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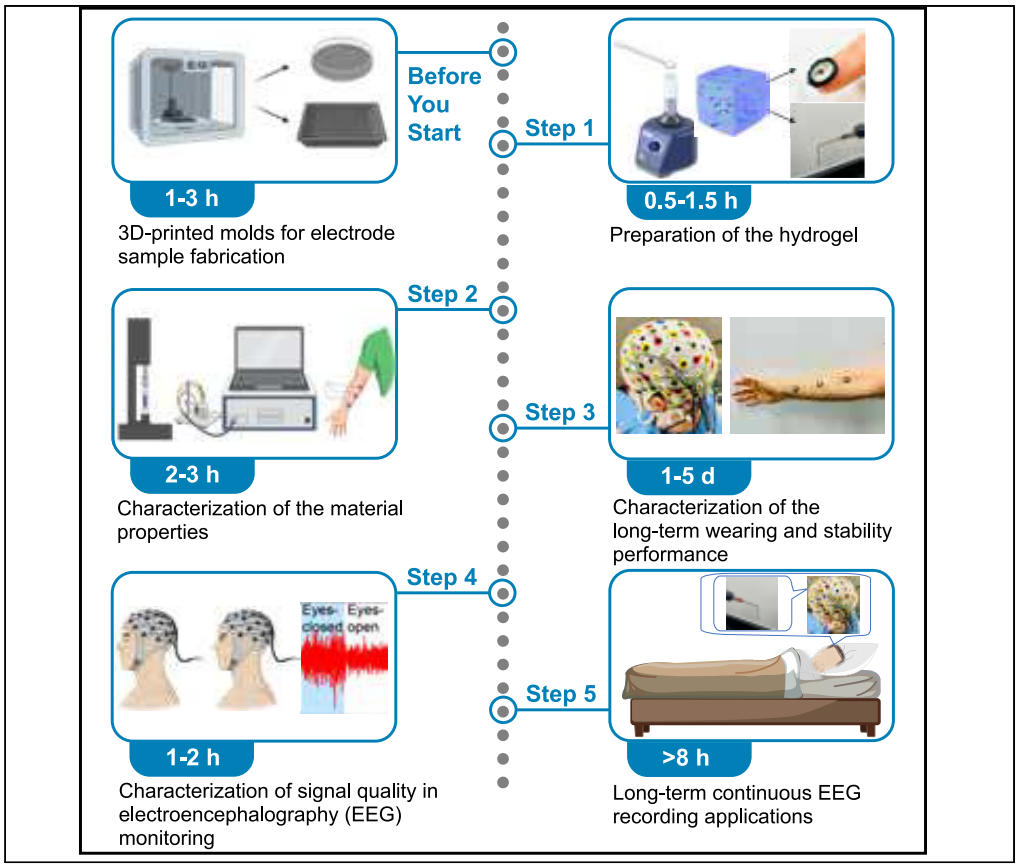
Protocol to fabricate a self-adhesive and long-term stable hydrogel for sleep EEG recording



Long-term continuous electroencephalogram (EEG) monitoring is crucial for neuroengineering but suffers from hardware limitations. Here, we present a protocol for EEG recording using a long-term stable and reagent-free-cross-linked hydrogel with configurable mechanical and adhesive properties. We describe steps for fabricating the hydrogel and performing material characterizations and stability tests. We detail procedures for setting up the EEG recording configuration and data analysis. This protocol can facilitate EEG recording experiments with the hydrogel, as well as other novel materials and devices.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Protocol to fabricate a self-adhesive and long-term stable hydrogel for sleep recording

Steps for characterizing hydrogel properties and performance

Instructions for setting sleep EEG recording experiments

Data analysis procedures for characterizing EEG signal quality and sleep EEG patterns

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Protocol to fabricate a self-adhesive and long-term stable hydrogel for sleep EEG recording

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SUMMARY

Long-term continuous electroencephalogram (EEG) monitoring is crucial for neuroengineering but suffers from hardware limitations. Here, we present a protocol for EEG recording using a long-term stable and reagent-free-cross-linked hydrogel with configurable mechanical and adhesive properties. We describe steps for fabricating the hydrogel and performing material characterizations and stability tests. We detail procedures for setting up the EEG recording configuration and data analysis. This protocol can facilitate EEG recording experiments with the hydrogel, as well as other novel materials and devices.

