Nondestructive and Real-Time Detection of Radiation Tissue Damage Using Surface Functionalization and Bioimpedance Spectroscopy

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1. Introduction

Radiation provides immense stress and incurable damage to exposed biological tissue by changing its biological and chemical properties [1]. Importantly, remote, real-time, sensitive monitoring of cellular stress in biological samples is crucial to detecting and preventing acute radiation damage [2]. However, most radiation-detecting methods are based on surface-restricted dosimeters [3], which do not reveal the internal condition of living materials, such as organs, cells, and organelles.

Here we demonstrate nondestructive and real-time detection of radiation damage in biological tissue using electronic impedance spectroscopy (EIS). Reactive vapor deposition (RVD) creates patterned conductive polymer coatings on the surfaces of plant leaves without destroying the tissue. The patterned polymer electrodes allow for tissue health analysis using EIS. UV irradiation bleaches plant leaves and this UV-induced damage can be clearly observed as a decrease in impedance and increase of phase of the specimen.

2. Methods and Results

2.1 Vapor-Deposited Conducting Polymer Electrodes for Impedance Spectroscopy

To monitor radiation damage of tissue using electronic impedance spectroscopy, we created patterned conducting polymer electrodes on plant leaves using reactive vapor deposition. The plant used in this study was *Patriot Hosta*, which is a shade-loving plant whose leaves are particularly susceptible to photolytic damage. As shown in **Fig. 1a**, picked and water-cleaned Hosta leaf was placed into the reaction chamber after masking. The leaf was masked with painters tape (used for its ease of removal) in a simple grid pattern, leading to electrodes of 50 mm width, 10 mm length and 3 mm separation.

For the conductive coating, a monomer, 3,4-ethylene dioxythiophene (EDOT), was polymerized to its polymer PEDOT by reactive vapor deposition in our custom-built quartz chamber as shown in **Fig. 1b** and our previous report [4]. The pressure of the chamber was maintained at 1 Torr during the whole deposition process. The chamber was heated with temperature-controlled fiberglass heating tape wrapped around the central quartz tube.

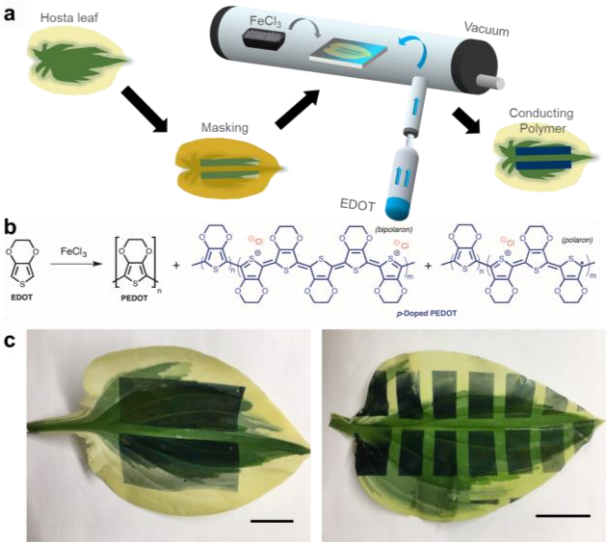

Fig. 1. (a) Scheme of the reactive vapor deposition on a Hosta leaf. (b) Polymerization reaction and structure of the conducting polymer. (c) Images of the polymer coated Hosta leaves. The size of scale bars is 10 mm.

The solid oxidant $FeCl_3$ was placed inside a ceramic boat and sublimed at 200-220 ^oC. For vapor coating of living and hydrated plant matter, only the part of the quartz tube containing the oxidant crucible was heated and the plant leaf was maintained at room temperature. The sublimed monomer vapor (heated to 80 ^oC) was introduced from a separate monomer tube into the evacuated chamber. The polymerization proceeded in the middle of the tube where monomer and oxidant met and could be recognized by the evolution of a dark blue color. The deposition was carried out for twenty minutes, on average. After the deposition, plant matter were dipped into a dilute acid solution (1 mM) and distilled water for 5 min each to remove residual monomer and oxidant. After removing the tape mask, conformally-coated polymer electrodes with crisp boundaries were obtained, as shown in **Fig. 1c**. The conductivity of films was as high as 1 S/cm.

