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Ultraconformable, Self-Adhering Surface Electrodes for Measuring Electrical Signals in Plants

Fabian Meder, Sirgi Saar, Silvia Taccola, Carlo Filippeschi, Virgilio Mattoli,* and Barbara Mazzolai**

The electrical signals in plant's physiological processes are of great interest in biology, biohybrid robotics, and sensors for interfacing the living organisms with an electronic readout and control. This paper reports on the application of conformable, self-adhering surface electrodes for the measurement and bidirectional stimulation of electrical signals in plants. The inkjet-printed poly(3,4-ethylenedioxythiophene) polystyrene sulfonate based electrodes are <3 µm thick, light-weight, soft and flexible, and can be easily and non-invasively transferred onto plant's outer organs for surface potential recordings due to their realization on tattoo transfer paper. The devices prove to be extremely versatile for analyzing electrical signals in *Dionaea muscipula*, *Arabidopsis thaliana*, and *Codariocalyx motorius* and for stimulating mechanical responses in *D. muscipula*. A benefit over traditional electrodes is the van der Waals self-adherence of the thin electrodes, their intrinsic flexibility and adaptation also on small leaves while providing excellent readout. The same electrode allows long-term multicycle measurements over at least 10 days and, moreover, straightforward recordings on fast-moving organs such as snapping fly traps and endogenously oscillating leaflets. The results confirm that self-adhering soft organic electronics are particularly suitable for plant electrical signal analysis when easy-application, self-adaptation, and long-term performance are required in plant science, biohybrid robotics, and biohybrid sensors.

1. Introduction

Electronic devices which join conformably with the outer surface of living organisms are extremely interesting for recording the organisms' physiology, for example, by measuring its electrophysiological signals.[1,2] In the last years, a new class of flexible organic electronic devices with physical properties similar to those of human epidermis are being developed.[1–4] Such devices enable non-invasive coupling with the complex features of the skin surface serving for subsequent sensing tasks. Next to systems developed for humans and related diagnostic devices, methods to analyze the electrical signals generated by living plants attract increasing interest across fields from biology to engineering.[5–10] Plants respond to different stimuli such as to touch, light, wounding or other stressors like drying with electrical signals.[6] The comparison of the fast long-distance electrical communication in plants compared to the slower biochemical signaling is a great research topic in plant biology and agriculture.[6,11–16] The electrical signals in plants originate on cellular and ionic level from a different mechanism compared to those in human and animal nerve cells (the depolarization in animal nerve cells is driven by an increased transmembrane influx of sodium ions, plant electrical signals, that is, action potentials, involve the influx of calcium and/or the efflux of chloride ions).[17] Further understanding and correlation of plants electrical signals with their physiology is necessary as it could become a tool, for example, for better growth control and systems that can respond to plant needs with fertilizer or pesticide application and light/water administration.

In addition, a different field, that tries to make use of the intrinsic functions of plants such as sensing, communication,

Dr. F. Meder, Dr. S. Saar, Dr. S. Taccola, C. Filippeschi, Dr. V. Mattoli, Dr. B. Mazzolai
Istituto Italiano di Tecnologia
Center for Micro-BioRobotics
Viale Rinaldo Piaggio 34, Pontedera, Pisa 56025, Italy
E-mail: fabian.meder@iit.it; virgilio.mattoli@iit.it; barbara.mazzolai@iit.it

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Dr. S. Saar
Department of Botany
Institute of Ecology and Earth Sciences
University of Tartu
Lai 40, Tartu 51005, Estonia

Dr. S. Taccola
Future Manufacturing Processes Research Group
School of Mechanical Engineering
Faculty of Engineering
University of Leeds
Leeds LS2 9JT, UK

growth, mechanical structure from a technological perspective for applications in robotics, energy harvesting, biosensing, and architecture is the research on biohybrid devices and biohybrid robotics.[18–27] A starting point for interfacing plants with reliable electronic components that adapt to the plant could indeed lead to systems that use the stimulus-responsive electrical signals from a technological perspective.[9,28] Different thin-layer sensors and electronic systems based on materials like conductive polymers and graphene operating on the plant surface have been developed for measuring for example humidity or concentrations of certain biomolecules with great potential for agricultural monitoring.[29–33] Especially thin film electrodes based on graphene and carbon nanotubes,[34–36] silver inks[37] and silver nanowires,[38] conductive polymers,[29,39] and liquid metals[40] have shown advantages for being patternable into various shapes, bearing an excellent applicability on plant surfaces, having physiological sensing capability, and some are transparent or semitransparent. Yet, the development of techniques for recording intrinsic electrophysiological signals on plants and using them in biohybrid technologies remains limited to a few examples whereas the long-established research on the biological aspects on plant's electrical signals started back in the 19th century by observations of electrical signals in Venus flytraps (*Dionaea muscipula*) by Sanderson.[41] Techniques like ink-jet printing of conductive polymers is a straightforward, cost effective, and up-scalable strategy for electrode fabrication developed in the last years. Yet, the traditional devices and the "gold standard" for measuring electrical signals in plants are typically hard electrodes (e.g., Ag/AgCl electrodes) puncturing the tissue of a single cell or, in configuration for surface potential measurements, the electrodes are dipping in a high-ionic strength hydrogel electrolyte (e.g., 0.01 M KCl) applied on the plant surface.[13,42] The setups can be restricted to certain laboratory conditions (micromanipulators holding electrodes, etc.) and, in addition, electrode insertion and the diffusion of ions from the gel electrolyte into the plant tissue may influence the measurements by provoking electrical signals itself or locally poisoning the tissue.[43–46] Easy-to-apply, non-invasive soft electrodes operating on the surface of the plant could provide tailorable tools for measuring the plants "electrome"[47] and assist the research on their relation in plant physiology. In addition, plant-conformable electronic systems will enhance the research and applications of a new generation of plant-hybrid robotic systems that try to exploit the plant functions and their intrinsic electrical signals in hybrid systems to access plant features like sensing, adaptability, self-healing, and the inherent environmental friendliness.