For complete details on the use and execution of this protocol, please refer to Hsieh et al.[1]

BEFORE YOU BEGIN

High-quality and continuous EEG monitoring is desirable for EEG-related research, including sleep monitoring and evaluating and treating sleep disorders.[2–4] Existing continuous EEG monitoring technologies suffer from fragile connections, long-term stability, and complex preparation for electrodes under real-life conditions. This protocol describes a technique to fabricate an adhesive, injectable, room-temperature (RT) spontaneous cross-linked, and conductive hydrogel (AIRTrode). AIRTrode features preparation-free, tunable mechanical and electrical properties and superior adhesiveness to achieve a variety of biopotential recording applications. The properties optimization strategy is described and discussed in this protocol. This protocol can also be applied to research on any other similar materials. Besides the hydrogel fabrication, we also describe the techniques to perform: 1. Material characterizations: a. Mechanical, b. electrical properties, and c. adhesion properties. 2. Long-term stability tests on a. Weight change under various environmental conditions, b. Skin-electrode interfacial impedance, c. Scalp-electrode impedance. 3. Overnight sleep EEG recording experiments, including a. Sleep EEG recording setup, b. EEG signal quality evaluation, and c. Sleep EEG analysis. Some techniques in the EEG recording setup section also apply to other experiments related to human EEG recording.

Institutional permissions

All human subject experiments are performed according to the protocol approved by the Institutional Review Board (IRB) at the University of Texas at Austin (STUDY00003980).

> *Note:* Acquiring permission from the relevant institutions is necessary to accomplish human subject experiments. Prepare all the required documents and protocols in advance, as the approval processes often take time.

Preparation before the experiment: 3D-printed molds for electrode sample fabrication

Timing: 1–3 h

1. Molds for 1) fabricating samples for electrical, mechanical, and adhesion properties characterization and 2) stability and on-skin impedance performance tests.
   a. Use the .stl files deposited at an open-source data repository, Zenodo (https://doi.org/10.5281/zenodo.10059079), for the molds used in our study,[1] or create your designs.
   b. If you use our files in the repository, download the .stl files and open them with a compatible 3D printing software (for example, PrusaSlicer) to your printer to generate G-Code files to print the mold.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS aqueous solution)	Heraeus	Clevios PH 1000
Glycerol (≥99%)	Fisher Scientific	CAS: 56-81-5
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	CAS: 67-68-5
2-acrylamido-2-methylpropane sulfonic acid (AMPS)	Sigma-Aldrich	CAS:15214-89-8
Other		
Force gauge	Torbal	FB20N
3D printer	Prusa Research	i3 MK3S+
Arduino UNO REV3 board	Arduino	UNO REV3
Potentiostat	BioLogic	SP-300
Analytical balance	Ohaus	PR 124
Corning LSE vortex mixer	Corning 6775	Catalog No. 10320807
Hydrogel mold	Lab-made	N/A
Headspace vials	Fisher Scientific	13-622-134
Glass plates	Unifrog	B0C1S3KJ85
Copper tape	3M	C614-CPR
Commercial EEG gel	Easycap GmbH	SuperVisc (1000 gr.)
Commercial EEG electrode	Cardinal Health	H124SG
Kapton film	McMaster-Carr	2271k41
Alligator clips	Romeda	B0995KJWR5
Waterproof medical covers	Houseables	B07VH2H151
10 mL Luer-lock syringe	BH Supplies	B07KW4KLG2
MATLAB	MathWorks	N/A
EEG amplifier	Brain Vision	actiCHamp Plus
EEG analysis algorithm	Hsieh et al.[1]	https://doi.org/10.5281/zenodo.10059079

MATERIALS AND EQUIPMENT

AIRTrode		
Reagent	Final concentration	Amount
AMPS	46.6 wt %	2 *g*
PEDOT:PSS	46.6 wt %	2 *g*
DMSO	2.1 wt %	0.09 *g*
Glycerol	4.7 wt %	0.2 *g*
Total	N/A	**4.29 *g***

