Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Sensitivity enhancement of impedance-based cellular biosensor by nanopatterned PEDOT:Nafion interface

Jong Seob Choi[a†], Byunggik Kim[b†], Gwangjun Go [a] and Deok-Ho Kim[a, b*]

[a] Department of Biomedical Engineering, Johns Hopkins University,
3400 N Charles Street, Baltimore, MD, 21218, United States

[b] Department of Mechanical Engineering, Johns Hopkins University,
3400 N Charles Street, Baltimore, MD, 21218, United States

† These authors contributed equally to this work.

* Author for correspondence: Deok-Ho Kim (E-mail: dhkim@jhu.edu)

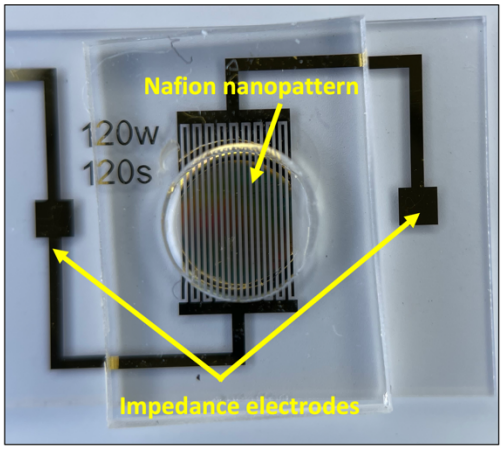

Fig. S1. A photograph of interdigitated electrode device with nanopatterned Nafion interface.

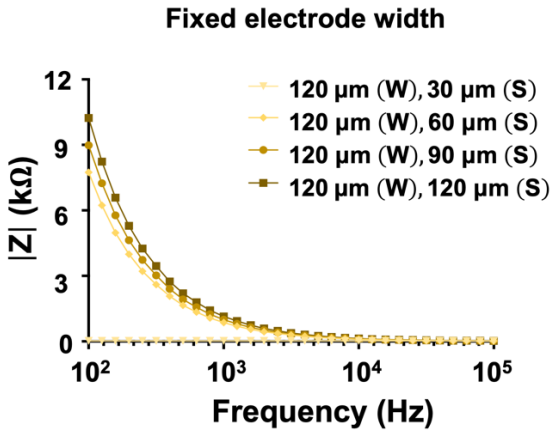

Fig. S2. The frequency-dependent impedance behavior of the interdigitated Au electrodes with varying space at fixed width.