2.2 UV irradiation on plant leaves

Among many radiation sources, UV is one of the most common and important sources to the living organism. In sunlight, a huge amount (~10 %) of UV is contained and especially, UVA having relatively longer

wavelength (320 nm to 400 nm) which can penetrate the skin and damage the inner tissue. To demonstrate this in the laboratory condition, UVA was irradiated using 365 nm UV source (OAI 500 W). As shown in **Fig. 2a** and **2b**, a water-filled Patri dish was placed between the UV lamp and sample to prevent thermal damage during the UV irradiation and filter UV of shorter wavelengths. The irradiation times were 2, 4, and 6 hours and the strongest peak intensities of UV light were measured by UV powermeter (OAI 306) and compared with sunlight in **Table 1**. The intensities of our experimental conditions were measured as 16.01 and 25.03 mW/cm^2, which are 5-6 times the peak sunlight intensities (measured on a sunny day at 1 PM in Massachusetts, United States, latitude 42.3°). Between those two wavelengths, 365 nm can be the main damage source due to its higher energy.

To compare the effect of UV irradiation on the tissue, one side of the leaf was masked with black insulation tape. The irradiated leaf is shown in **Fig. 2c**. After six hours of UV irradiation, the tissue of unmasked area was bleached to white/yellow color while the PEDOT electrodes remained unchanged. This color change is commonly shown on the Hosta exposed to the sun damage.

Table I: Comparison of UV intensity at different conditions

Intensity	365 nm (mW cm^{-2})	400 nm (mWcm^{-2})
UV	16.52	26.40
+ Water	16.01	25.03
Sunlight	2.50	5.32
Clouds	0.28	0.52

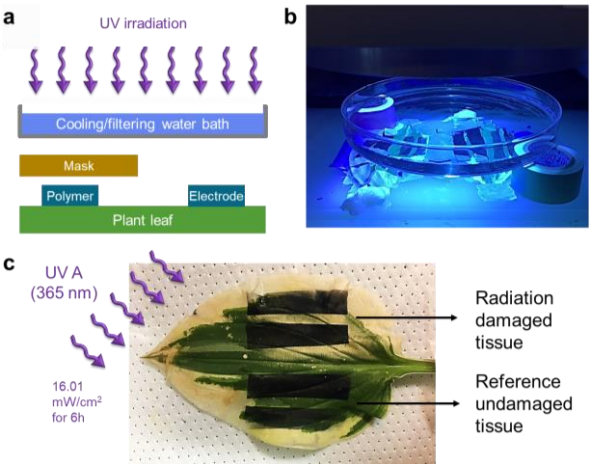

Fig. 2. (a) Scheme of the UV irradiation on the polymer-coated Hosta leaf. (b) Image of the UV irradiation. (c) Image of Hosta leaf after six hours of UV irradiation. The upper part of the leaf is much severely bleached.

2.3 Bioimpedance spectroscopy of UV irradiated tissue

Even though the UV damage of the plant leaf can be visually exhibited by the color change, it is inappropriate for the characterization of the inner tissue. Also, this method is inappropriate for the real-time monitoring of the sample. As one of the alternatives, EIS is used to characterize the health condition of the tissue. When the AC voltage is supplied between two electrodes, the charge passes through the tissue composed of capacitor-like membranes and resistor-like fluids (**Fig. 3a, b**) [5]. Because high-frequency signal passes through the membrane but low-frequency signal cannot pass through the membrane, we could know the health condition by the phase and impedance change as the frequency dependence.