Here, we describe easy-to-apply conformable, self-adhering electrodes for measuring and stimulating electrical signals in living plants. The printed devices are based on conductive polymer poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) pads in combination with stretchable silver conductor paste (Ag ink) interconnections realized on common tattoo transfer paper.[48] Our integrated electrode assembly (electrode, transfer paper, interconnections to external connectors) is an improved system which allows reliable and long-term stable interconnections to external connectors which often remains as a challenge for these types of electrodes. The devices can be easily and non-invasively transferred to the plant without specialized equipment or vacuum infiltration procedures such as reported previously for realizing conductive polymer electrodes on plants. Yet, they enable recording of the electrical signals with the accuracy of traditionally used Ag/AgCl surface electrodes avoiding however complex setups and highly concentrated electrolyte bridges like KCl hydrogels. We prove that the devices operate long-term over a period of at least 10 days after application on different plant species, that is, *D. muscipula*, *A. thaliana*, and *C. motorius*. These species were selected either being well-known model species with known triggerable electrical signals (i.e., mechanically triggered signals in *D. muscipula*[49] and wounding-induced electrical responses in *A. thaliana*[13]). Moreover, the species have fast moving organs such as the mechanically snapping trap of *D. muscipula*[49–52] and the endogenously oscillating leaves of *C. motorius*[53] (both motions occur in relation to electrical signals). Due to their light-weight, thin-film structure, and adaptability, the electrodes perform extremely well during challenging measurements on fast moving leaves. Moreover, we show that the same electrodes can also be used to electrically trigger a mechanical response such as the snapping of the Venus flytrap as also shown by Volkov et al. using Ag/AgCl electrodes.[54] Hence, the conformable electrodes provide a multifunctional tool for recording and bidirectionally stimulating plant physiology as a soft-organic-electronic alternative for traditional electrodes with potential cross-disciplinary relevance from plant science to biohybrid electronic and robotic systems due to their straight-forward applicability, adaptability, accuracy, and long-term performance.

2. Results and Discussion

2.1. Structure and Leaf-Transfer of the Conformable, Self-Adhering Electrodes

Figure 1a shows the structure of the conformable, self-adhering surface electrodes for registering the electrical signals. A circular PEDOT:PSS electrode (5 mm diameter, thickness <500 nm)[56] was ink-jet printed on a tattoo transfer paper (<2 µm thick) similar to our previous human-electrode interfaces.[48,55,56] Electrical connection was made by screen-printing a soft Ag-ink track on the tattoo paper substrate (containing the PEDOT:PSS electrode) and using a flexible polyimide–copper printed circuit board (PCB) strip as an intermediate interconnection layer between the PEDOT:PSS thin film electrode and the external cable for data acquisition (Figure 1b). A drop of Ag ink was placed between the track on the tattoo transfer paper and the one on the PCB providing the linkage. The glue sheet of the temporary tattoo paper kit was first laser cut, and then placed on top of the device, leaving the PEDOT:PSS electrodes uncovered for initial temporary fixation on the plant surface supporting the transfer of the device onto the leaf as well as for providing an electrical isolation of the silver-ink connector from the plant surface. Transferring the PEDOT:PSS layer onto the leaf is done by dissolving the sacrificial layer between the tattoo paper onto which the PEDOT:PSS layer is printed and the thicker paper substrate which is removed after transfer. Eventually, the PEDOT:PSS electrode self-adheres permanently onto the plant surface by physical forces, that is, van der Waals adhesion. Video S1, Supporting Information, shows the extremely facile procedure of applying the conformable electrodes onto a leaf. The electrodes can be fabricated in different dimensions