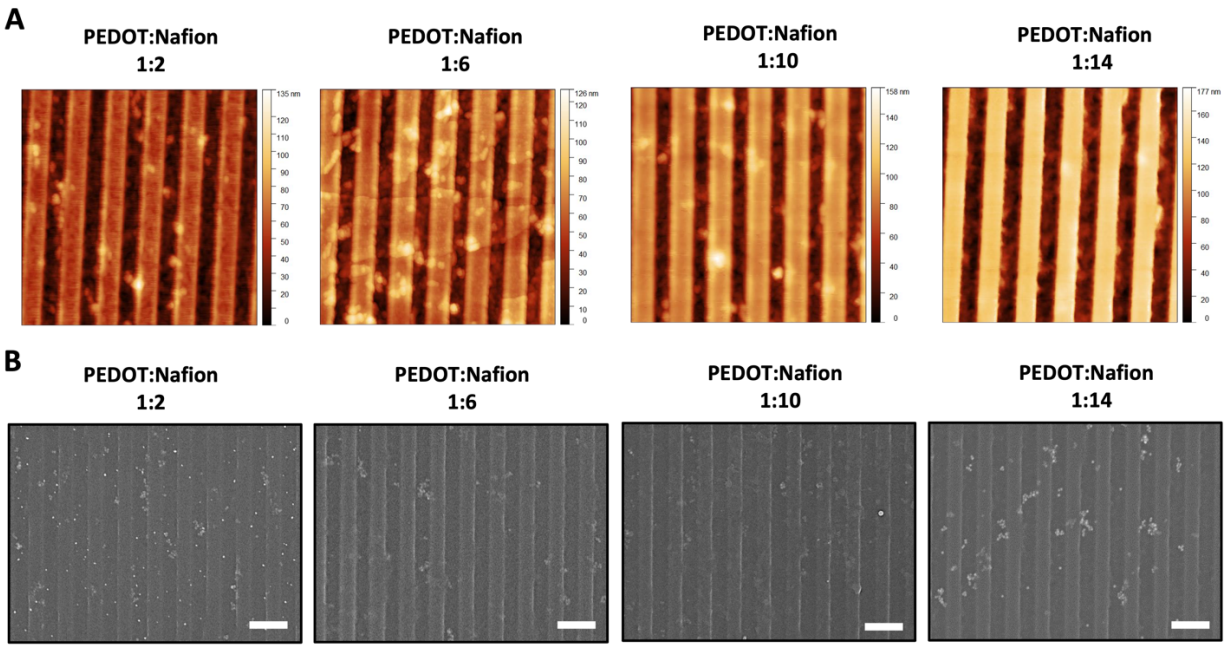

Fig. S3. Nanopattern fidelity of PEDOT:Nafion composite with varying mixing volumes. (A) 2D representation of the AFM topographic analysis, (B) representative SEM images for composite nanopatterns prepared at different volumetric mixing ratios (Scale bars: 2 µm)

Experimental Methods

Microfabrication of nanopatterned Nafion combined interdigitated electrodes:

The inter-digited electrode array was microfabricated via photolithography. A negative photoresist for lift-off was patterned on a glass substrate to define the electrode patterns. Then, 200 Å of chrome (Cr) and 2,000 Å of gold (Au) were deposited in sequence by a thermal evaporator. A final interdigitated Au electrode was obtained after 24 hours by lift-off of the photoresist with acetone. In order to confine the PEDOT:Nafion nanopattern and phosphate-buffered saline (PBS) solution on the active area, a Polydimethylsiloxane (PDMS) sheet with 5 mm in thickness was prepared by thoroughly mixing PDMS prepolymer and curing agent in a weight ratio of 10 to 1. The two reagents were then degassed to remove any bubbles, and finally placed in a convection oven for 24 hours to cure. The PDMS chamber was prepared by punching a 6 mm diameter hole in the PDMS sheet and attaching it to the electrode to define the working electrode area. Then, 3-5 µL of PEDOT:Nafion solution was placed inside the chamber and was pressed by a previously prepared nanopatterned PDMS stamp. Finally, the device was let dry. It solidified in 24 hours to form a PEDOT:Nafion composite at room temperature. Different mixing ratios of PEDOT solution to Nafion were tried; 1 to 14, 1 to 10, 1 to 6, and 1 to 2.

Cell culture and plating on nanopatterned interdigitated electrodes:

Human breast epithelial cell lines MCF10A and MCF7 were maintained in Dulbecco's Modified Eagle's Medium (DMEM)/F12 (1:1) and DMEM (Thermo Fisher Scientific, USA), respectively. The MCF10A medium were supplemented with 5% horse serum, 20 ng/mL EGF, 10 ug/mL insulin, 0.5 mg/mL hydrocortisone, and 100 ng/mL cholera toxin (all supplements were from Sigma-Aldrich) while the MCF7 medium was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were incubated in a 95% air, 5% CO_2 humidified atmosphere at 37 °C. After peeling off the PDMS stamp from nanopatterned interdigitated electrodes, the nanopatterned Nafion layer was sterilized in UV under a tissue-culture hood for 30 min prior to seeding. MCF10A or MCF7 cells were seeded at 10^5 cells per well. The cells developed a monolayer and covered the whole electrode area on Day+3 of seeding. For the human cerebral endothelial cell line (hCMEC/D3) culture, cell maintenance was referenced to the manufacturer's standard protocol. Brain endothelial cells were cultured and maintained in EndoGRO™-MV Complete Media Kit (Cat. No. SCME004) supplemented with 5 ng/mL FGF-2.

Electrochemical Impedance Spectroscopy (EIS) measurement:

Impedance characteristics of nanopatterned PEDOT:Nafion layers were measured on an Autolab PGSTAT302N potentiostat equipped with an EIS module. The frequency spectrum for the measurements ranged from 1 MHz to 100 Hz with an AC amplitude of 10 mV. Electrodes were prepared by conventional photolithography as described above. To pattern PEDOT:Nafion thin films onto the electrodes, the solution was dropped inside each chamber and pressed with a prepatterned PDMS mold. After curing at room temperature for 24 hours, the PDMS mold was carefully removed from the surface, leaving behind a nanopatterned PEDOT:Nafion thin film formed on the electrodes. The PDMS chamber was filled with 1 M PBS (Sigma-Aldrich) to submerge the effective electrode area. Then, a micro-tip probe was placed on the electrode and the open-circuit voltage was stabilized to verify the conformal contact between the probe and the electrode.