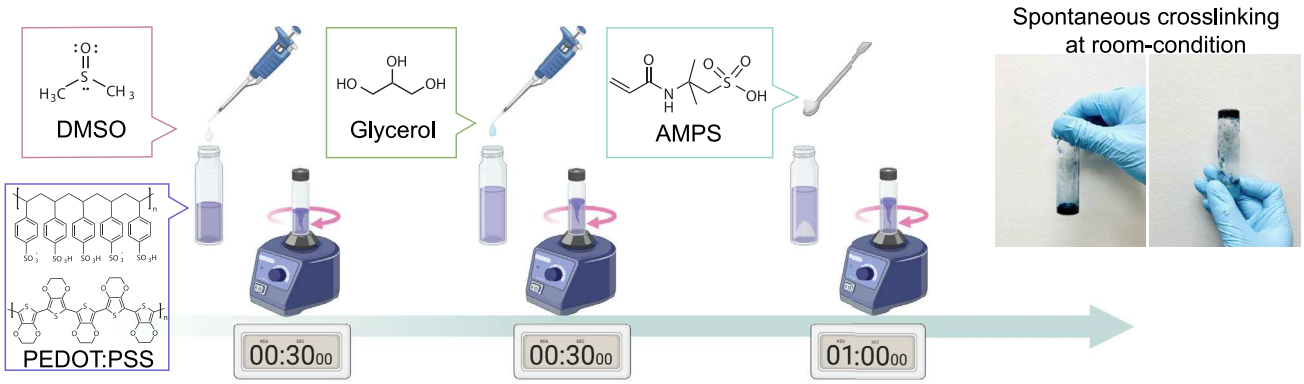

Figure 1. The fabrication process of the AIRTrode

The AIRTrode precursor is prepared through a sequential blending of pristine PEDOT:PSS with DMSO, glycerol, and AMPS. Subsequently, the mixture spontaneously undergoes cross-linking at ambient conditions without the need for external interventions or additives.[1]

Note: Despite the PEDOT:PSS can tolerate up to a week outside of the refrigerator, PEDOT:PSS is recommended to be stored at temperature of 4°C–8°C. AIRTrode can have a long shelf lifetime (> 1 month) if stored at a high relative humidity (60% RH) condition.

⚠ CRITICAL: AMPS is an acidic chemical with powder-state and is very lightweight. Irritating sensation is expected if inhaled.

STEP-BY-STEP METHOD DETAILS

Preparation of AIRTrode

Timing: 15–85 min

This section describes the fabrication method of the AIRTrode hydrogel electrode in detail.

Figure 1 shows the step-by-step scheme diagram of the method.

1. Prepare PEDOT:PSS/DMSO solution.
   a. Weigh 2 *g* of poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) into a 20 mL vial with a microbalance.
   b. Add 0.09 *g* DMSO with a 100 µL pipette into the PEDOT:PSS vial on a microbalance.
   c. Mix the two materials with a vortex mixer for 30 s.

   Note: The weight of DMSO depends on the weight of PEDOT:PSS. (4.5 wt % relative to PEDOT:PSS) Change the amount of DMSO according to the weight of PEDOT:PSS you add.

2. Prepare the PEDOT:PSS/DMSO/glycerol solution.
   a. Add 0.2 *g* glycerol with a 1 mL blunt syringe on a microbalance.

   Note: Depending on the desired electrode properties, the weight of glycerol is suggested to range from 10–30 wt % relative to the PEDOT:PSS you add to obtain a balanced final product.

   b. Mix the mixture with a vortex mixer for 30 s.

Note: Using a pipette to take glycerol can be challenging due to the viscosity of glycerol. To tackle this, we use a 1 mL syringe with a broader caliber opening compared to pipette tips.

3. Add AMPS to PEDOT:PSS/DMSO/glycerol solution to obtain AIRTrode precursor.
   a. Weigh 2 *g* AMPS with a weighing paper on a microbalance.

   Note: Depending on the desired electrode properties, the weight of AMPS can range from 73 - 100 wt % relative to the amount of PEDOT:PSS you add to obtain a balanced final product.

   b. Mix the mixture with a vortex mixer for 60 s.

   ⚠ CRITICAL: Do not add more than 2 *g* AMPS to a vial per time. Use a wide neck vial instead of a narrow one to prepare the AIRTrode precursor for a more straightforward AMPS powder-adding process. When adding AMPS powder to the vial, the process should be quick and steady to obtain a uniform precursor. Troubleshooting, problem 1.

4. Prepare the AIRTrode to desired shapes and formats with prepared molds or syringes.
   a. Pour the AIRTrode precursor into the mold and wait for 7–82 min to obtain the AIRTrode hydrogel, depending on the glycerol and AMPS loadings in the precursor.
   b. To use the AIRTrode hydrogel as an injectable hydrogel electrode, extract the precursor with an arbitrary volume luer lock syringe with a blunt tip needle. The hydrogel will be formed spontaneously and can be used after the gelation process.

   Note: If you want to shorten the curing time further, bubbling inert gas into the precursor would help. However, you do not need to if you are comfortable with the curing time frame. The gelation process will occur spontaneously without any treatments (external stimuli or cross-linking agents).