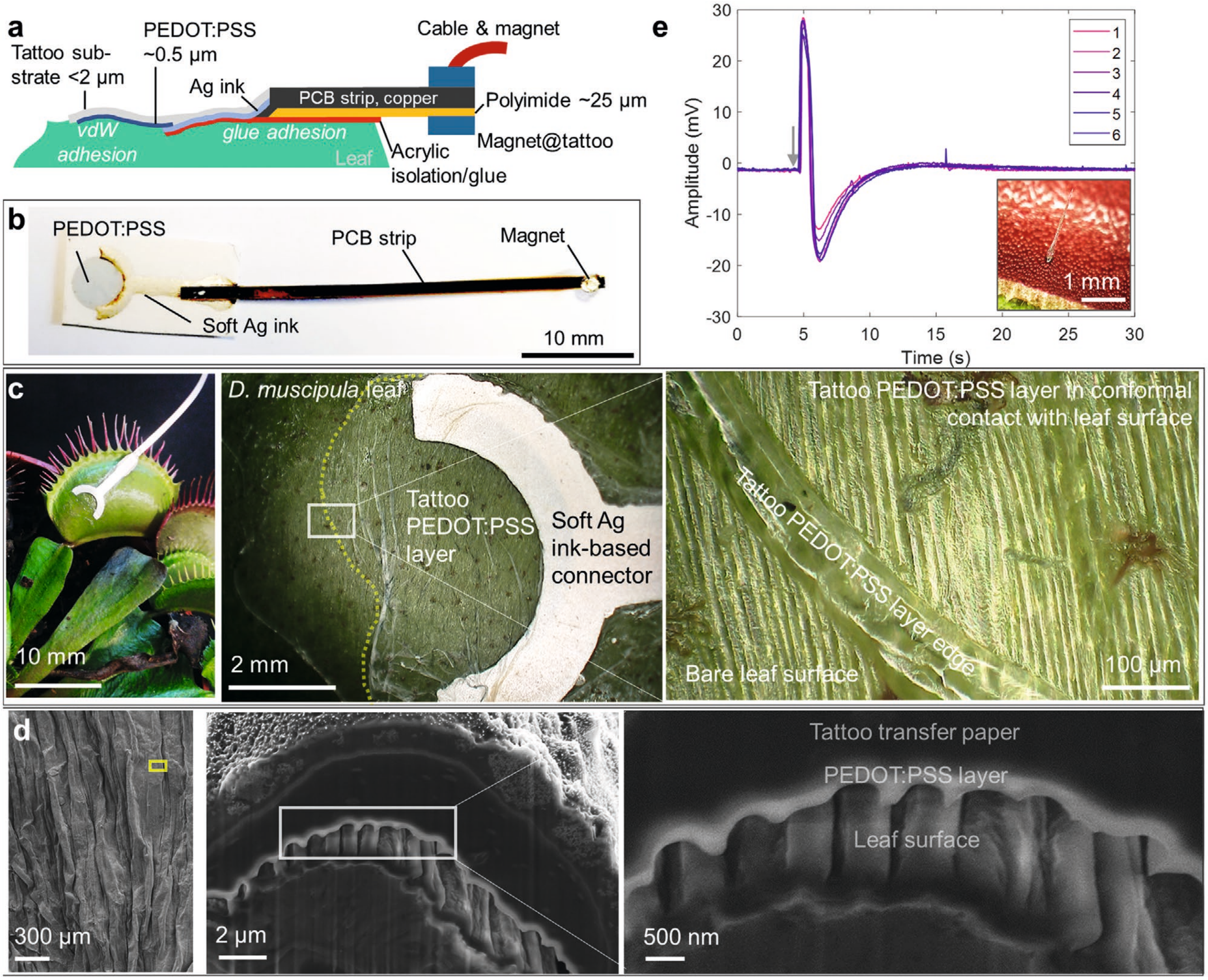

Figure 1. Overview of the assembly and structure of the conformal, self-adhering electrodes. a) Illustration of the assembly of the conformal ink-jet printed PEDOT:PSS layer on a tattoo paper support, the soft Ag-ink-based connectors, and the magnetic cable connectors for measuring electrical signals of plants. b) Image of the electrode in a typical configuration used in this study. c) Left: Photograph of the tattoo electrode on *D. muscipula leaf* (Video S1, Supporting Information, shows the straightforward assembly procedure) and low (central image) and high (right image) magnification digital microscopy images of the conformable electrode layer adapting and adhering to the leaf surface. The dotted yellow line in the central image indicates the edge of the PEDOT:PSS layer. d) Left: SEM image of *D. muscipula* leaf surface covered with the electrode. The yellow square indicates an area where an ion beam cross-section was cut. Center and right: Overview and zoom-in image, respectively of the ion-beam cross section visualizing the different layers and the adaptation of the electrode to sub-microscale leaf features. e) Repeated measurement of action potentials of the same *D. muscipula* leaf recorded with the conformal electrodes after six individual stimulations of a trigger hair-like trichome as shown in the image inset. The grey arrow in the graph indicates the timepoint of the mechanical stimulation.

and Figure 1b shows the typical shape and size used in this study. Figure 1c depicts the conformable, self-adhering electrodes on a *D. muscipula* leaf. The digital microscopy image shows a magnified view of the electrode on the leaf and the zoom-in, high-resolution image reveals that the conductive polymeric thin film is capable to adapt to the leaf structure and conformably wraps also around uneven structures like the ripples on the outer *D. muscipula* leaf surface. Moreover, the microstructure of the electrode-leaf assembly and the excellent self-adaptation to sub-microscale leaf features are given in the scanning electron microscopy (SEM) images after ion beam cross-sectioning the electrode coated leaf (Figure 1d) indicating a thickness of the PEDOT:PSS layer of about 300–400 nm.

