.9 Signal characteristics of flame-induced potential changes on tobacco stems. **a)** Scatter plot of transmission speed of potential signals as a function of distance from point of wounding. **b)** Scatter plot of peak amplitude as a function of distance from point of wounding. Lines connect two points collected in a single wounding event from two electrodes positioned at E1 and E2, indicated in Figure 5.8a and b.

EPC-interfaced surface electrodes were also used to monitor signal transmission within

tobacco stems (**Figure 5.8** and **Figure 5.9**). The measured signal intensities (33 ± 13 mV) and durations (40 – 50 min) match those obtained using inserted microelectrodes in a similar study (**Figure 5.10**).^[7] Moreover, the recorded waveforms generally resemble those reported previously, where a sharp spike is first elicited followed by continuous depolarization, repolarization, and a slowed repolarization.^[7] Analysis of 13 data points does not show obvious correlation between signal transmission speed and distance from point of injury (Figure 5.9a) with an average speed of 1.0 cm s^{-1} , unlike the slowed transmission in sunflowers as signals travels away from the site of stimulus.^[2] More interestingly, the peak amplitude seems to increase, instead of decrease, with increasing distance from the point of injury (Figure 5.9b). This contradicts the normal understanding of VPs, which are believed to attenuate as the signals transmit.^[8] This observation deserves further research to elucidate the mechanisms.

Figure 5.10 Electric potential recording of tobacco plant (*Nicotiana benthamiana*) using inserted glass electrodes in a previous study. **a)** Image of the setup. Letters b and c represent reading glass electrode and reference glass electrode respectively. **b)** Three electric potential signals propagated after burning injury was inflicted on first (left side signal), second (middle signal) and third leaf (right side signal) of the plant. Arrows indicate potential spikes and dashed circles indicate observed slowing of signal repolarization rate. Adapted with permission.^[7] Copyright 2018, Taylor & Francis Group.

5.6 Conclusions

This chapter discussed the fabrication and application of surface electrodes interfaced with

plants through various hydrogels for plant electrophysiological measurements. The design of plate-shaped surface electrodes greatly simplified electrode setup and application procedure, which allowed plug-and-play integration between electronics and plants. Moreover, EPC thermogel demonstrated comparable signal acquisition capability as conventional hydrogels on non-hairy plants, and improved signal fidelity on hairy plants. Specifically, the adhesion failure of agar gel and PAAm hydrogel on tobacco stems underlined the imperative of interfacial adhesiveness and deformability during gel application for facile electrode placement and robust mechanical bridging. The flaming experiments demonstrated non-invasive electrodes interfaced via EPC thermogel, almost imperceptible to plants, were competitively capable of faithful recording compared with invasive measurement. In conclusion, the conformable and adhesive thermogel ionic interface can deliver improved electrophysiological recording performance on hairy plants than conventional hydrogels.

References

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Chapter 6

Mechanistic Study

This chapter describes the detailed study on the mechanisms for the superior performance of EPC thermogel ionic interface. Surface activity of EPC polymers contributes to improved wetting on plant cuticles and enhanced interfacial bonding with plants and electrodes, which is absent in agar polymers. Sol-gel transition benefits conformal coverage while the reversible hydrophobic associations result in superior cohesive mechanical properties when compared to adhesive polymer pastes. As for agar gelling solutions, trade-offs exist between rheological and adhesive properties, making agar less optimal for practical handling. Overall, EPC thermogel is the most practically advantageous ionic interface for plant electrophysiology compared to other hydrogels and polymer solutions, originating from its unique molecular composition.

6.1 Introduction

Chapter 4 and Chapter 5 have respectively presented the properties and practical superiority of EPC thermogel as an ionic interface between hairy plants and metal electrodes. In particular, the outstanding conformability and adhesiveness contribute to the effective electrical and mechanical coupling between the disparate plant tissues and metallic electrodes. It is clear that the sol-gel transition allows liquid-phase application and thus intimate contact between gel and plant, when compared to solid hydrogels like PAAm. However, it is not well understood the origin of the adhesiveness between thermogel and plant, when compared with agar gel, which also experiences sol-gel transition, and how EPC compares to other adhesive polymers which do not experience sol-gel transition. In this chapter, these questions will be investigated through a series of experiments and analyses.

6.2 Experimental Methods

6.2.1 Surface Tension and Contact Angle Measurements

Surface tension of agar solutions and EPC solutions, as well as their contact angles on various types of surfaces were measured using an OCA 15 Pro (DataPhysics Instruments GmbH) equipped with a single direct dosing system SD-DM.

Surface tensions of physiological saline, agar (0.01% to 0.5%) and EPC (0.01% to 3%) in physiological saline were measured using the pendent drop method by dispensing 10 μL liquid from a needle of outer diameter 0.91 mm. Young-Laplace fitting of a droplet shape gave its surface tension. Higher concentrations of agar solution ($>0.5\%$) were not successfully measured due to pre-solidification of solutions in dispensing syringes. Ambient temperature during testing was 22 – 23 $^{\circ}\text{C}$ and humidity was within 50 – 60%.

For contact angle measurement, low polymer concentration (0.03%) was used to ensure fluidity at testing temperature, 22 – 23 $^{\circ}\text{C}$, so that solidification in dispensing syringes and

during wetting was avoided. Substrates included four types of polymer films – polydimethylsiloxane (PDMS, made from SYLGARD™ 184 Silicone Elastomer Kit, 10:1 prepolymer/crosslinker mixing ratio, 60 °C overnight curing), low density polyethylene (LDPE, cut from ziplock bags), polyethylene terephthalate (PET, thin film), and polyimide (PI, Kapton®, Tesa 51408 flame retardant polyimide masking tape), two types of fresh leaves – Swiss cheese plant leaf (*Monstera deliciosa*) and red button ginger leaf (*Costus woodsonii*), and two types of metal electrodes – Pt plates (99.99%, Zhi Feng Yi, China) and Ag/AgCl plates (prepared according to the protocol introduced previously). All substrates were cut and pasted on glass slides to ensure flatness. Before testing, polymers and metals were cleaned with ethanol and blowing N₂, and leaves were cleaned with blowing N₂ only. During measurement, 6 µL liquid was dispensed from a needle of 0.51 mm outer diameter. Immediately after this, the substrate (initially located right underneath the dispensing needle) was slowly brought into contact with the droplet and caught it off the needle. This is to eliminate influence of fast dropping on wetting behaviors.^[1-2] The droplet was left undisturbed for 3 min before an image was captured and contact angle was measured. A waiting time shorter than 3 min would result in a larger measured contact angle, and a longer duration would cause observable droplet shrinkage due to water evaporation.

6.2.2 UV-Vis Spectrometry

Dye solubilization experiment was conducted according to previous reports.^[3-6] First, 0.6 mM 1,6-diphenyl-1,3,5-hexatriene (DPH) was dissolved in methanol. Next, 30 µL of DPH solution was added to 3 mL saline (0.9% NaCl) with or without 11% EPC (denoted as saline-EPC-DPH and saline-DPH respectively). The mixture was left at 4 °C for 2 days to ensure homogeneous mixing before testing. Absorbance spectra of saline-DPH and saline-EPC-DPH were recorded (at ~0 °C) using a UV-Vis spectrophotometer (SHIMADZU, UV-2550) from 330 nm to 430 nm with 1 nm step size. Pure saline without DPH served as the baseline sample. Each scan was completed within 30 s from sample being taken out from icy water bath to minimize error due to temperature increase.

6.2.3 Confocal Microscopy

Fluorescent dye-labelled EPC solution and agar solution were imaged at room temperature by confocal microscopy (LEICA DMI8) to visualize the internal structure.

Fluorinated glass slides were used as the substrates during imaging to mimic the hydrophobicity of plant tissues at the same time ensure flatness and transparency for imaging. First, a cover slip was thoroughly cleaned using ethanol and underwent 5 min of plasma treatment at 150 W, 50 kHz and oxygen flow rate of 50 sccm (CUTE plasma system, Femto Science), to render hydrophilicity and –OH groups on the glass surface. Next, ~10 μ L trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich, 448931) was dropped on a glass slide and placed beside the plasma-treated cover slip in vacuum for an hour, to allow fluorination to take place.

Labelling of EPC solution (0.01%, 1%, and 11% in 0.9% saline) follows the same procedure as described for UV-Vis spectroscopy, except that the concentration of DPH in methanol was 1 mM. 10 μ L DPH-labelled EPC solution was dropped onto fluorinated glass and allowed to stabilize and gelate for 3 min before imaging. Water evaporation was minimized using a plastic cap. Images were captured using 405 nm excitation laser and counting emission from 420 to 480 nm. Z-stack scanning was done at a step length of 0.4 μ m.