AIRTrode fabrication

Steps	Temperature	Time
Vortex PEDOT:PSS/DMSO solution	22°C (roughly)	30 s
Vortex PEDOT:PSS/DMSO/glycerol solution	22°C (roughly)	30 s
Vortex PEDOT:PSS/DMSO/AMPS solution	22°C (roughly)	60 s
Molding	22°C (roughly)	7–82 min

Material characterizations: Mechanical properties

Timing: 1 h

Note: All mechanical characterizations of the hydrogel were performed using a custom-designed motorized test stand comprised of a force gauge (FB20N, Torbal), an Arduino board (UNO REV3, Arduino)-controlled motor, and a 3D printed test platform with a PLA 3D printer (i3 MK3S+, Prusa Research). The force gauge had a maximum load capacity of 20 N, and the uniaxial strain was applied at a controlled ramp rate of 68 mm/min. The mechanical test can also be performed with other commercially available universal mechanical testing systems. (Figure 2).

5. Specimen preparation for strain-stress tests.
   a. Prepare all samples to have the exact dimensions to ensure consistency in specimen size for the experimental evaluation. For example, all samples have dimensions of 35 × 20 × 1.5 mm.
   b. Carefully remove any imperfections or irregularities that could affect the test results.
6. Mounting the specimen.

Figure 2. The image of customized motorized mechanical testing systems

The test stand consisted of a force gauge, an Arduino board-controlled motor, and a 3D-printed test platform. The force gauge had a maximum load capacity of 20 N, and the uniaxial strain was applied at a controlled ramp rate of 68 mm/min.

a. Use non-stretchable tape on the longitudinal ends of the samples to facilitate mounting onto the force gauge and the platform. The effective length of all samples is suggested to be maintained to facilitate the data processing and calculation of Young's modulus for the samples.
b. Place the specimen securely in the grips of the tensile testing system, ensuring that it is centered and aligned properly. The grips should hold the specimen firmly without causing any damage or slippage during the test.

7. Zero, calibrate, and record.
   a. Calibrate and zero the load cell to ensure accurate force measurements.
   b. Gradually and uniformly start applying a tensile force to the specimen using the testing machine.
   c. Record the specimen's applied force (load) and corresponding elongation (displacement).
   d. Stop the experiment after the sample reaches the failure point.

⚠ CRITICAL: To ensure the quality of the data, you need to avoid sudden shocks or fluctuations during the measurements.

Note: To calculate Young's modulus, see quantification and statistical analysis.

Material characterizations: Adhesion properties

Timing: 1 h

Note: In this work, we performed tensile adhesion and 90°-peeling tests to characterize the adhesion properties of the hydrogel. The experiments evaluate the hydrogel's bonding strength with the targeted substrates under different conditions and using scenarios.

8. For the 90°-peeling test.
   a. Prepare the sample with a backing layer.

Note: To ensure the force recorded is solely contributed by the adhesion strength of the material, the elastic materials need to sit on a non-stretchable substrate during the experiment. The non-stretchable substrate for the elastic material serves as a backing layer here.

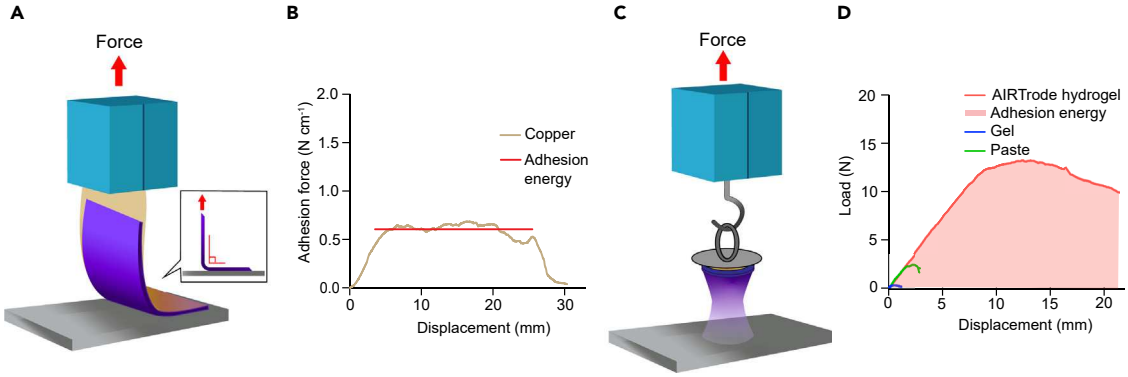

Figure 3. The characterization setup and data analysis of adhesion properties

(A) The setup of the 90°-peeling test.

(B) The adhesion energy can be calculated from the plateau value of the adhesion force-displacement plot.

(C) The setup of the tensile adhesion test.