Numerical simulation study of electrical current flow pathway and density on the nanopatterned PEDOT:Nafion composite thin film on Au interdigitated electrodes:

A 2D numerical current flow simulation was conducted via COMSOL Multiphysics Modeling Software. To analyze the electric flow pathway, the nanopatterned electrode with Nafion layer interface was modeled to consist of a glass substrate, gold electrodes (thickness: 120 nm and space: 120 nm), nanopatterned film (thickness: 2μm), and PBS. The material properties of Au electrodes, the glass substrate, and PBS were determined from the material library of the COMSOL software. The electrical conductivity of nanopatterned films was set to 9 S/m (Nafion), 31 S/m (PEDOT:Nafion mixing ratio of 1:14), and 80 S/m (PEDOT:Nafion mixing ratio of 1:2), respectively[1]. To model all electrical interfaces at the intersection of the composite, Au electrode, PBS media, and an electrical double layer (thickness: 2 nm and electrical conductivity: $2.710*10^{-9}$ S/m^2) were applied as boundary condition as shown in **Fig. S3**. The size of the nanopatterns was determined to be 800 nm ridges, 800 nm grooves, and 800 nm depth. Alternating voltage with a peak-to-peak of 1-Volt in the frequency range 1 kHz to 100 kHz was applied to the electrode.

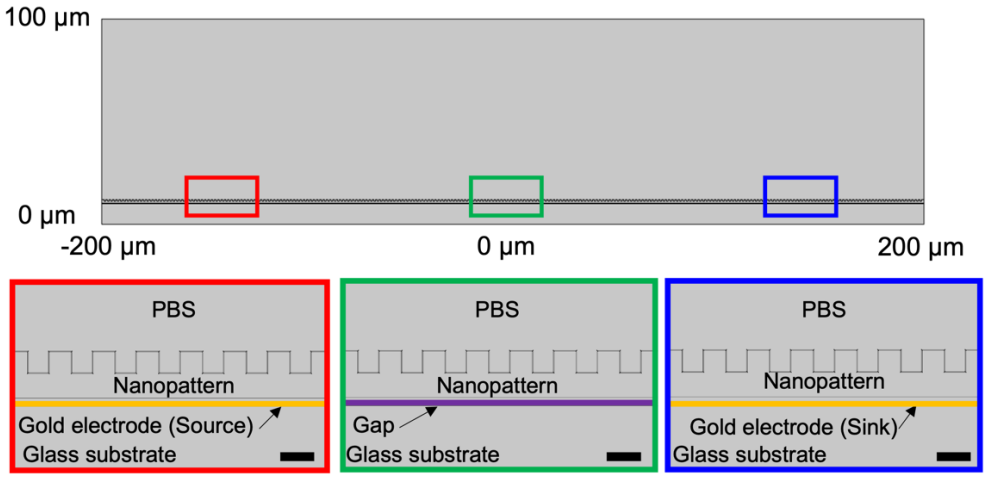

Fig. S4. Computational modeling of PEDOT:Nafion thin films on IEA. Multiple layers consist of a glass substrate, gold electrode pairs represented by source and sink for electric current, nanopatterned conductive thin film, electrical double layers, and PBS or cell culture media (Scale bars: 1 µm).

References

1 S. Carli, M. Di Lauro, M. Bianchi, M. Murgia, A. De Salvo, M. Prato, L. Fadiga and F. Biscarini, *ACS Applied Materials and Interfaces*, 2020, **12**, 29807-29817

2. A. Norlin, J. Pan and C. Leygraf, in *Biomolecular Engineering*, 2002, **19**, 67-71

Polarization resistance measurement by Electrochemical Impedance Spectroscopy (EIS) to study a potential mechanism of sensitivity enhancement using PEDOT:Nafion composites:

Polarization resistance is defined by the following equation:

$$\text{Polarization Resistance } (R_p) = \left(\frac{\Delta E}{\Delta i}\right)_{\Delta E \to 0}$$

Where, ΔE is a variation of the applied potential around the polarization potential and Δi is the resulting polarization current. Polarization resistance behaves like a resistor in a circuit and can be calculated by taking the inverse of the slope of the current potential in the linear region of a potentiodynamic scan as shown in the **Fig. 4A**.