Agar polymer was labelled with carboxylate-modified microspheres (ThermoFisher, F8888) of 2 μ m diameter. First, 50 mg mL⁻¹ *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (Sigma-Aldrich, E1769) in water was mixed in equal volume with the microsphere dispersion (2% solids) and ultrasonicated for ~30 min. The mixture was dropped in hot agar solution (0.5% in 0.9% saline, pH adjusted to 5 using HCl) at 10 vol%. The final mixture was stirred at 60 °C for 2 h before dropping (10 μ L) on fluorinated glass for confocal microscopic imaging. Complete gelation of the droplet was ensured before imaging. Water evaporation was minimized using a plastic cap. Images were captured using 405 nm excitation laser and counting emission from 520 nm to 580

nm. Z-stack scanning was done at a step length of 0.4 μm .

6.2.4 Preparation and Characterization of Adhesive Polymer Solutions/Pastes

To prove the necessity of sol-gel transition for mechanically robust bridge between hairy plants and electrodes, viscous solutions and pastes made of polymers that are commonly used in liquid glue were prepared and compared with EPC thermogelling solution in terms of rheological and adhesive properties. Poly(acrylic acid) (PAA) of 130 kDa M_n (Sigma-Aldrich, 09612JH) was dissolved in 0.9% saline at room temperature with agitation; polyvinyl alcohol (PVA) of 13 – 23 kDa M_n , 98% hydrolyzed (Sigma-Aldrich, 348406) was dissolved in 0.9% saline at 95 °C with stirring. A range of concentrations were explored for both polymers, until similar rheological properties to 11% EPC saline thermogelling solution in sol and gel states were obtained, respectively. The M_n of PVA was chosen to be comparable to that of EPC (~ 25 kDa), and M_n of the used PAA was the closest to EPC among available variants of PAA.

As-prepared PVA and PAA solutions and pastes underwent rheological testing in temperature ramp, with the same parameters used for characterizing EPC solutions, i.e., 5 rad s^{-1} frequency, 1% amplitude, 3 $^{\circ}\text{C min}^{-1}$ ramp rate from 0 to 50 $^{\circ}\text{C}$. Polymer pastes with sufficient mechanical integrity were also exposed to amplitude sweeps at 25 $^{\circ}\text{C}$ (5 rad s^{-1} frequency, 0.1 – 3000% amplitude) to study yield behavior. Attempts to measure shear adhesive strength of viscous polymer solutions in lap-shear configuration were made by dropping polymer solutions in between two adherends separated by a distance of ~ 1 mm and held vertically by clamps of the mechanical tester. Measurement of shear adhesive strengths of polymer pastes was done by spreading the paste with pressure (to ensure full coverage) on adherends before clamping samples on the mechanical tester.

6.2.5 Tensile Adhesion Tests of Agar Gel

To investigate the adhesion property of agar gel in more depth, tensile adhesion tests were conducted on agar gels of varying concentrations. Agar gels at concentrations of 0.5%, 1%,

2% and 3% in 0.9% saline were tested according to the procedure illustrated in **Figure 6.1**. Gelling solutions were stored at 60 °C to improve ease of injection. Approximately 130 μL of solution was injected so that after gelation a complete coverage of Ag/AgCl plates could be obtained. 3 min of solidification (before first stretch) and 1 min of stabilization (before second stretch) were practiced and a strain rate of 6 mm min⁻¹ was used. Two adhesion forces were recorded from such tests: in-situ tensile adhesive strength and ex-situ tensile adhesive strength.

Figure 6.1 Schematics of procedure for tensile adhesion tests of agar gel. Distance between the two Ag/AgCl plates is 1 mm.

6.3 Wetting and Interfacial Adsorption

As EPC thermogelling solution and agar gelling solution are just dropped on plants, they behave like viscous liquids. Surface wetting of these liquids on plants is crucial for conformal coverage, especially considering the fact that most plant surfaces are hydrophobic.^[7] Furthermore, interfacial adsorption is critical to adhesion because only when the polymers in solution have direct contact with the substrate can robust adhesion occur. Previous literature also suggests that sole physical adsorption at the interface is sufficient for considerable adhesion.^[8-9] Moreover, the presence of interfacial water preventing the direct interaction between polymer and substrate is a major cause of poor

adhesion between hydrogel and wet tissues as well as underwater adhesives.^[10-11] Lastly, polymer affinity to plant surface affects the interfacial bonding strength between gel and plant. Taking these factors in account, studying the surface activity of EPC and agar polymers will shed light on the adhesion properties of corresponding gels.

Due to the disparate molecular compositions of EPC thermogel and agar gel, their behaviors in surface wetting and interfacial bonding are likely to differ. Specifically, as a flexible block copolymer with hydrophilic PEG and hydrophobic PPG and PCL segments (Figure 3.1),^[12] EPC can form micelles in water.^[3, 5] In contrast, agar polysaccharides (agarose and agarpectin) are primarily hydrophilic, alternating and random copolymers with conformationally inflexible sugar backbone, and arrange in stiff double helices once gelate (Figure 2.9).^[13-14]

To systematically study the interfacial behaviors of EPC and agar polymers, a series of experiments were conducted. First, surface tension and contact angle measurements were done using dilute solutions to confirm the surface activity (tendency of interfacial adsorption) of EPC polymer. Next, surface activity of EPC polymer at higher concentration and at lower temperature was verified using UV-Vis spectrometry and confocal microscopy, respectively. This evidence suggests that during the practical application of EPC thermogelling solution, interfacial adsorption indeed occurs, which results in enhanced adhesion compared to agar gelling solution. Therefore, the surface activity of EPC polymer offers an attractive feature to enhance interfacial adhesion.

6.3.1 Dilute Solutions at Room Temperature

Surface tension and contact angle are direct indication of surface activity. However, they can only be measured at around room temperature because of equipment limitation. Hence it is not possible to directly study the properties of gelling solutions at application temperatures (i.e., 4 °C for EPC solution and 40 °C for agar solution). Alternatively, dilute solutions of EPC and agar polymers were used to avoid undesired gelation within dispensing syringes. Such tests can be used to study the intrinsic properties of polymers

and to infer properties at higher concentrations and different temperatures.

Figure 6.2 Surface tension of agar and EPC solutions. **a)** Surface tension of 0.9% NaCl droplets in air ($n \geq 3$) with addition of agar and EPC polymers at increasing concentration. Error bars, s.d. **b)** Enlarged view of surface tension of agar solutions (mean $\pm$ s.e.) with statistical analysis. *** $p < 0.001$; unpaired two-tailed t-test. ns, not significant.

Surface tension was first measured for saline added with varying amounts of polymers (**Figure 6.2**), and contrasting results were obtained for EPC solutions and agar solutions. For instance, only 0.1% EPC added can reduce the surface tension of a saline droplet from 71 mN m⁻¹ to 49 mN m⁻¹, but the maximum reduction in surface tension by agar is only 1.6 mN m⁻¹ at 0.03% (Figure 6.2a). The reduction in surface tension by EPC polymer comes from physical adsorption at the liquid-air interface with hydrophobic segments pointing outward and hydrophilic segments pointing to the droplet interior. In contrast, the minor reduction in surface tension by agar polymer suggests a much lower density of physical absorption at the interface, due to rigid molecular structure and lack of amphiphilicity. Subsequent recovery (increase) in surface tension back to the level of pure saline at agar concentrations higher than 0.03% (Figure 6.2b) may be an indication of aggregation of agar polymers within a droplet, leaving a negligible number of polymers absorbed at the liquid-air interface. It should be noted that both 0.3% and 0.5% agar solutions can gelate at room temperature; therefore, it is very likely that assembly of agar polymers has already started during solution dispensing and image capture, although the droplets appeared liquid during experiment. Moreover, previous evidence points to pre-gel clustering at very low

concentrations.^[14] In other words, dilute agar solutions that do not gelate at room temperature (0.1% agar in this experiment) can still form double helix assemblies or other intermediate forms of assemblies. The above analyses all support the hypothesis that aggregation of agar polymers before complete gelation results in negligible influence on droplet surface tension in air. The concentration of 0.03% was selected for the following contact angle measurement to avoid such pre-assembly effect, where the maximum surface activity is observed.

Figure 6.3 Histogram showing contact angles of saline, agar and EPC solution droplets on solid surfaces (mean $\pm$ s.e.). * $p < 0.05$, *** $p < 0.0001$; unpaired, two-tailed t-test. ns, not significant. PDMS = polydimethylsiloxane, LDPE = low density polyethylene.