(D) The adhesion energy can be calculated from the area under the curve (AUC) in the load-displacement plot.

i. Prepare all samples to have the exact dimensions to ensure consistency in specimen size for the experimental evaluation. For example, all samples have dimensions of 35 × 20 × 1.5 mm.

ii. The backing layer can be any flexible thin-film materials that can be applied to the sample. For example, polyethylene terephthalate (PET) sheet or polyimide (Kapton) tape.

⚠ CRITICAL: The backing layer must stay adhered to the sample the entire time during the experiment to ensure the accurate recording of the data.

b. Mount the sample to the testing system.

i. Securely mount the sample on the targeted substrate.

Note: For the cyclic 90-peeling adhesion test, prior to each test, the sample should be gently pressed onto different substrates to ensure the sample and the substrate are well-bonded.

ii. Securely mount one end of the sample with the backing layer to the grips of the testing system, ensuring that the bonded interface is aligned perpendicular to the direction of peeling. (Figure 3A).

c. Zero, calibrate, and record.

i. Calibrate and zero the load cell to ensure accurate force measurements.

ii. Gradually and uniformly start applying a tensile force to the specimen using the testing machine.

iii. Record the applied force (peeling strength) and the corresponding displacement of the sample.

iv. Stop the experiment after the sample reaches the failure point.

⚠ CRITICAL: To ensure the quality of the data, you need to avoid sudden shocks or fluctuations during the measurements.

d. Data Analysis:

i. Calculate the adhesion energy using the following equation[5] (Figure 3B):

Adhesive energy (J m^{-2}) = plateau value of load/width (N m^{-1}).

9. Tensile adhesion tests.

a. Specimen preparation.

Note: Suppose the hydrogel sample is fluidic and gel-like. In that case, the sample can be effectively confined within a 3D-printed ring with a diameter of 12 mm and a thickness of 2 mm in dimension during the application process onto the subject's skin. Upon application, the ring can be gently removed. Following application, a handle can be attached to the hydrogel to ensure that the tensile test system's grip securely holds the sample. (Figure 3C).

Note: If the hydrogel sample is in a stand-alone form, you can mold it into a diameter of 12 mm and a thickness of 2 mm before applying it to the subject's skin. Following application, a handle can be attached to the hydrogel to ensure that the tensile test system's grip securely holds the sample.

b. Zero, calibrate, and record.
   i. Calibrate and zero the load cell to ensure accurate force measurements.
   ii. Gradually and uniformly start applying a tensile force to the specimen using the testing machine.
   iii. Record the applied force (load) and the corresponding elongation (displacement) until the detachment occurs.
c. Data analysis:
   i. Tensile adhesion at the bonded interface can be calculated with the formula: Tensile adhesion = Force / Bonded area.
   ii. The adhesion energy was calculated based on the area under the load-displacement curve. (Figure 3D).

Material characterizations: Electrical impedance of the hydrogel

Timing: >12 h

To evaluate the electrical properties of the hydrogel, you can test the hydrogel with two different methods: One is to measure the impedance of the hydrogel, and the other is to measure the interfacial impedance.

10. Method 1: Impedance measurement of the hydrogel.
    a. Sample preparation:
       i. Fabricate cylindrical AIRTrodes with a diameter of 12 mm and a thickness of 2 mm.
       ii. Sandwich cylindrical AIRTrodes between two glass plates with copper tape.
    b. Impedance measurement setup:
       i. Connect the extended part on the copper tapes of the sample to the potentiostat using alligator clips.
       ii. Turn on the potentiostat and open the corresponding software on the computer.
       iii. Select potentiostatic electrochemical impedance spectroscopy (PEIS) as your method.
       iv. Set the sweeping range to 0.1–100 kHz.
       v. Start the PEIS sweep.
    c. Investigation of impedance change over time.
       i. Monitor weight loss by recording the sample's weight on a microbalance to quantify changes in weight over time.
       ii. Measure impedance at specific time points during stability tests to evaluate the on-skin impedance changes, such as 0 h, 2 h, 4 h, 8 h, 12 h, and so forth.
11. Method 2: Long-term skin-electrode impedance evaluation.
    a. Setup:
       i. Use the standard three-electrode method.[6] (Figure 4).