2.2. In-Depth Evaluation of Electrical Signal Acquisition in *D. muscipula*

Venus flytraps (*D. muscipula*) were chosen as a model organism to evaluate the performance of the conformable electrodes as the species is producing well-defined action potentials upon mechanical trigger of the hair-like trichomes in the inside of the trap (inset in Figure 1e).[49,51] Figure 1d shows recordings of six action potentials using the electrode on a *D. muscipula* leaf. As signal acquisition hardware, a commercial open-source device further described in Figure S1, Supporting Information, was used. The mechanically triggered signals in *D. muscipula* were used to generate multiple action potentials to characterize the electrodes in detail. Typically, a series of six action potentials with reproducible amplitude was recorded within about 12–15 min (Figure 1d). Between the different stimuli, a 2-min interval of no stimulus was maintained. Note that when two mechanical stimuli occur together (within ≈30 sec), this triggers the closure of the trap which hinders further stimulation due to restricted access to the trigger hairs (the trap reopens usually only within 12 h to several days[51]). However, when the time interval between two stimuli is sufficiently large (>30 sec), only one action potential is produced without closure of the trap.[51,57] In this manner, it was possible to preserve the leaves in an opened configuration while enabling multiple measurements with the same leaf and electrode. The signals show a reproducible shape in magnitude and time domain and only slight variations occur even if the plant-produced action potentials may naturally not always produce exactly the same amplitude. To further evaluate the performance of the conformable electrodes, we compared them with signals recorded by traditional Ag/AgCl surface electrodes prepared as described in Ref. [13] and installed at the same leaf. The Ag/AgCl electrode is brought in contact with the leaf surface using a standard 10 mM KCl gel electrolyte and both electrodes were applied at the same leaf (see inset in **Figure 2**a) to avoid variances occurring from signals generated in different leaves. The leaf was stimulated at intervals of 3 min and measurements were performed alternatingly with both electrodes (first conformable electrode, then Ag/AgCl etc.). The performance of both electrode types is depicted in Figure 2a and was found to be very similar resulting in almost the same amplitudes and time domains. As a control, the leaf was touched at the outer surface where no trigger hairs exist and the signal was recorded. Expectedly, no action potentials were generated and no signals measured with either of the electrodes (Figure 2b). This confirms that conformable PEDOT:PSS electrodes are capable of measuring action potentials with similar accuracy to Ag/AgCl electrodes and no significant noise signals are recorded from, for example, touching the plant.

A clear advantage of the light-weight electrodes (total weight of 20 mg in the configuration as depicted in Figure 1b including magnet) adhering on the leaf arises from the fact that no additional glue, clamps, manipulators, and no high-concentration electrolyte are necessary to keep the electrode in the desired position for short and long-term measurements as further described below. This leads to the intrinsic capability of the flexible electrode to move with the leaf without losing connection to the data acquisition device which may occur with Ag/AgCl electrode dipping in the electrolyte (KCl) gel on the leaf causing signal interruption as depicted in Figure 2c and Video S2, Supporting Information, during fast motions such as the snapping of *D. muscipula*. Further mechanical characterization of the same electrode arrangement reported by us in a separate

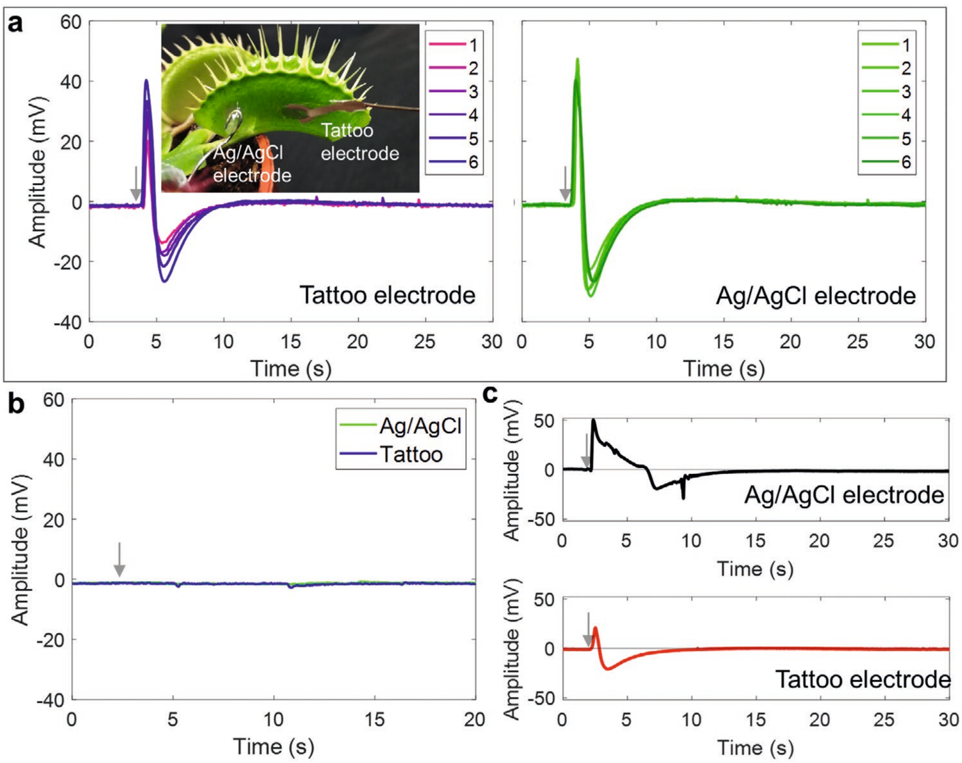

Figure 2. Conformable, self-adhering electrodes in comparison with Ag/AgCl electrodes. a) Surface recordings of action potentials of *D. muscipula* measured on the same leaf with the conformable tattoo electrodes (left graph) and Ag/AgCl surface electrodes (right graph) indicating almost identical behavior. To generate an action potential, the trigger hairs were mechanically stimulated. b) Control treatment was performed on the outer leaf surface (which does not have trigger hairs). No signals were recorded with either of the electrodes confirming that the mechanical stimulation does not create noise in the measurements. c) Measurement of the action potential of *D. muscipula* during closure of the fly trap (see Video S2, Supporting Information). The signal for the Ag/AgCl electrode interrupts as the fast-closing trap moves the electrode out of the gel electrolyte. In contrast, the conformable electrodes self-adhere to the leaf surface and move smoothly with it so that the action potential can be fully recorded. The grey arrows in the graphs indicate the timepoint of the mechanical stimulation.

study[48] proved high stability toward stretching (up to 10%) in over more than 100 000 cycles providing excellent mechanical properties for measurements on plants and plant motions.