Figure 6.3 summarizes the results of contact angle (CA) measurement on synthetic polymers, fresh leaves, and metal-based electrodes. In general, addition of EPC causes statistically significant and drastic changes in CA of saline sessile drops, whereas addition of agar induces either insignificant or small changes, which suggests stronger surface activity of EPC compared to agar, consistent with surface tension results. The changes in CA displayed in Figure 6.3 can be classified and explained in three categories: CA reduction on initially hydrophobic surfaces, CA increment on initially hydrophilic surfaces, and CA reduction on initially hydrophobic surfaces.

With reference to the tension analysis at the liquid-solid-vapor contact line illustrated in Figure 3.8, it can be easily deduced that if an initially hydrophobic surface (when in contact with saline) sees a reduction in CA (when in contact with agar or EPC solution), γ_{SL} must be reduced no matter whether γ_L is unchanged or decreased (by addition of polymers). Similarly, if an initially hydrophilic surface sees an increment in CA, γ_{SL} must be increased no matter whether γ_L is unchanged or decreased. The first scenario applies for PDMS, LDPE, and Swiss cheese leaf surfaces, and the second scenario applies for Pt and Ag/AgCl surfaces. Therefore, adsorption of EPC polymers on hydrophobic surfaces causes reduction in interfacial tension, suggesting favorable physical interactions,^[15-16] which should contribute to interfacial bonding, based on prior evidence that reducing interfacial tension improves bond strength of solid-state polymer adhesives.^[17-18] On the other hand, EPC polymers increase interfacial tension between metal-based electrodes and aqueous solution. This is reasonable because water as a highly polar solvent can bond strongly with metallic surfaces through interaction of outer shell electrons^[19] and with ceramic surfaces which are often charged^[20], but polymers are less polarizable thus energetically less stable when in contact with these surfaces. Nevertheless, interfacial adsorption still occurs in lowering the free energy of the whole system. The final scenario, on red button ginger leaves, does not lead to definite conclusion on interfacial tension. The reduction in CA by EPC addition may be a result of decreased γ_L , decreased γ_{SL} , or the combined effect from the former two. Despite the inconclusiveness of the final scenario, results on other samples provide compelling evidence that substantial adsorption of EPC polymers on both hydrophobic and hydrophilic surfaces occurs, but minimal adsorption occurs for agar polymers.

Table 6.1 Calculation of solid-liquid interfacial tension change due to addition of 0.03% agar or EPC polymers on various types of surfaces. Orange shading indicates calculated results, and green shading indicates constant values for all surfaces. The unit for angle is degree (°) and for surface tension is N m⁻¹.

	γ_{saline}	γ_{agar}	γ_{EPC}	$\Delta\gamma_{SL}^{agar}$	$\Delta\gamma_{SL}^{EPC}$
PDMS	111	108	96.6	-4.09	-18.8
LDPE	96.6	95.4	81.5	-1.66	-16.4

Swiss cheese leaf	97.5	97.4	72.1	-0.377	-26.4
Red button ginger leaf	82.2	95.1	71.3	15.7	-8.41
Pt	55.2	61	69.1	7.02	20.2
Ag/AgCl	50.8	52.7	56.6	3.04	13.7
γ_L^{saline}	70.5	γ_L^{agar}	68.5	γ_L^{EPC}	56.1

To quantitatively compare the effect of EPC and agar polymers on saline-solid contact, changes in solid-liquid interfacial tension

$$\Delta\gamma_{SL}^{polymer} = \gamma_{SL}^{polymer} - \gamma_{SL}^{saline} \quad \text{Equation 6.1}$$

where *polymer* denotes either agar or EPC solution, are calculated using Young equation ($\gamma_L \cos\theta = \gamma_S - \gamma_{SL}$ Equation 3.12), assuming γ_S is a constant for a certain solid and γ_L takes the value measured from surface tension experiments (Figure 6.2). Parameters used for calculation and calculation results are tabulated in **Table 6.1**

Calculation of solid-liquid interfacial tension change due to addition of 0.03% agar or EPC polymers on various types of surfaces.. It is clearly seen from the table that changes in interfacial tension induced by EPC is drastically larger than those by agar polymers, except for red button ginger plants, which might be caused by large data variation instead of true difference since the leaves are highly hairy.

Figure 6.4 Histogram showing mean contact angles of saline and EPC solution (1%) at 0.9%

NaCl ($n \geq 3$) on various polymers and corresponding reduction of contact angles by addition of EPC. Error bars, s.d. PDMS = polydimethylsiloxane, LDPE = low density polyethylene, PET = polyethylene terephthalate, and PI = polyimide.

Contact angle measurements suggest favorable physical interactions between EPC polymers and hydrophobic surfaces including leaves. Thus, it is meaningful to investigate whether there is specific affinity between EPC polymers and plant tissues, which will be helpful in establishing strong gel-plant adhesion. Contact angles of EPC solution on different polymers shed light on the possible physical interactions between EPC polymers and plant tissues. As seen in **Figure 6.4**, the overall trend of contact angle reduction by EPC addition has a negative correlation with hydrophilicity of polymeric surfaces, i.e., more substantial reduction is seen on more hydrophobic surfaces. This is reasonable in that the interfacial tension between liquid droplet and polymeric surface is higher for more hydrophobic surfaces and more easily reduced by adsorption of hydrophobic segments of EPC polymers. However, one polymer that does not follow this trend is LDPE, which, among all polymers tested, shows the highest reduction in contact angle despite not being the most hydrophobic polymer. This means the liquid-solid interfacial energy is reduced substantially by adsorption of EPC, implying some special affinity between LDPE and EPC.

Figure 6.5 Molecular structures of polymers used in contact angle measurement. a) PDMS, b) LDPE, c) PET, and d) PI.

This affinity may be explained by the similar molecular structure and greater chain mobility of LDPE and EPC, both of which possess flexible, non-chemically crosslinked carbon chains, but other polymers do not (**Figure 6.5**). The greater chain mobility facilitates

conformational rearrangements for maximal contact in a lowest energy state between LDPE and EPC at the interface.

Figure 6.6 SEM images of polymers used in contact angle measurement at two magnifications. Cracks visible in high resolution images are from sputtered Au coating (20 nm thickness) to enhance contrast. Impurities are for ease of focusing.

On the other hand, SEM reveals a possible morphological origin of the abnormality of LDPE. Seen from **Figure 6.6**, all polymers other than LDPE have relatively flat and smooth surface topography, while LDPE possesses the roughest surface with irregularities of ~100 nm lateral feature size. Moreover, Atomic Force Microscopy (AFM) measured the largest average surface roughness of LDPE among all polymers (**Table 6.2**). This increased surface roughness will result in smaller apparent contact angle of EPC solution than the theoretical Young contact angle, as explained by the Wenzel equation:

$$\cos\theta^* = \tilde{r}\cos\theta \quad \text{Equation 6.2}$$

Here, θ is the Young contact angle introduced in Section 3.3.5, θ^* is the apparent contact angle observed macroscopically on a rough surface, and $\tilde{r}$ is the roughness ratio of the wet area, i.e., the ratio of the real surface in contact with liquid to its projection onto the horizontal plane (apparent area). An important conclusion from the Wenzel equation is that inherently smooth hydrophilic/hydrophobic surface will be more hydrophilic/hydrophobic when riffled.^[21] Notice that the rough LDPE initially behaves hydrophobically when saline is dropped on top, but it turns hydrophilic when EPC solution is dropped. These observed angles are in fact “amplifications” of those on smooth surfaces, i.e., increased contact

angles for saline (θ_{sal}^*) and decreased contact angles for EPC solution (θ_{EPC}^*), which means the difference between the two has been amplified by surface roughness. In other words, increased surface roughness results in larger contact angle reduction.

Table 6.2 Surface amplitude parameters of polymers, measured by AFM. R_q = root mean square (RMS) roughness, R_a = average roughness.^[22]

Polymer	R_q (nm)	R_a (nm)
PDMS	0.9	0.7
LDPE	11	8.5
PET	4.4	3
PI	3.9	2.9

In summary, molecular structure and surface morphology could be the origin of improved wetting on LDPE surfaces by addition of EPC. Intriguingly, the outmost surface of plant tissues is covered by waxes of similar molecular compositions (**Table 6.3**) with rough surface topography.^[7, 23] Therefore, the improved wetting on plants by EPC solution (Figure 6.3) may not only stem from surface activity of EPC polymer (adsorption on hydrophobic surfaces in general), but also be attributed to a greater physical affinity between EPC polymer and plant waxes through van der Waals forces. In conclusion, EPC thermogelling solution is effective in wetting and physically interact with plants thereby establishing conformal contact and strong interfacial bonding, suitable for plant-interfacing applications.

Table 6.3 Main classes of waxes present in most plant cuticles and their chemical structure. Adapted with permission.^[24] 2009, Springer Netherlands.