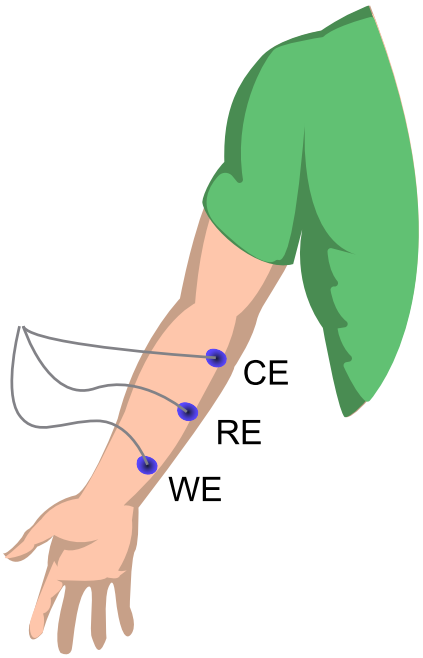

Figure 4. The standard three-electrode placement for on-skin interfacial impedance measurements

This method involves the utilization of three electrodes, namely the working electrode (WE), reference electrode (RE), and counter electrode (CE).[1]

Note: The standard three-electrode method for testing electrical impedance is commonly used in electrochemical impedance spectroscopy (EIS). This method involves the use of three electrodes: a working electrode (WE), a reference electrode (RE), and a counter electrode (CE).

Note: WE is where the electrochemical reaction of interest occurs. It is often the electrode where the material under study is deposited or immobilized.

Note: RE has a stable and well-defined electrochemical potential. It is a reference point for measuring the working electrode's potential.

Note: CE completes the electrical circuit. It provides a pathway for current to flow between the working electrode and the reference electrode.

ii. Place three pre-formed AIRTrode electrodes on the subject's forearm with equal spacing between one and another.

Note: You can use Ag/AgCl electrodes for RE and CE.

b. Measurement frequency: Measure electrode-skin interfacial impedance values every morning and evening for five consecutive days.

Material stability under various environmental conditions

Timing: 6 h

In this section, you can explore the material stability of hydrogel under diverse environmental conditions by investigating the hydrogel's stability and performance across a spectrum of environmental humidity factors.

12. Electrodes preparation.

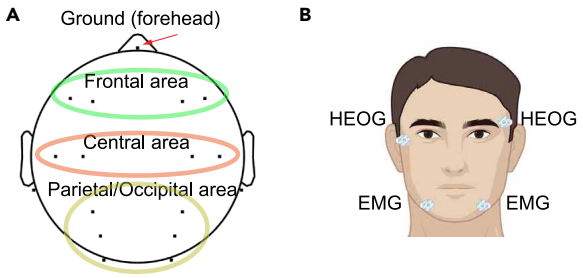

Figure 5. The electrode placement for continuous sleep EEG recordings
(A) An example EEG montage and (B) HEOG and EMG electrode placements.

a. The electrode preparation is the same as the cylindrical electrode in the last major step.

13. Setup the controlled environments:
    a. Room condition: Measure the relative humidity (RH) and temperature and place electrodes under ambient conditions. (~22°C, ~45% RH) Wait for 1 h before testing.
    b. Low-humidity conditions:
        i. To achieve a low-humidity environment, a customized system was built following the system reported by Chen et al.[7] The system allows gas to flow through a desiccation chamber containing sodium hydroxide before entering the chamber, creating an environment with ~10% RH. The samples were placed in this environment for the experiments.
        ii. Allow electrodes to sit for 2 h before testing.
    c. High-humidity conditions:
        i. Water trays or containers: Placing open containers or trays of water in the environment can increase humidity as the water evaporates into the air.
        ii. Transfer electrodes to high-humidity conditions (83.0% RH, 30°C).
        iii. Allow electrodes to sit for 2 h.
        iv. Measure the electrical impedance of the electrodes in high-humidity conditions.
14. Measure the electrical impedance values of the electrodes.
    a. The step is the same as the procedure of impedance measurement of the hydrogel used in the last major step.

Eight-hour "overnight" sleep EEG recording

Timing: >9 h

This protocol outlines procedures for conducting an 8 h ("overnight sleep") EEG study to: 1. Evaluate the performance of the newly developed EEG electrodes, 2. analyze brain activity during different sleep stages, and 3. assess the agreement between sleep stage hypnograms recorded by the electrodes with statistical methods.

15. Sleep EEG setup:
    a. EEG caps are configured according to Long-term scalp-electrode impedance evaluation.
    b. Two types of electrodes, AIRTrode and commercial EEG gel, are applied to proximity locations of each selected channel in the 10–20 EEG system.
    c. The montage can be designed according to the applications. For example, if the goal is to cover as much of the area of the brain as possible while keeping the number of channels low, you can select two to three channels from each lateral side of the frontal, central, and occipital areas. (Figure 5A).
    d. Monitoring procedures:

Note: Signals from both types of electrodes are recorded simultaneously for subsequent analysis.

i. Pre-formed AIRTrodes are placed on the facial area to record eye and chin muscle movements (horizontal electrooculography (HEOG) and electromyography (EMG)) to facilitate accurate sleep staging. (Figure 5B).