2.3. Long-Term on-Plant Measurements

Figure 3a shows recordings of the initial electrode directly after application, 2 h after application and after wetting the PEDOT:PSS layer. The results show that action potentials of this leaf could be initially recorded but no signals could be measured 2 h later with the same electrode. However, simply placing a drop of deionized water on the PEDOT:PSS layer while still attached to the leaf, immediately restored its measurement capability. To analyze this behavior in detail, we performed the following analysis. For successful measurement, it is necessary that the impedance between electrode and plant tissue does not exceed certain thresholds. We thus analyzed the resistance R_x of the conformable electrode on the leaf as a function of time using a Voltage divider circuit (Figure 3a). This shows that R_x is stable (typically 100–2000 kΩ measured between electrode and soil reference electrode) for about 40 min, then a continuous increase in the resistance occurs until the electrode-plant tissue electrical connection disrupts leading to high resistances (>10–20 MΩ) as seen in Figure 3b, upper graph. However, slightly wetting the electrode with a 25 µl drop of deionized water applied on the top of the tattoo paper film completely restored the measurement capability of the electrode. To further elaborate what causes this effect, we recorded the resistance of the electrode upon wetting using different liquids, that is, ethanol and water (Figure 3b). In both cases, subsequent to application of the liquids, the resistance immediately decreases and after a given time interval that depends on the type of liquid used for wetting, the resistance increases. Then a "drying transition" stage begins with a liquid type-dependent duration indicating when the electrode would require rewetting for further measuring. The results show that it is a transient effect that is furthermore related to the type of the liquid. Samples treated with ethanol become significantly faster highly resistive, after ≈3 min, compared to ≈40 min when applying water. As the PEDOT:PSS electrode is also conductive when dry (see Figure S3, Supporting Informaion), the

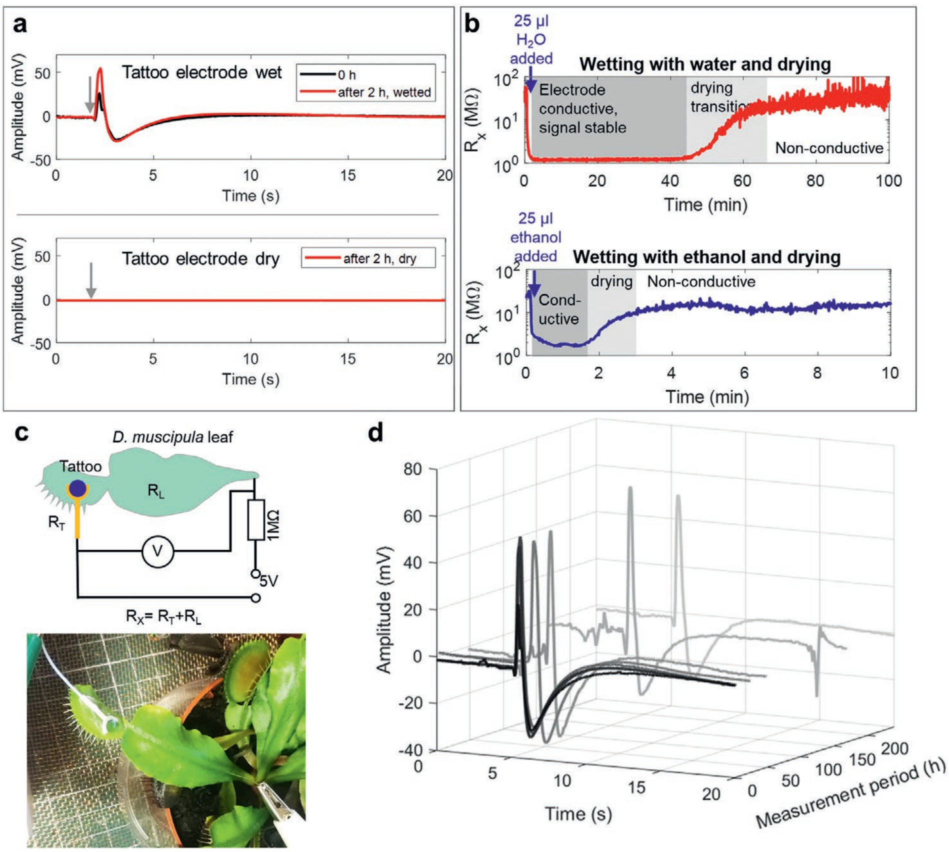

Figure 3. Multicycle and short- and long-term behavior of the conformable electrodes. a) Effect of electrode drying and rewetting on the recordings of action potentials in *D. muscipula*. The upper panel shows the electrical signals recorded with an electrode directly after transfer to the leaf and the same electrode after 2 h and wetting the PEDOT:PSS layer with 25 µl of deionized water. The lower panel shows that no signal could be measured 2 h after application due to drying of the electrode layer. b) Time-dependent resistance of the tattoo electrodes on the *D. muscipula* leaf measured using the c) voltage divider circuit. The upper panel in (b) shows the behavior when the leaf is wetted with deionized water and the lower panel when wetted with pure ethanol. d) Long-term measurements of the action potentials of *D. muscipula* recorded over a period of 10 days (time intervals = 0 h, 1 h, 2 h, 3 h, 24 h, 2 days, 7 days, and 10 days) confirming reliable signal acquisition during the entire period. The measurements were conducted with the same electrode applied on a single leaf and a droplet of deionized water was applied before each measurement.