Component	Chemical structure	Range	Main constituents
Hydrocarbons	$\text{CH}_3(\text{CH}_2)_n\text{CH}_3$	$\text{C}_{21}\text{--}\text{C}_{35}$	C_{29} , C_{31}
Ketones	R_1COR_2	$\text{C}_{25}\text{--}\text{C}_{33}$	C_{29} , C_{31}

Secondary alcohols	$R_1CH(OH)R_2$	$C_{29}-C_{33}$	C_{29}, C_{31}
β -diketones	$R_1COCH_2COR_2$	$C_{27}-C_{33}$	C_{29}, C_{31}, C_{33}
Monoesters	R_1COOR_2	$C_{30}-C_{60}$	$C_{44}, C_{46}, C_{48}, C_{50}$
Primary alcohols	RCH_2OH	$C_{12}-C_{36}$	C_{26}, C_{28}
Aldehydes	$RCHO$	$C_{14}-C_{34}$	C_{24}, C_{26}, C_{28}
Carboxylic acids	$RCOOH$	$C_{12}-C_{36}$	C_{24}, C_{26}, C_{28}

6.3.2 Gelling Solutions at Low Temperature

Surface tension and contact angle measurements have confirmed the surface activity of EPC polymers at room temperature, but since PPG has a LCST in water in the range of 10 – 30 °C (as introduced in Section 3.2.1), it cannot be directly inferred that surface activity of EPC polymers persists at low temperature where EPC gelling solutions are in a sol state. It is possible that at 4 °C, PPG segments at the optimized concentration (11% EPC, corresponding to 1.9 wt% PPG) are also hydrophilic, and the low weight ratio of PCL (1.3 wt% of polymer weight, corresponding to 0.14 wt% in solution) might not be enough to drive effective micelle formation or surface adsorption. Hence, to find out whether surface activity still exists at low temperature (around application temperature 4 °C) and at optimized gelling concentration (11% EPC-0.9% saline), dye solubilization experiment was conducted on such formulation using DPH.

Figure 6.7 UV-Vis absorption spectra of DPH solution (6 μ M in 0.9% saline) with and without

11% EPC at $\sim 0^{\circ}\text{C}$.

Samples were stored in icy water before testing. A temperature of 0°C is easy to achieve and maintain and can avoid temperature increase to higher than 4°C during scanning. Moreover, if surface activity is confirmed at such low temperature, it will present at higher temperatures as well. **Figure 6.7** displays the absorption spectra of DPH solution with and without EPC addition. It is clear that with the addition of EPC, hydrophobic environment is formed around the dye DPH, which should be attributed to micelle formation by EPC.^[3-5] This proves the surface activity of EPC polymers at slightly higher than 0°C . Now one has confidence to conclude during sol application on plants, the surface activity of EPC polymers indeed participates in the gel-plant interaction, contributing to wetting and adhesion.

6.3.3 Gelling Solutions at Room Temperature

To give a clearer picture of the molecular arrangement within gelling solutions of EPC and agar, and to illustrate any difference between the two gelling systems, confocal microscopy was used on samples labeled with fluorescent dyes. Images from two viewing angles were extracted from z-stack scanning: top view and side view (**Figure 6.8a**).

For DPH-labeled EPC solution imaging, an excitation wavelength near the absorption peaks in UV-Vis spectrum (**Figure 6.7**) was used, and so only when DPH is present in a hydrophobic environment (micelle interior) can fluorescent emission be detected. Seen in **Figure 6.8b**, EPC micelles preferably accumulate at the three-phase contact line, in line with the contact angle results (**Figure 6.3** and **Figure 6.4**). 0.01% and 1% EPC solutions exist in sol state at room temperature, which is reflected in the absence of signal from interior of droplets in the confocal images. In contrast, fluorescent signals are prevalent across the droplet of 11% EPC solution, indicative of micelle formation throughout the gel bulk, confirming the associated micellar structure proposed in a previous study.^[5]

Figure 6.8 Setup and images for confocal microscopy of EPC sol/gel (with 10 μM DPH) and agar gel (with 0.1 wt% fluorescent beads) at room temperature. **a)** Setup and viewing angle. **b)** Top view and side view of droplets composed of EPC saline solution at varying EPC concentrations. **c)** Top view and side view of droplets composed of saline and 0.5% agar-saline. **d)** Side view of saline and agar droplets with outline highlighted (orange dashed lines). The green shading in agar images denotes blank area where no fluorescent signal was observed even when adjusting image brightness and contrast. Scale bars, 50 μm .

On the contrary, no preferable interfacial adsorption occurred in agar droplets (Figure 6.8c), in agreement with previous results of surface tension and contact angle. The accumulation of microbeads at the saline droplet circumvent should be a result of the coffee ring effect,^[25]

where evaporation and capillary flow of water bring particles towards the rim.^[26] Additionally, as the microbeads are made of polystyrene and surface functionalized with carboxyl groups, they may possess some surface activity thus tending to migrate to air-droplet interface. However, this phenomenon disappears in agar droplets, indicating uniform attachment of microbeads on agar polymers as well as the absence of surface activity in agar. One interesting observation from many images captured during the investigation is that agar droplets always appear “shorter” in z-axis (by about 20 μm) as compared to saline droplets of equal volume, despite expected reduced water evaporation rate (Figure 6.8d). This should be attributed to syneresis, i.e., extruding water on standing.^[27] Agar gel is known to experience severe syneresis, during which the gel network shrinks and water is expelled from any exposed surfaces (and sometimes sealed surfaces as well^[28-29]). This can have a significant impact on adhesion properties, as will be discussed in the following section.

To summarize Section 6.3, adsorption on water-air and water-solid interfaces is substantially more drastic for EPC polymer than agar polymers, stemming from their contrasting molecular structures. Adsorption of EPC on plants is through favorable physical interactions, whereas adsorption on metal electrodes occurs despite less favorable interactions than water, and both interfaces establish bonding through van der Waals forces. As a consequence, wetting of hydrophobic plant surfaces by gelling solutions is enhanced, and interfacial bonding between gel and both plant and electrode is improved. In addition, the wetting and bonding enhancement might be amplified by the molecular resemblance between plant waxes and EPC, as well as plant surface roughness. Such surface activity is also potentially helpful in achieving conformal contact with superhydrophobic biological surfaces (e.g., lotus leaf), of which pure saline poorly follows the asperities.^[2] By contrast, agar polysaccharides have minimal interfacial adsorption and thus affinity on solid surfaces, partly explaining the poor adhesiveness observed in Section 4.6.

6.4 Cohesion and Adhesion

6.4.1 Cohesive Integrity

Performance of EPC thermogel has been compared with solid chemical PAAm hydrogel, agar gelling solution, and viscous agar solution. In addition, given the adhesive properties of EPC thermogel, it is also meaningful to compare it with viscous polymer solutions and pastes that are commonly used in liquid glue (e.g., PVA, PAA) but do not undergo sol-gel transition. Although, to our knowledge, viscous polymer solutions/pastes have not been used in plant electrophysiology study, such comparison might provide further evidence on the unique advantages of EPC thermogel.

After surveying a range of concentrations, 20% PVA solutions at 25 °C possess similar viscosities to 11% EPC gelling solution at sol state, and 43% PVA paste and 30% PAA paste at 25 °C possesses similar viscosity to 11% EPC gelling solution at gel state, but these polymer solutions and pastes do not undergo sol-gel transition in response to temperature change (**Figure 6.9a**). Attempts to measure shear adhesive strength of 20% PVA solution using lap-shear test failed due to large liquid characteristics (**Figure 6.9b**). Moreover, $\tan\delta$ of 30% PAA paste is larger than 1 at all temperatures, indicating absence of stable gel network despite high viscosity of the mixture. This implies that PAA polymer chains are highly entangled at a high concentration of 30%, giving rise to high viscosity, yet inter-chain interactions that are strong enough to resist chain motion (i.e., crosslinks) are lacking.

Figure 6.9 Mechanical properties of viscous polymer solutions and pastes, compared to 11% EPC thermogelling solution. **a)** Viscosities and $\tan \delta$ of 20% PVA solution, 43% PVA paste, and 30% PAA paste. The number suffixes indicate wt% of polymers. **b)** Images within 10 s after applying 20% PVA solution between two Ag/AgCl plates with glass backing in a lap-shear configuration. **c)** Results from amplitude sweeps of 43% PVA paste and 11% EPC thermogel at room temperature around 25 $^{\circ}\text{C}$. **d)** Shear adhesive strengths of 43% PVA paste and 11% EPC thermogel on Ag/AgCl electrodes and *Gloxinia* hairy leaves, measured by lap-shear tests. **e)** Photographs of 43% PVA pastes on *Gloxinia* leaves (left, side view) and Ag/AgCl plates (middle and right, side and front view respectively) after shear tests. Scale bars in (b) and (e), 5 mm.