Note: Depending on the protocol, monitor the EEG signal and fix any disconnected electrodes to maintain data integrity.

ii. Check the impedance value for each electrode/ channel. Make sure all electrodes are showing low impedance (<30 kΩ) to ensure great contact quality.
iii. Monitoring the participant's sleep: Monitor the participant over the experiment period according to the study protocol.
iv. Post-night checks: Re-check impedance values for all electrodes. Ensure the EEG signal quality remains consistent throughout the night.
v. Additional data collection: Collect any additional data required for the study protocol, such as subjective sleep quality reports from the participant.

16. Eyes-open and -closed task EEG signal analysis: Time-frequency and PSD analyses are conducted in MATLAB using the Signal Processing Toolbox and EEGLAB. For the detailed analysis of the sleep EEG data, see quantification and statistical analysis.
17. Overnight Sleep EEG Analysis.

Note: Assess the similarity of measured EEG signals between the two types of electrodes. (The evaluated new electrodes (AIRTrode) and the gold-standard electrodes (Ag/AgCl commercial gel EEG electrode)). The similarity of measured EEG signals between two types of electrodes can be assessed with appropriate measures.

a. Preprocessing: preprocess both EEG signals with 2^{nd} order Butterworth filter in [0.1, 100] Hz and notch filter at 60 Hz to remove any artifacts noise that could affect the similarity assessment.

Note: Depending on the requirement of signal quality for the following analysis, additional baseline correction and artifact removal techniques can also be included.

b. Segmentation: Segment both EEG signals into smaller epochs of equal length, typically ranging from a few to tens of seconds.

Note: This allows for the comparison of corresponding segments between the two signals.

c. Evaluation: Apply the chosen similarity measure to the normalized feature vectors and calculate the similarity score between the two EEG signals.

Note: For example, Pearson correlation coefficient, which measures the linear correlation between two sets of variables. Higher similarity scores indicate greater similarity between the signals.

18. Evaluate the statistical agreement of sleep stage hypnograms between the two electrodes within the same subject.
    a. Sleep staging is performed according to American Academy of Sleep Medicine guidelines and manual scoring by human sleep experts. The agreement between hypnograms from the electrodes can then be assessed using Cohen's K value and Pearson's correlation coefficient.

Note: Cohen's K value is calculated using the formula:

$$\kappa = \frac{P_o - P_e}{1 - P_e}$$

Where P_o is the percentage of observed agreement, and P_e is the expected agreement due to chance.

19. Investigate the similarity of slow-wave spatial patterns during the slow-wave stage between the two types of electrodes.
    a. Preprocessing: Apply a Butterworth bandpass filter with a passband of [0.5, 4] Hz to isolate the slow-wave activity.
    b. Segmentation: Segment the filtered EEG signals into epochs corresponding to the slow-wave stage.

    Note: You can identify the slow-wave stage using established criteria, such as the presence of slow waves (0.5–4 Hz) during non-REM sleep.

    c. Similarity measure: Use an appropriate similarity measure to quantify the similarity of spatial patterns between the two types of electrodes.

    Note: One common measure is the correlation coefficient between the spatial patterns (images).

20. Visualization: Visualize the spatial patterns of slow-wave activity using a topographic plot (topoplot) for each electrode type.

    Note: You can use MATLAB's topoplot function for this purpose.

EXPECTED OUTCOMES

In this protocol, detailed instructions for the fabrication of AIRTrode hydrogel electrodes are provided. Adhering to the procedures described allows for precise control over their properties, such as adhesiveness, stretchability, and conductivity. Therefore, through this protocol, AIRTrode electrodes tailored to the desired specifications for specific applications are expected to be successfully prepared.

Through mechanical and adhesion tests described in the protocol, the material properties of the hydrogel electrodes can be characterized accurately. This includes determining parameters such as Young's modulus and bonding strength, which are crucial for assessing the material's performance and suitability for various applications. The protocol includes methods for evaluating the electrical impedance of the hydrogel electrodes over time and under different environmental conditions. By conducting these tests, insights into the conductivity and stability of the electrodes are accessible. Finally, following the protocol and the setup for overnight sleep EEG recording should enable accurate recording of the overnight long-term continuous EEG recordings. Overall, the experiment following this protocol is expected to yield valuable data and insights into the properties and performance of AIRTrode hydrogel electrodes, as well as brain activity during sleep. The same characterization methods can also be applied to other newly developed hydrogel electrodes for bioelectrophysiological signal recordings.