fact that electrode wetting reestablishes reliable signal acquisition on leaves is suggested to be due to two effects. First, the outer leaf surface, the cuticle, is a dielectric polymeric phase separating the inner cellular tissue from the surface electrode and for establishing an electrical connection, coupling through openings on the leaf surface such as the substomatal cavities is required.[13] Here, wetting may help by further adapting the thin film to microscale leaf features. When the PEDOT:PSS film instead dries, small air-gaps between the electrode and the leaf may form reducing the effective electrode area that makes electrical contact with the inner tissue. Second, small amounts of ions in the plant tissue and from the electrode dissolving in the water could be sufficient to locally increase the conductivity without the need for an additional electrolyte. Consequently, no high-ionic strength electrolytes are required as for Ag/AgCl electrodes to establish an electrical connection between the inner leaf tissue and the electrode.[13] Alcohol treatments are also known to increase the conductivity of PEDOT:PSS,[58] however, the conductivity was higher when applying water in the special combination with the leaf. Ethanol evaporates significantly faster compared to water due to its higher vapor pressure which may cause the shorter duration of the conductive period. In addition, applying ethanol may stress the plant and lead to effects like closure of the stomata[59] which may reduce the electrical contact to the apoplast. Further analysis of the effect by impedance spectroscopy of the dry and moistened PEDOT:PSS electrodes on the leaf are given in Figure S3, Supporting Information, and comparison of the separate electrodes suggests that effective contact to the inner plant tissue plays a major role for increasing/decreasing the resistance. Drying out over time is a critical drawback for traditional electrodes requiring high ionic-strength hydrogel electrolytes as evaporation of the liquid phase allows limited applications time frames of typically less than 12 h next to potentially adverse effects of high salt stress.[10] However, such electrodes may not be easily restored by wetting with deionized water as in our case. Indeed, repeatable and accurate long-term data acquisition was possible with the same ultraconformable surface electrodes as follows.

Simply wetting the electrode film using deionized water (no addition of extra electrolyte required) before the measurement restores its signal acquisition capability and hence enables repeatable long-term measurements as shown in Figure 3d. The graph shows action potentials of *D. muscipula* recorded on the same leaflet at different intervals ($t = 0$ h, 1 h, 2 h, 3 h, 24 h, 2 days, 7 days, and 10 days). Within a period of 10 days, the conformable, self-adhering electrode remained attached on the same leaf and the acquisition of action potentials was possible after prior wetting of the electrode resulting in comparable amplitudes and signal time domains. Electrode delamination or other processes that could lead to the separation of the electrode from the leaf surface were not observed and the electrodes remain strongly adhered to the leaves.

As wetting the electrode improves the performance in particular during long-term measurements, effects from watering the plants and even spraying water on leaves and electrodes, are not expected to affect the measurement. Moreover, the electrodes are applied on the cuticle which is an additional barrier that naturally protects the inner plant tissue from possible effects of external materials and hence the electrodes on the cellular tissue. Indeed, we did not observe any obvious adverse effects on the plants while the electrodes have been applied.

2.4. Measurements on Further Species

We then tested the electrodes on two additional species. **Figure 4**a shows the conformal electrode applied on an *A. thaliana* leaf to record the well-known wounding-induced electrophysiological responses. The electrode can be tailored to the leaf size and it adapts to the leaf surface including protruding structures like the trichomes which do not hinder making sufficient electrical contact for measurements on the leaf surface. Wounding of the same leaf by cutting it with scissors (grey arrow indicates timepoint of the wounding event), resulted in the electrical signals depicted in Figure 4b proving the capability of registering wounding induced signals on this species.

We furthermore measured the electrical signals in *C. motorius* to confirm that the light-weight, conformable, self-adhering electrodes are well suited for recording signals in plants executing fast movements. The different leaves of this species, also known as the dancing plant, continuously move: The larger leaves perform nyctinastic leaf folding movements and fold and elevate upon day/night transition. However, each larger leaf has two fast-moving small lateral leaflets at its base that continuously perform endogenous oscillatory movements requiring only about ≈150 s for a full rotation. Video S3, Supporting Information, shows the leaf movements. Figure 4d shows the conformal electrode attached to the pulvini or "joints" of the larger leaves which enable the leaf motion. The plant was exposed to either light stimulus or the leaf was wounded by cutting. Both stimuli triggered electrical signals. The overall signal magnitude and time domains vary with the type of stimulus indicating that the manifestation of the electrical responses is a function of the stimulus. Figure 4d instead shows measurements with electrodes attached onto the small, oscillating leaves. After attaching the electrode, the motion of the leaf with the electrode is slightly impacted but not completely obstructed, whereas the second leaf of the pair can freely move (Video S4, Supporting Information). The electrical measurements show negative peaks in intervals of ≈60–140 s which correspond to the interval required for one full rotation of the small leaf (≈170s, see a time domain analysis of the mechanical motion in Figure S4, Supporting Information).[53,60] This suggests that the signals measured are connected to the oscillatory movements of *C. motorius* which can be easily recorded with the conformable electrodes. The experiments confirm the versatility for signal recording on various species without the need for additional gluing to fix the electrodes or electrolytes to establish sufficient connection.