On the other hand, although 43% PVA paste has similar viscosity and viscoelasticity to EPC thermogel (at 25 $^{\circ}\text{C}$), it has inferior mechanical properties, including smaller yield stress and strain, and smaller storage modulus (Figure 6.9c). This inferior cohesive

mechanical integrity results in poorer adhesive performance (Figure 6.9d), because PVA paste also fails cohesively as EPC thermogel. This suggests the hydrophobic associations present in EPC thermogel are more effective in strengthening the gel than hydrogen bonding within PVA paste responsible for its gel-like appearance. In addition, when applying the PVA paste on adherends, difficulty was encountered to ensure full and uniform coverage. The PVA paste always existed in lumps, hard to spread evenly (Figure 6.9e), a result of lack of sol-gel transition and strong hydrogel bonding between PVA polymers. Therefore, significant amount of pressure had to be applied, which is not desirable for practical application.

The application of liquid glue to bond porous materials relies on the interfacial locking between polymers and adherends instead of the bulk mechanical strength of gluing polymers. Therefore, a thinner layer of liquid glue normally results in better adhesive strength than a thicker layer. Moreover, liquid glue although are applied in liquid form, must be completely dried to function. These conditions do not apply in gel-based ionic interface for electrical coupling, which should bear a certain thickness to accommodate plant surface texture (hairs of several mm long in this study) and maintain moisturized to attain conductivity. Consequently, although PVA and PAA are effective liquid glue component, it is not suitable for bridging hairy plants with electrodes. Furthermore, the high polymer mass ratio of PVA and PAA pastes is more prone to drying when exposed to air, which will result in increased salt concentration and eventually loss of conductivity, detrimental to plant ionic balance and electrical signal acquisition, respectively.

In summary, viscous polymer solutions cannot serve as an effective mechanical bridge between hairy plants and metal electrodes; a gel with sufficient mechanical robustness is imperative for stable plant-electrode interfacing. Although increasing polymer concentration can produce a gel-like material with similar viscoelasticity to EPC thermogel, the lack of sol-gel transition, inferior mechanical properties, and much higher polymer loading render adhesive polymer paste inferior to EPC thermogel.

6.4.2 Interfacial Bonding

In Chapter 4, the adhesive properties of EPC thermogel and agar gel were characterized in lap-shear configuration, and it was concluded that EPC thermogel has strong interfacial bonding and its cohesive strength is the limiting factor in shear adhesion failure, whereas agar gel has poor interfacial bonding, leading to adhesive failure at a lower strength. The possible reason for the contrasting interfacial bonding behavior is multiply: polymer mass ratio, physical interactions with adherends, gel syneresis, etc. The following discussions are to fully unravel the underlying mechanisms.

Figure 6.10 Rheological characterization of agar gelling solutions at 0.5% and 3% concentrations in the temperature range of 0 – 50 °C, as compared to 11% EPC solution. **a)** Viscosity and storage modulus comparison. **b)** $\tan \delta$ comparison.

Figure 6.11 Rheological characterization for gelation time estimation. **a)** EPC thermogelling solution was stabilized at 4 °C before quickly heating up to 25 °C, whereas agar solutions were stabilized at 50 °C before quickly cooling down to 25 °C. The different slope is due to varying heating/cooling rate of equipment (faster heating than cooling). **b)** Storage and loss moduli of 11% EPC, 0.5% agar, and 3% agar solutions under the temperature conditions in (a).

Since the difference in polymer mass ratio is huge between agar gel (0.5%) and EPC thermogel (11%), it was the first parameter studied, and the first step was to identify a proper range of concentration for adhesive strength measurements. Gelation temperature of agar solutions (in 0.9% saline) increases with agar concentration, which means there exists an upper limit of concentration where agar solution gels adequately slow at room temperature to leave ample time for manipulation. Agar solutions at weight ratios of 0.5% up to 11% were prepared and a concentration equal to or higher than 5% failed to produce

sufficient fluidity at 50 °C to allow withdrawal and dispense through a plastic pipette (dropper), and a concentration of 3% is barely enough to allow pipette handling. Further rheological characterization as temperature ramping from 50 °C to 0 °C gives more quantitative description of the gelation behavior of 3% agar solution (**Figure 6.10**). When compared with 0.5% agar solution, the viscosity of 3% agar in sol state (>40 °C) increases by nearly two orders of magnitude and becomes comparable to that of 11% EPC solution at <10 °C (Figure 6.10a), and the gelation temperature shifts from 34 °C to 42 °C (Figure 6.10b), which narrows the operational window for agar application. Gelation time estimation also confirms the short operational window of 3% agar solution (**Figure 6.11**), whose storage and loss moduli match those of 11% EPC and 0.5% agar solutions at 25 °C even before the equipment cools down to 25 °C (within 30 s from start of cooling). This problem was encountered during the later adhesion tests, where swift pipetting must be carried out in the shortest time possible, otherwise the solution will gelate inside pipette tips. In addition, compared with 11% EPC solution, 3% agar gel behaves more elastic and much stiffer (a smaller $\tan\delta$ and larger storage modulus).

Figure 6.12 Tensile adhesive strengths of agar gels at varying concentrations, Ag/AgCl as the adherends, and the corresponding differences between in-situ and ex-situ adhesive strengths. One-way ANOVA with Tukey correction. Error bars, s.d. ns, not significant.

Based on the rheological properties of agar solutions, 3% was selected as the upper limit

for adhesive strength investigation. Agar solutions at concentrations of 0.5%, 1%, 2%, and 3% were tested for tensile adhesive strength in the procedures described in Figure 6.1, and two adhesive strengths were recorded: in-situ strength and ex-situ strength. Note that adhesive failure always occurs for agar gel. Therefore, in-situ strength originates from interfacial bonding established in sol state, whereas ex-situ strength measures the ability to establish interfacial bonding in gel state. As seen from **Figure 6.12**, the in-situ strength follows a linear function with a y-intercept of $0.0086 \pm 0.1637 \approx 0$, suggesting that the interfacial bonding from sol state depends on bond density at the interface, which is proportional to polymer mass ratio. Therefore, ideally, if higher concentrations of agar gel could be prepared and applied easily, very high in-situ adhesive strength could have been achieved. For instance, 11% agar gel (same concentration as EPC thermogel) would have a tensile adhesive strength of 25 kPa. However, this extrapolation never occurs in reality, because very high temperature is required to maintain such highly concentrated solution in sol state (higher than 100 °C for 11% agar solution).

Since increasing agar mass ratio can enhance interfacial adhesion, agar gel with higher agar concentration should produce a higher shear adhesive strength as well. Therefore, 3% agar gel was tested in lap-shear configuration to measure its shear adhesive strength, results plotted in **Figure 6.13**, with reference to previous measurements of 0.5% agar gel and 11% EPC thermogel (Figure 4.10). It seems that by increasing the concentration of agar, agar gel can outperform EPC thermogel in shear adhesion on both metal-based electrodes and hairy leaves. Nevertheless, it is important to notice that these values of shear adhesive strength were measured after in-situ gelation, meaning the adherends were fixed in place while pre-gel solution was injected into the slit and gelates. However, during plant electrophysiology study, pre-gel solution was firstly dropped on plants followed by electrode placement, and there was normally 10 – 20 s interval between the two steps, when pre-gel solution was undergoing gradual gelation. It is thus meaningful to look at the ex-situ adhesion properties of agar gel and EPC thermogel.

Figure 6.13 In-situ shear adhesive strengths of 0.5% and 3% agar gels and 11% EPC thermogel on Ag/AgCl electrode and hairy leaf surfaces. Error bars, s.d.

Coming back to the tensile adhesion tests shown in Figure 6.12, for all concentrations of agar gel, the ex-situ strength is smaller than the corresponding in-situ strength by ~ 1 kPa, independent of agar concentration. This reduction in adhesive strength should be attributed to poor chain flexibility and syneresis. Upon detachment of agar gel from the adherend, the exposed gel surface may experience morphological changes due to sudden relaxation of stress from interfacial bonding, which makes the surface no longer match perfectly with the surface roughness on the adherend. Slight water evaporation will also cause surface deformation. Hence when the two surfaces are brought into contact again, voids are likely to exist, reducing effective bond density. In addition, because agar polymer chains are relatively rigid, molecular motion to fill these voids is limited. Furthermore, syneresis generates a layer of free water covering exposed gel surface, which further hinders interfacial bonding.^[10] The amount of water extruded is inversely related to agar concentration,^[27] and indeed during experiment, severe syneresis of 0.5% and 1% agar gels were observed but not for 2% and 3% agar gels. This will result in a smaller ex-situ strength measured for lower-concentration agar gels.

In contrast to agar gel, EPC thermogel failed cohesively in tensile adhesion tests, and the tensile adhesive strength remains constant for in-situ and ex-situ adhesion (**Figure 6.14a**).