QUANTIFICATION AND STATISTICAL ANALYSIS

Young's modulus calculation

Young's modulus is a measure of the stiffness of a material. It is the ratio of stress (force per unit area) to strain (unit deformation) in the linear elastic region of the material's stress-strain curve. Mathematically, Young's modulus (E) can be calculated using the formula:

$$E = \frac{Stress}{Strain}$$

Where:

Stress (σ) is measured in units of force per unit area (such as Pascals or N/m^2). Strain (ε) is a dimensionless quantity representing the deformation ratio to the original dimension. Young's modulus can also be expressed in terms of force (F), original length (L), and change in length (ΔL) as:

$$E = \frac{F \cdot L}{A \cdot \Delta L}$$

Where:

A is the cross-sectional area of the material. To calculate Young's modulus, you need to measure the stress applied to the material and the resulting strain within the linear elastic region. Then, you can use either of the formulas above depending on the available data.

Overnight EEG data analysis

EEG signal quality analysis

For all EEG signal analysis mentioned in this section, you can perform using MATLAB with Signal Processing Toolbox and EEGLAB. Other general computing languages, including but not limited to Python and C++, are applicable as well.

For the eyes-open/closed paradigm, signal-to-noise ratios (SNRs) are computed for eyes-closed conditions for each of the electrodes. The frequency range of interest is defined as 8–12 Hz (i.e., the alpha band), while background EEG activities are defined as spectral responses of 5–30 Hz, with excluded frequencies of interest.

SNRs are calculated using the formula:

$$SNR = 10 \times log_{10}\left(\frac{mean\ PSD\ of\ SOI}{mean\ PSD\ of\ BG}\right)$$

Where:

PSD is power spectral density, SOI is signal of interest during eye-closed periods, and BG is background activities during eye-closed periods.

LIMITATIONS

In the protocol, the spontaneous crosslinking process replies by incorporating PEDOT:PSS in the AMPS polymerization system to increase the amount of reactive sulfonic acid groups to prompt the catalytic effect to polymerize AMPS to form the backbone of the hydrogel. Therefore, the fabrication process may not be applicable to other combinations of polymers.

TROUBLESHOOTING

Problem 1

The incomplete gelation process may occur due to the hydrogel precursor's failure to form a uniform dispersion. This usually happens when the AMPS powder is added to the PEDOT:PSS/DMSO/glycerol mixture at an inconsistent rate while the mixture is not being vortexed.

Potential solution

To ensure proper mixing, the AMPS powder should be added consistently and quickly while stirring or vortexing the mixture.

Problem 2

In adhesion force tests, the backing layer materials (e.g., non-stretchable tapes) can sometimes fall or be detached from the hydrogel during the experiments, resulting in the incompletion of the experiments or inaccurate readouts.

Potential solution

To ensure a strong connection between the hydrogel samples and the backing layer, you can use super glue. Alternatively, you can consider using a different material for the backing layer that is more suitable for your specific sample. It is important to ensure that the adhesion force between the backing layer and the hydrogel is always stronger than the adhesion force between the hydrogel and the targeted substrate.

Problem 3

In the EEG recording experiment, the HEOG and chin-EMG electrode placement can cause inaccurate sleep recordings if the electrodes are not placed at a suitable position.

Potential solution

After placing the electrodes, below are some ways to examine the electrodes' recording accuracy.

HEOG: Kindly ask subjects to look at both sides of their vision horizontally. If you can see the opposite waveform when the subject looks to the left and the right from the EOG signal readout, it means that the electrodes are ready.

EMG: To check if the electrodes are ready for EMG, kindly ask the subjects to clench their jaw or talk. If you can see the signal generated during these activities from the EMG signal readout, it means that the electrodes are ready.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Huiliang Wang (evanwang@utexas.edu).

Technical contact

Technical questions on executing this protocol should be directed to and will be answered by the technical contact, Ju-Chun Hsieh (jchsieh@utexas.edu).

Materials availability

This study did not generate any new unique reagents.

Data and code availability

Data and code used in this protocol are deposited at an open-source data repository, Zenodo (https://doi.org/10.5281/zenodo.10059079).

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AUTHOR CONTRIBUTIONS

Conceptualization, J.-C.H., H.W.; methodology, J.-C.H., B.B.; software, J.-C.H.; validation, J.-C.H., M.Y., B.B.; formal analysis, J.-C.H., B.B.; writing – original draft, J.-C.H., M.Y., H.W.; writing – review and editing, J.-C.H., M.Y., B.B., H.W.; visualization, J.-C.H., M.Y.; project administration, J.-C.H., H.W.; resources, B.B., H.W.; funding acquisition, J.-C.H., H.W.

DECLARATION OF INTERESTS

The authors declare that a patent application relating to this work has been filed.

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