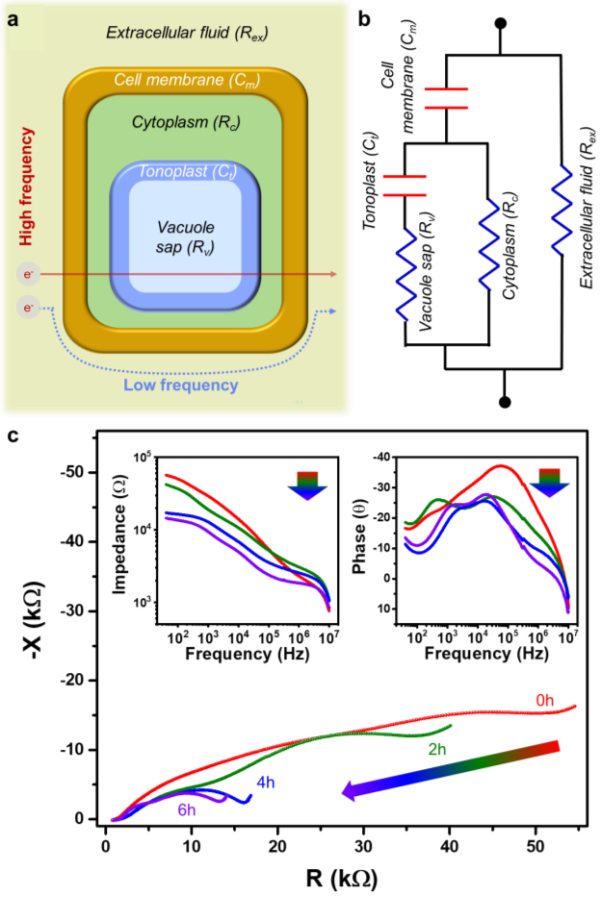

Fig. 3. (a) Scheme of the components of the plant cell. (b) The equivalent electronic circuit. (c) Measured impedance signal of Hosta leaf after 0 h, 2 h, 4 h and 6 h UV irradiation. Nyquist (real and imaginary parts of the impedance) and Bode (impedance and phase as a function of the frequency) plots are demonstrated in and as insets, respectively.

The nondestructive and real-time features of the impedance spectroscopy enabled it to be used in many biomedical fields including human health monitoring, plant stress test, and food science [6]. To apply this powerful technique on the tissue, appropriate

preparation of electrodes by the patterned deposition of conductive material is demanded. However, previous materials and deposition methods are not possible to make conformal contacting on rough and hydrophobic plant surfaces and human skin, and frequently these methods cause damage to both epidermal and inner tissue [7, 8]. Instead, we made conformally coated electrode with minimizing the damage on the plant tissue, by using vapor phase and relatively higher pressure (~1 Torr).

For the EIS measurement, the electrodes were connected to an impedance analyzer (4294A, Agilent) using a droplet of salt water solution (0.1 M NaCl) and steel needles. Relatively hydrophilic PEDOT restricted the solution on the electrode area and made the contact equivalent at each measurement. Through the contact, the electronic signal passes through the tissue of the Hosta leaf as shown in **Fig. 3a** and this can be simplified by the equivalent circuit of **Fig. 3b**. The impedance signal was measured for every two hours and demonstrate in **Fig. 3c** and its insets.

As UV exposure time increased, the impedance signal moved closer to zero and became more circular. This is shown by the decrease of impedance and increase of the phase on most ranges of the frequency. This change in impedance signal is especially evident after 2 to 4 hours of UV exposure. The loss of capacitive components in plant tissue can be explained by UV-induced damage: aqueous cell fluids generate reactive oxygen species (ROS) upon UV exposure and this degrades cell membranes [9]. The damaged cell membrane is not normally functioning as the separation wall of the inner and outer liquids, and shown as the decreased capacitor properties in the EIS [10].

The first two-hour irradiation showed a slightly different trend from other changes and this phenomenon can be attributed to the epidermal conditions of the Hosta leaf (moisture, wax). Relatively small change on four to six hours can be rationalized by the saturation of radiation damage on the tissue. These results demonstrate that bioimpedance spectroscopy can successfully and sensitively measure radiation damage in tissue, while maintaining the technical advantages of nondestructive, real-time, and in-depth characterization. Further, it is expected to investigate the more detailed change of each plant cell components by comparing the signal with the equivalent signal and get the electronic value of each cell component.

3. Conclusions

In this study, we nondestructively functionalized plant tissue using reactive vapor deposition, and applied it for real-time monitoring of UV damage in plants. Our unique coating method enabled the conformal and damage-free preparation of conductive electrodes on the plant surface. The effect of UV irradiation is demonstrated by the bleaching of the leaf and by the decrease of capacitor-properties in impedance spectroscopy.

Based on the generality and simplicity of our method, we expect to extend this technique to the short- and long-term monitoring of damage caused by other kinds of radiation sources. We anticipate that this unprecedented study can be broadly applied to monitor radiation damage in agriculture, environmental engineering, radiotherapy, medical imaging, and biotechnology.

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