This indicates that in gel state, EPC thermogel can still form effective interfacial bonding with adherends, and the gel coherent strength does not change even after gel fracture and reattachment (self-healing ability). These behaviors can be explained by the high chain mobility and high tendency of viscous flow, as well as the reversibility of physical crosslinks responsible for gel cohesive strength (resonating with large yield strain), as discussed in Section 4.4. Moreover, surface activity (Section 6.3) may further contribute to both in-situ and ex-situ interfacial bonding with solid adherends.

Figure 6.14 Tensile and shear adhesive strengths of EPC thermogel and agar gels on Ag/AgCl plates. **a)** Tensile adhesive strengths of 11% EPC thermogel. Paired t-test. Error bars, s.e. ns, not significant. **b)** Shear adhesive strengths of 3% agar gel and 11% EPC thermogel. ****** $p < 0.01$; unpaired, two-tailed t test. Error bars, s.e.

After understanding the different behaviors of agar gel and EPC thermogel in in-situ adhesion and ex-situ adhesion, the gels were subjected to shear adhesion tests that simulate the real situation during plant electrophysiology study to compare their performance. In such tests, pre-gel solutions were dropped on one piece of adherend (Ag/AgCl electrode in this case), and after brief gelation (not fluidic), clamped on the mechanical tester and brought into contact with another piece of adherend. Gentle pressure was then applied to facilitate adhesion, and tests were started after ensuring nearly zero force was exerted on the laminated gels. It was found that deterioration of interfacial bonding in ex-situ adhesion of agar gel is more prominent in shear adhesion tests than tensile counterpart. Shear adhesive strength of 3% agar gel on Ag/AgCl electrode decreases from 1.4 kPa for in-situ

adhesion to 54 Pa for ex-situ adhesion (Figure 6.14b). Such orders of magnitude reduction in adhesive strength, in contrast to the small change from 6.9 kPa to 5.5 kPa in tensile adhesive strength (Figure 6.12), is a result of smaller contact area between convex surface of agar gel (formed naturally from a sessile drop) and flat surface of Ag/AgCl electrode in ex-situ gelation. Shear adhesion now becomes more like a friction problem, and hydrogel is known for effective lubrication^[30] especially when agar gel has a layer of free water covering the surface (syneresis). Consequently, the ex-situ shear adhesive strength of 3% agar gel (54 Pa) is significantly smaller than that of 11% EPC thermogel (180 Pa) (Figure 6.14b), which is closest to the realistic situation during plant electrophysiology experiments considering the fast gelation of 3% agar gel presented in Figure 6.11.

Finally, the contrasting adhesive behaviors of 11% EPC thermogel and 0.5% agar gel observed in Chapter 4 can be explained based on the abovementioned experiments and analyses. First, low mass ratio in agar gel indeed contributes significantly to the poor interfacial bonding due to low bond density, but higher concentration (>3%) is not permitted due to inappropriate rheological properties. Second, since ex-situ adhesion is more likely to occur during practical electrode application on plants, small contact area due to surface morphology mismatch between gel and electrode as well as syneresis results in further reduction in interfacial adhesive strength. Furthermore, rheology and adhesion face trade-off in agar gel formulation: a lower concentration allows higher possibility of in-situ gelation that enhances adhesion but meanwhile it reduces bond density, which deteriorates interfacial adhesion; a higher concentration although can increase bond density and thus boost adhesive strength but once ex-situ adhesion occurs it makes negligible difference to the final adhesion strength. By tuning agar concentration within the range of 0.5% to 3%, it is possible to get an optimized concentration where adhesive strength is maximized and perhaps exceeds that of EPC thermogel. However, this causes great inconvenience during practical application because the agar gelling solution may need to be stored at high temperature with short time window for operation, in order to prevent early solidification. From practical point of view, EPC thermogel is more advantageous, since it gels gradually and is still adhesive after gelation, giving ample time and confidence to operators. In addition, EPC thermogel is self-healing with unchanged cohesive strength, so accidental

gel fracture due to careless operation would not cause much trouble.

6.5 Conclusions

In this chapter, the mechanisms for the superior performance of EPC thermogel as an ionic bridge between hairy plants and metal electrodes were investigated in detail. On the one hand, surface activity of EPC polymer was proved using various techniques and believed to promote conformability and interfacial adhesion, especially on rough waxy plant surfaces. On the other hand, gel mechanical properties including cohesiveness and adhesiveness are advantageous for practical application over adhesive polymer pastes and agar gels. These superior properties originate fundamentally from the flexible amphiphilic molecular structure and hydrophobic associations as crosslinks, as well as sol-gel transition, which is a thermodynamic consequence of EPC molecular composition.

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Chapter 7

Conclusion and Future Work

This chapter provides reflections and discussions on the results and proposes directions for future work. The hypotheses proposed at the beginning of the thesis were proven through a series of experiments and analyses presented in the previous chapters. Implications of the current work include contribution to the knowledge of thermogelling polymers and hydrogel materials, inspiration for soft material utilization in bio-electronic integration, and effective tool for plant electrophysiological study, etc. Future work can be done in aspects such as improving thermogel properties (mechanical strength and water retention) towards practical application, consolidating a generalizable theory for bioelectrode conformability on rough surfaces, and exploring plant-electronic integration for advanced sensing and controlling functions.

7.1 Discussion and Conclusion

7.1.1 Testing Hypotheses

The two hypotheses proposed previously include:

Hypothesis 1: Thermogel can establish a conformal and adhesive ionic interface between plants and electrodes.

Hypothesis 2: Thermogel ionic interface can deliver improved signal quality and greater operational convenience than conventional hydrogels in surface contact electrophysiology on hairy plants.

The hypotheses were proven through the following experiments and results. Firstly, a thermogelling polymer solution with salt additive (EPC thermogel) was optimized to satisfy application requirements, and microscopic observation and adhesion characterization proved the conformability and adhesiveness on hairy plants and metal electrodes. Secondly, rheological, mechanical, and electrical characterizations of EPC thermogel demonstrated more promising properties than conventional hydrogels in surface contact electrophysiological measurement. Thirdly, plant electrophysiology studies were carried out on hairy plants using a simplified electrode design to previous studies, with EPC thermogel and other control gels as the ionic interfaces; mechanical bridging was the most robust with thermogel and statistical analyses of signal amplitudes revealed improved signal fidelity from EPC thermogel as well. Finally, detailed investigations into the molecular origins of the observed properties uncovered more practical advantages of EPC thermogel.

7.1.2 Major Findings

Major findings of the study are summarized below:

1. Liquid-phase application of pre-gel solution results in conformal coverage of hydrogel on hairy and rough plant surfaces, and surface activity of EPC polymer further enhances wetting on waxy plant surfaces.
2. Physical adsorption of EPC polymer on solid surfaces including plants and electrodes contributes to interfacial adhesion. In particular, adsorption on hydrophobic surfaces is accompanied with favorable physical interactions, which may further enhance interfacial bonding.
3. Low polymer mass ratio is the main contributor to poor adhesion of agar gel, but higher mass ratio is not permitted due to rheological tradeoff. The lack of surface activity and thus interfacial adsorption also contribute to the poor adhesion. Additionally, ex-situ gelation further deteriorates interfacial adhesion, resulting from poor chain mobility and syneresis.
4. EPC thermogel remains adhesive to solids even after ex-situ gelation due to high chain mobility and surface activity. High chain mobility as well as reversible hydrophobic association also results in self-healing capability. These two characteristics are helpful in practical application.

7.1.3 Implications of Current Work

The study described in this thesis is multidisciplinary with a material focus and has an engineering goal yet generating scientific insights. As a result, implications from this work are multiply.

From materials perspective, the current work systematically studies the properties of EPC polymer and EPC thermogelling solution, in terms of surface wetting, interfacial interaction, rheology, sol-gel transition, conductivity, adhesiveness, etc. Adhesive properties of agar gel are also studied as a control sample, which has not been examined before. Knowledge generated through these investigations will contribute to the field of

polymer materials, in particular hydrogel materials. Furthermore, this work proposes a new application of thermogelling polymers, which are traditionally used for tissue engineering and drug delivery. This attempt will inspire future exploration of soft materials in integrating with biological objects to achieve advanced and diverse functions.

From plant biology and bioelectronics point of view, this work provides a feasible solution for accessing plant electrical signals non-invasively on hairy surfaces with greatly simplified experimental setup and improved operational convenience. This will equip plant biologists with better tools to study the electrophysiology of a wider array of plant species, and potentially enable plug-and-play integration between plants and electronics for advanced applications such as sensing and actuation, pioneering a new direction in bioelectronics.