2.5. Electrical Stimulation of Plant's Mechanical Responses by the Conformal Electrodes

Next to recording plant's electrophysiology, it is interesting to bidirectionally electrically stimulate the plant for studying and provoking responses of plants to the stimuli. This enables plant-biohybrid devices such as biohybrid sensors and plant-based

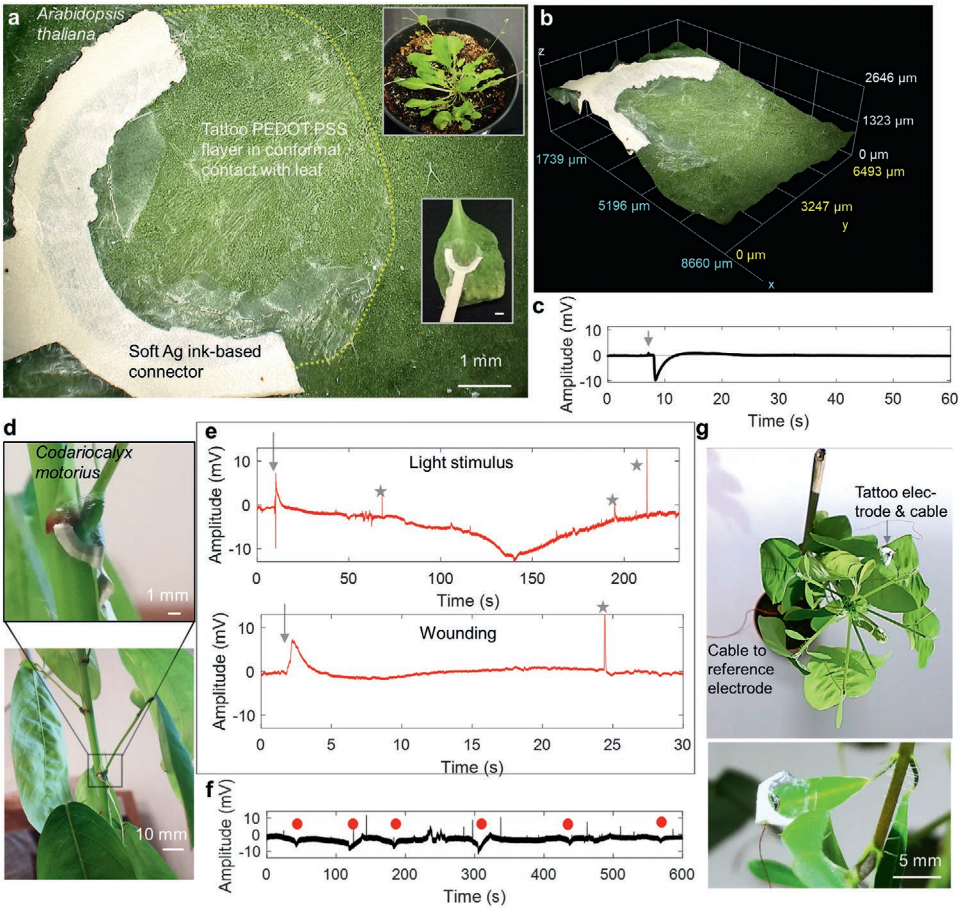

Figure 4. Application of the conformable electrodes for measuring light- and wounding-induced electrical signals. a) Digital microscopy image of conformable tattoo electrode on *A. thaliana*. The insets are photographs of the whole plant and a leaf with the tattoo electrode. The dotted line (yellow) indicated the edge of the PEDOT:PSS layer. b) High resolution 3D focus stacking digital microscopy image of the tattoo electrode on the *A. thaliana* leaf showing conformal adaptation of the PEDOT:PSS electrode layer to the leaf surface. c) Typical leaf wounding-induced electrical signals recorded with the tattoo electrode in *A. thaliana*. The grey arrow indicates the timing of the wounding event. d) Photographs of the conformal electrodes applied on the pulvinus of the large lateral leaves of *C. motorius*. e) Light-stimulus (upper panel) and wounding (lower panel) induced electrical signals recorded in *C. motorius*. The grey arrows indicate the timepoint of the stimulus. The grey stars highlight short duration noise signals that were caused by interference of an air conditioning system in the plant growth chamber which has not been removed from the data for showing the clear difference to the longer-duration plant-caused signal. f) Electrical signals recorded with the conformable electrode at a small oscillating leaflet of *C. motorius*. The negative peaks highlighted by the red dots occur in intervals of ≈60–140 s which correspond to the interval required for one full rotation of the small leaf (≈170s, see Figure S4, Supporting Information). Video S3, Supporting Information, shows the endogenous leaf oscillation. g) Photographs of the *C. motorius* with the tattoo electrode.

actuators that can be electrically controlled. Indeed, the same electrode assembly as used for measuring the mechanically-triggered signals in *D. muscipula* can also be used to trigger a mechanical response (trap closure) when a voltage is applied between the electrode on the leaf and the reference electrode (Video S5, Supporting Information).