7.2 Future Work

7.2.1 Improvement of Thermogel Properties for Plant Study

The formulation used in this study though was optimized still suffers from some drawbacks, such as higher salinity than plant internal environment, low cohesive strength, dehydration during long-term measurement (>1 h), and limited tolerance to temperature variation. To overcome these issues towards practical utilization of thermogel in plant electrophysiology study, the following efforts may be made.

7.2.1.1 Salt concentration reduction

The salt concentration used in this study is higher than those in reported studies. To avoid any damage to plant cells during long-term measurement, salt concentration should be lowered. Preliminary examination of EPC thermogel added with 10 mM KCl, according to the protocol for *Arabidopsis thaliana*,^[1] shows comparable rheological and mechanical properties to the optimized formulation used in this study, and sufficient conductivity (**Figure 7.1**).

Figure 7.1 Rheological, mechanical, and electrical properties of 10% EPC thermogel added with 10 mM KCl (labeled as EPC10-KCl0.01), in comparison with optimized formulation used in this study, 11% EPC-0.9% NaCl (labeled as EPC11-NaCl0.9). **a)** Storage and loss moduli as a function of temperature. **b)** Gelation time comparison when thermogelling solutions are heated from 4 °C to 25 °C. **c)** Yield behavior comparison in amplitude sweep. **d)** Histogram of conductivity of 10 mM KCl without and with addition of 10% EPC polymers (mean $\pm$ s.e.). * $p < 0.05$; unpaired, two-tailed t test.

Another way to fully circumvent the safety concern of high salt concentration is to improve hydrogel conductivity using electronically conductive fillers, such as carbon materials, metal nanomaterials, and conductive polymers.^[2-3] Recent works on conductive hydrogels have made substantial progress in achieving conductivity up to $\sim 50 \text{ S cm}^{-1}$.^[4-5] Since the requirement of conductivity for plant electrophysiology is not highly demanding ($\sim 1 \text{ S cm}^{-1}$), addition of a small amount of conductive fillers might be sufficiently effective.

7.2.1.2 Rheological and mechanical improvement

Mechanical properties may be improved through molecular composition adjustment and inclusion of additives. Specifically, higher mass ratio of PCL segments may be incorporated, or more hydrophobic polymers such as PHB can be used to substitute PCL to promote hydrophobic association, bringing down critical gelation concentration^[6] so that reduced polymer concentration can be used in the formulation to improve sol fluidity; conductivity may be improved as well. Furthermore, a higher volume of hydrophobic associations might result in higher gel strength.

A more effective way to improve gel cohesive strength is to include reinforcement particles, such as silica nanoparticles, nanoclay, and carbon-based materials, which are reported to effectively improve the mechanical properties of hydrogel composites at low concentrations.^[7] As thermogel is physically crosslinked, interactions between materials within gel networks determine the rheological and mechanical properties. Therefore, special attention should be paid to possible interactions between thermogelling polymers and reinforcement particles to ensure low sol viscosity, appropriate gelation temperature, and high gel strength. For example, due to strong hydrophobicity, graphene might act as a core for micellization and gelation, lowering gelation temperature. Substantial increase in sol viscosity may be seen if large aggregates form around graphene flakes. In contrast, carbon nanotubes are likely to produce less increase in sol viscosity because of high aspect ratio. Hydrophilicity of carbon materials can be easily modified through surface functionalization, which provides more possibilities to achieve the desirable rheological and mechanical properties. Furthermore, nanoclay particles bearing negative charges may be feasible as well, serving as salt-out ions to precipitate thermogelling polymers, thereby lowering critical gelation concentration and improving gel strength with mild influence on sol viscosity. Additionally, they are advantageous over ionic salts in low risk of penetration through plant cuticle.

7.2.1.3 Dehydration mitigation

Dehydration is a problem for monitoring longer than ~1 h (observed in the current study), where signal quality deteriorates as impedance of a drying gel increases (**Figure 7.2**). To solve this issue, elastomeric encapsulation can be applied, as this strategy was previously shown to effectively delay hydrogel dehydration.^[8] This can be done in a sprayable coating format, like liquid bandage (e.g., Nexcare™ No Sting Liquid Bandage, Hansaplast Spray Plaster). Liquid bandage is applied in a liquid form, through either spraying or brushing, and solidifies to a flexible film of shield on wounds. This film is waterproof and claimed to last for several days on skin. It is suitable for wounds of awkward shape because of liquid-form application. Based on its durability, waterproof property, shape-adaptability, and biocompatibility, liquid bandage might be suitable for sealing surface electrodes on plants. Commercial liquid bandage products can be purchased for trial, with further modification of formulation if necessary. Ecoflex™ might also be a feasible choice since a fast-curing (within 5 min) version (Ecoflex™ 00-35 FAST) is available and it is certified safe when curing against the skin, so it should be biocompatible on plants as well.

Figure 7.2 Dehydration of thermogel (11% EPC, 1% NaCl). **a)** Setup for measuring the conductivity of thermogel. Sample was placed in fume hood for air drying. **b)** Percent of water loss (by mass) with time. **c)** Conductivity drops with water loss and reaches a threshold value for effective electrophysiology recording (according to conductivity of 10 mM KCl used in previous reports^[1,9]) at around 70 wt% water loss. **d)** Conductivity change with time on a logarithm scale. At the current setup and drying conditions, it takes around 3 hours for thermogel to reach the threshold conductivity. In real plant measurement, this time varies.

Another potential solution to the dehydration problem is to replace some water in the thermogel with glycerol, a non-volatile, biocompatible solvent, which has been demonstrated to effectively suppress hydrogel dehydration.^[10] However, rheological and electrical properties might be altered with the partial substitution of water, which needs experimental verification.

7.2.1.4 Temperature variation tolerance

One limitation of the current electrode setup is that it cannot sustain large temperature variation in service, because a drop in temperature below gelation temperature will cause liquification of thermogel and consequent mechanical failure of electrode. Hence, although the current electrode is sufficient for fundamental study in labs with well controlled environmental conditions, it cannot be directly applied in outdoor environment with largely varying temperature (e.g., 10 °C temperature difference within a day).

Figure 7.3 Holistic encapsulation to improve temperature tolerance and mitigate dehydration of thermogel-based electrode.

Possible solutions to this issue include holistic encapsulation that seals the thermogel-plant interface so that liquification and dehydration can be overcome concurrently (Figure 7.3). To fully seal the electrode on hairy plants, such encapsulation should also be applied in a liquid form, establish robust adhesion with plants, and mechanically secure the metal conductor and wire. Common elastomer precursors (silicones) have low surface energy,^[11] so they should have good contact with hydrophobic plants. Short-term liquification of thermogel should not be a problem for electrical signal measurement as long as the encapsulation is effective, since gel conductivity will not decrease in this scenario.

7.2.2 Theory for Conformability on Rough Surfaces

Conformability is one of the key parameters for bioelectronics attached on biological surfaces and most biological surfaces are rough with feature size at different scales. The hairy plants studied in the current work represent a rough surface with large feature size (mm scale), but the metal-plate electrodes have microscopic irregularities (**Figure 7.4**). Conformability on these two types of rough surfaces was investigated in this study and two conclusions concerning with the criteria for conformability were drawn: 1) liquid-phase

application allows conformability on hairy plants; 2) high chain mobility at the adhesion interface allows microscopic roughness to be followed. The two conclusions can be further summarized using a unified concept: large-scale deformation (e.g., polymer chain relative displacement) is required for conforming to macroscopic surface asperities (e.g., beyond mm scale), and small-scale deformation (e.g., chain segment motion) is sufficient for microscopic irregularities (e.g., below 10 μm scale). Therefore, depending on the surface topography of a surface to be attached to, rational design of the interfacing material can be drafted accordingly. The thermogel ionic interface between hairy plants and electrodes in the current study is a good illustration of this concept. On the one hand, sol application permits large-scale deformation to allow gel conformability on plants; on the other hand, high chain mobility in gel state permits interfacial chain arrangement to conform to electrodes thereby enhancing adhesion.

Figure 7.4 Surface height profile of Ag/AgCl (a) and Pt (b) plate electrodes.

Despite the usefulness, the unified rule mentioned above is rather qualitative, without numerical description of material specifications. What rheological and mechanical properties (e.g., viscosity, modulus) described mathematically in relation to surface topography parameters (e.g., roughness, asperity height and width) are required for a material to conform to a given surface? What is(are) the assumption(s) for wettability? What is the kinetics of wetting/conforming process? What is the influence of external pressure? All these questions remain open for further study, requiring knowledge in

mechanics of soft materials and surface/interface science. If a general quantitative rule could be derived, it would be a fundamental guideline for future bioelectronics design.

7.2.3 Plant Electrophysiological Study and Plant-Electronic Hybridization

With the thermogel interface as a handy tool, many plant electrophysiological studies that are not permitted previously can be carried out. For example, tobacco though being an important economic crop, has rarely been studied systematically on its electrical signaling behavior.^[12-13] The plant shoot is small in size with vulnerable leaves and hairy stems, making testing troublesome. In the current work, it has been demonstrated that non-invasive recording on tobacco stem using thermogel has comparable signal quality to invasive methods. Hence further research on tobacco behavior such as multi-leaf wounding, response to light, mechanical wounding, etc., and plant-plant communication can be conducted. Interestingly, signal transmission within stems with speed and amplitude characteristics contradictory to conventional understanding was observed, which should be studied thoroughly in the future.

Furthermore, in the sunflower wounding experiment described in this thesis, small peaks were collected using thermogel with higher peak amplitude than solid PAAm hydrogel. The meaning behind these small peaks is unknown. Now that thermogel can help to capture these peaks more effectively, further investigation into the feature of potential fluctuation waveforms might be carried out. Additionally, the novel observation of voltage-dependent signal intensity difference between EPC thermogel and PAAm hydrogel-based electrodes has been explained in Section 5.4 by different transmission pathways of VPs and APs near trichomes. The hypothesis needs more in-depth study that entails more accurate electrode positioning and larger sample size. Results from such studies would provide more precise description of the transmission pathways of electrical signals within plants.

Beside fundamental study on plant electrophysiology, the knowledge generated should be utilized to modulate and instruct plant physiology, to achieve goals like promoting growth and defending against pests, which eventually could lead to crop yield improvement. In

addition, the environmentally sensitive and responsive nature of plants can be accessed through electronics for smart sensing of environmental changes, which may be invisible to or slow to capture by other forms of sensing/forecast. To achieve these futuristic functions, innovative device design and deeper understanding of plant electrophysiology are pivotal.

Overall, plant electrophysiology is still a small branch of plant biology yet will be of growing importance as the issues of climate change and food security escalate with time. There is plenty of unknown to be discovered and unsolved problems to be overcome, which could help provide solutions to future sustainable growth. The work presented in this thesis is an effort to address one of the problems encountered in plant electrophysiological study and plant-electronic integration, which hopefully will inspire more research to be conducted in this area in the future.

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APPENDIX

1 Types of Electrical Signals in Plants

The term ‘electrical signals’ can be used to refer to any alteration of membrane potential caused by ion flux cross the plasma membrane. For the sake of analyzing plant behavior at the organism level, normally signals that can travel relatively long distance are the focus of study. These signals include action potentials (APs) and variation potentials (VPs, also known as slow wave).^[1]

Action potentials in plants are very similar to those in animals. They are self-propagating, obey the all-or-nothing rule, transmit with constant velocity and amplitude, and rely on voltage-gated ion channels (VGICs).^[2] The resting membrane potential of plant cells is negative on the inside, and once an AP is evoked (either by external stimuli or self-initiated), Ca^{2+} flow into the cell through VGICs, causing depolarization of the cell membrane. The following Cl^- efflux further intensifies the depolarization, and finally the membrane potential is brought back to the resting level by K^+ efflux. APs in vascular plants mainly transmit in bundles of phloem,^[1] although living cells of xylem (protoxylem, metaxylem) and the entire organ (trap) of insectivorous plants can be pathways of transmission as well.^[2] Intriguingly, plants do not have cell types that specifically perform the generation and transmission of APs, like neurons and cardiomyocytes in animals. Any cells with VGICs can potentially participate in the generation and transmission of APs. However, it is still unclear why APs are confined in living vascular bundles but not the surrounding tissues.^[1] The firing of APs can be spontaneous, i.e., initiated by the plant itself (whose function is not well understood but may be associated with growth progression),^[3] triggered by electrical stimulation,^[4] or other forms of non-damaging stimuli (mechanical touch, light/dark alterations, pollination, etc.).^[1]

Variation potentials are fundamentally also perturbations in membrane potential caused by ion fluxes, but they are very different from APs in terms of transmission characteristics and mechanisms. VPs attenuate in amplitude and transmission speed with further distance from

the point of generation, and thus do not rely on VGICs. Instead, they are believed local changes in membrane potential caused by another type of signal passing by. Pressure changes and chemical species are the most possible types of such source signals, and to evoke VPs in nearby tissues, mechanosensitive channels and ligand-activated channels are proposed to be the basic machinery. VPs primarily transmit in xylem guarded by dead cells, and they are often triggered by damaging stimuli such as heating and severe wounding.^[1]

Knowing the distinct characteristics of APs and VPs, to identify them in recorded waveforms is, however, nontrivial, because in many cases the measured signals are a combination of APs and VPs superimposed in time and amplitude (such as in heat-wounding). It has been suggested that stimuli that can evoke VPs are also capable of triggering APs, and the passing of VPs can potentially initiate a wave of APs.^[1] These complications make signal analysis very challenging, and consequently, plant electrical signals are generally poorly understood.

2 Functions of Electrical Signals in Plants

Electrical signals as a rapid, long-distance signaling channel, is crucial to plants' response (to environmental danger) that requires fast and coordinated functioning of different cells and tissues. They also serve other indispensable communication functions at organism level for plants' normal physiological activities, with or without supplement from other types of signals (e.g., pressure, chemicals).

2.1 Preying

Electrical signals are firstly discovered in insectivorous plants.^[1] Plants such as Venus flytraps, waterwheel plants, sundews, and butterworts, demonstrate fast movements of the trap organ to constrain insects once the preys land on the mechanosensory parts (e.g., trigger hairs). Electrical signals (APs) are evoked by mechanical stimuli from the insects through mechano-responsive ion channels, transmit across the entire trap, and instruct the actuation of trap cells. In addition, APs are also found to signal the secretion of enzymes

to digest the trapped insects, and to be positively related to enzyme secretion.^[2]

2.2 Fertilization

APs are triggered once a pollen sets on the stigma of the pistil, and the ovary respiration rate increases afterwards indicating its readiness for pollination. It is also found in several species that only when a second AP, triggered mostly probably by some kind of chemical stimulus induced by pollen recognition, travels down the style, the ovary responds. The detailed mechanism and process have yet to be unraveled, but it reflects the intelligence of plants in requiring additional information for making the right decision.^[2]

2.3 Thigmonastic Movements

The movements of plant parts in response to mechanical stimulation is termed thigmonastic movements (independent of stimulus direction). Besides insectivorous plants that are capable of trap closure, several other species like *Mimosa pudica*, *Biophytum sensitivum* and *Incarvillea* spp. also exhibit thigmonastic movements. In these plants, a large number of cells are capable of mechanical sensing. For example, the entire leaflets of *Mimosa* are responsive to touch. Electrical signals are evoked in mechano-responsive cells, transmit through leaves, petioles, and sometimes stems, which cause water loss in cells at specific locations and result in macroscopic deformations and movements, such as leaf folding and petiole bending. The purpose of thigmonastic movements is however not fully understood.^[2]

2.4 Environmental Adaptation

Plants are exposed to constantly changing environment in their habitats, and they should be well informed of environmental conditions such as temperature, brightness, humidity, and water content in soil for adjustment of physiological activities in order to adapt to the environment. Generation of electrical signals has been associated with many of these environmental factors and may play a role in plant physiological modulation.

For example, rapid cooling of plant parts was observed to trigger APs and VPs in many species, and signal generation is believed to involve Ca channels. AP-induced pre-adaptation has already been successfully provided for some plants. Therefore, electrical signaling is likely to play a part in temperature sensing. Furthermore, wetting roots with water was also observed to trigger electrical signals and suggested to mediate physiological adaptation to water availability in soil. Moreover, light/dark switch-induced APs have been observed in many species, believed to be closely related to photosynthesis, but the exact generation mechanism has yet to be deciphered. Although there is study showing fast changes in root activity by shining light on shoots, whether electrical signals play an indispensable role in light/dark signaling is still unclear.^[2]

2.5 Defensive Response to Danger

Electrical signals are strongly related to the defense response of plants to noxious stimuli (burning, freezing, mashing, cutting, and pathogen invasion).^[2] Severe damage of a plant part can trigger systemic response in gene expression and resultant physiological modulation. Such response often involves growth arrest, photosynthesis drops, enhancement of respiration, induction of ethylene emission, jasmonic acid biosynthesis and reactive oxygen species generation, which are all associated with danger perception.^[2] It is easy to understand the indispensable role of electrical signaling in danger sensing, because this is the situation where rapid, systemic responses are mostly needed and critical for plant survival. It is often the case that VPs and APs are evoked simultaneously by a damaging stimulus, which makes the signal deciphering challenging. Moreover, electrical signals might only be part of the defense signaling pathway, where chemical signaling is likely to play a role as well.^[2]

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