3. Conclusion

Our research describes the application of conformable, self-adhering electrodes for recording and bidirectionally stimulating electrical signals in different plants triggered by stimuli such as touch, light, and wounding or by applying a potential to the electrode. The soft, flexible, self-adhering electrodes are designed to be easy-to-apply without the need for further gluing or additional electrolyte on the tissue and our in-depth analysis of plant electrical signal acquisition, impedance, and long-term behavior proved that they are accurate (similar to standard Ag/AgCl electrodes), enable surface potential measurements on leaves and stems of plants for at least 10 days while being excellently suited also for recordings on fast moving plant organs. The electrodes enable furthermore bidirectional operation in terms of measuring plant intrinsic signals and electrically stimulating trap snapping in *D. muscipula*. The platform hence may advance the linking of plant physiology and biohybrid technology (robotics, sensors, actuators etc.) that could make use of plant's intrinsic trigger-induced electrical signals in future biohybrid devices. Moreover, the tool may promote the overall understanding of plant electrical signals by opening up new measurement scenarios through high adaptability to different plant species and straightforward, reliable measurements. Further characteristics during application during human thorax motion and related details on design and fabrication are reported in a separate publication.[48]

4. Experimental Section/Methods

Materials: PEDOT:PSS aqueous dispersion (Clevios PJet 700) was purchased from Heraeus, Germany. Stretchable Ag conductor paste (CI-1036) was purchased from Engineered Materials Systems, USA. Temporary transfer tattoo paper kit (Silhouette Tattoo Paper) was purchased from Silhouette America, USA. Deionized water was obtained from a Milli-Q water purification system with a conductivity >18.2 MΩ cm^{-1} (Merck, Germany). Absolute ethanol, KCl, and HCl were obtained from Merck, Germany. Silver wire electrodes were obtained from Good Fellow Ldt., UK.

Plants: *Dionaea muscipula* was purchased at a local plant nursery. *Arabidopsis thaliana* Est-1 was grown in soil mixed from commercial potting soil and vermiculite using standard protocols. *Codariocalyx motorius* was grown from seeds (Asklepios Seeds, Germany) in pots (diameter 15 cm) using a 3:1 mixture of commercial potting soil and vermiculite with addition of 50 mL clay soil per pot. *D. muscipula* and *A. thaliana* were watered daily with deionized water. *C. motorius* was watered every second day and once in every 2 weeks fertilized with fertilizer for green plants (14-10-27, Mondo Verde S.r.l., Italy). All plants were kept in a phytochamber (Nuova Criotecnica Amcota S.N.C., Italy) with lighting in a 16 h day and 8 h night cycle. Temperature was set to 20 °C and air humidity at 80%. Mechanical stimuli were applied in *D. muscipula* by touching a single trigger hair with a plastic stick as shown in Video S2, Supporting Information. Wounding-stimuli were performed by cutting the leaf 1–2 mm with scissors. Light-stimulus was applied by keeping the plant in a dark chamber for at least 1 h and then switching on a 400 W halogen lamp.

Fabrication of the Ultraconformable, Self-Adhering Electrodes: The electrodes were prepared as described in ref. [48]. In short, the surface of the decal transfer paper of the tattoo paper kit was gently washed with deionized water and dried using a compressed-air gun. In order to avoid its wetting, the back of the paper was covered with an aluminum foil and the edges were sealed using an impermeable adhesive tape. Then the PEDOT:PSS electrodes were deposited by inkjet printing the PEDOT:PSS aqueous dispersion in a mixture with 10 vol% glycerol after being filtered through a membrane filter with 0.20 µm pore size (Minisart, Sartorius, Germany). Inkjet printing was carried out with a Dimatix DMP-2800 system (Fujifilm Corp., Japan) endowed with a 10 pL cartridge (DMC-11610). The interconnecting Ag tracks paper were deposited by screen printing of the stretchable Ag conductor paste using a homemade manual setup, composed of a mask (laser-cut stencil, laser cutter model VLS3.50, Universal Laser Systems, USA) and a blade for squeegeeing and uniformly distributing the paste. After the deposition, the substrates were baked at 120 °C for 10 min. A flexible polyimide-copper PCB strip (0.05 mm thick, 1 mm wide, RS Components, UK) was employed for the external electrical connection. During the assembly of the two parts, the contact pad was glued by a drop of the Ag paste between the tracks and the pads followed by baking at 120 °C for 15 min. The acrylic glue sheet of the temporary transfer tattoo paper kit was first laser cut, and then placed on top of the device for providing additional tattoo-substrate-adhesion leaving the PEDOT:PSS parts uncovered thus acting as protective insulating layer preventing direct contact of the Ag interconnection lines with the plant leaf. One neodymium magnet (0.5 mm thick, 2.5 mm diameter) was fixed with glue on the opposite side of the silver pad obtaining the final electrode assembly.

Characterization and Electrophysiological Measurements: Digital microscopy images were recorded using a KH-8700 digital microscope (Hirox, USA). Voltages in the voltage divider circuit were measured using the data acquisition hardware USB-6009 (National Instruments, USA). For recording electrical signals, a commercial open-source-based platform was used (Plant Spikerbox, Backyard Brains, USA.) driven with a DC power supply operated at 9 V and simply connecting the electrode to the input channel. As a reference electrode, an Ag/AgCl electrode dipping in a 10 mM KCl gel in a conical plastic tip (pipette tip) inserted in the soil was used. The acquisition device is further described in Figure S1, Supporting Information. Impedance spectroscopy was performed using an E4980A Precision LCR Meter (Keysight Technologies, USA). A typical measurement consisted of six individual stimulations of the electrical signals and comparing the amplitudes and time domains. SEM imaging and ion beam cross-sectioning were performed with a Helios NanoLab 600, (FEI, Italy) using an air-dried leaf-electrode specimen.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

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

Keywords

biohybrid robotics and sensors, electrophysiology, organic electronics, plant's electrical signals, soft electronics

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