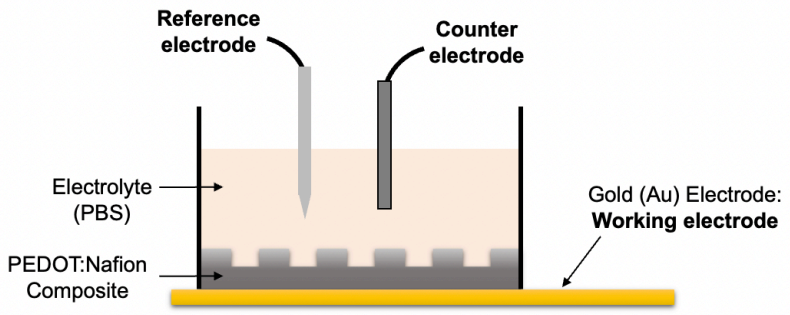

Fig. S5. Schematic illustration of the experimental setup to measure polarization resistance (R_p) of the PEDOT:Nafion composites on microelectrode arrays.

The application of the proposed assay as a quantitative method to monitor cell proliferation on PEDOT:Nafion composites:

A commercially available IEA system (Maestro Edge, Axion Biosystems, US) has been utilized to monitor the cellular proliferation of two cell lines; T47D (human breast cancer cell line) and human cerebral endothelial cells (hCMEC/D3). We coated PEDOT:Nafion on the effective electrode area (CytoView-Z 96, Axion Biosystems, US) using capillary force lithography to form a nano-topographic pattern, then cured the system for 24 hours at room temperature. Prior to seeding cells on the PEDOT:Nafion nanopatterned IEA platform, the plate was UV sterilized, and then the electrode baseline measurement was taken with culture media filled wells. 5×10^4 cells of each cell line were then seeded in the wells. Each experimental condition was duplicated (n=2). The impedance behavior of cellular proliferation was observed for 45 hours and microscopic images were taken at 24 hours and 48 hours after seeding.

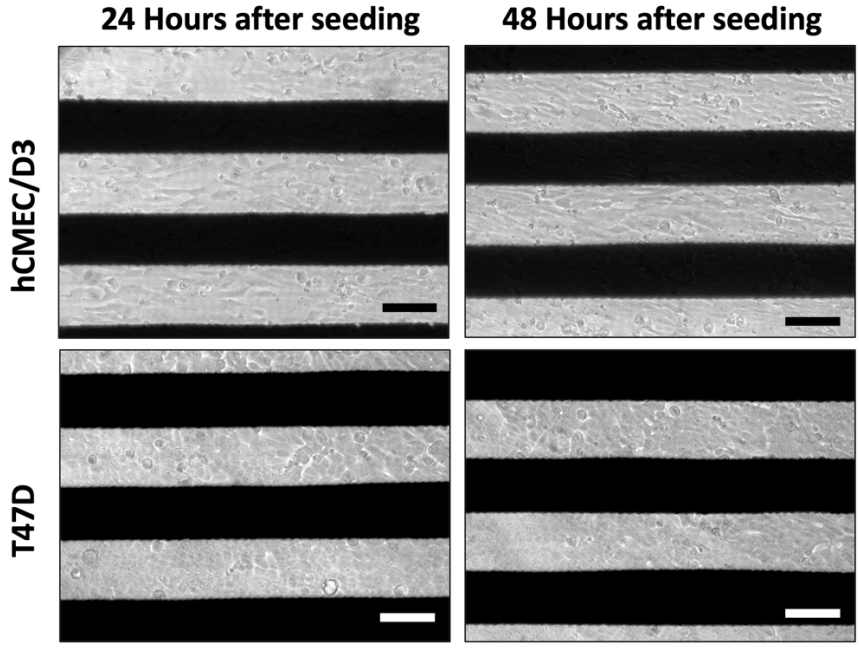

Fig. S6. The microscopic images of cells seeded on the PEDOT:Nafion IEA electrodes after 24 and 48 hours. Cells seeded (T47D; a human breast cancer cell line and hCMEC/D3; a human cerebral endothelial cell line) on PEDOT:Nafion composite, after proliferating for 48 hours, showed topographic alignment. (Scale bars: 100 μm)

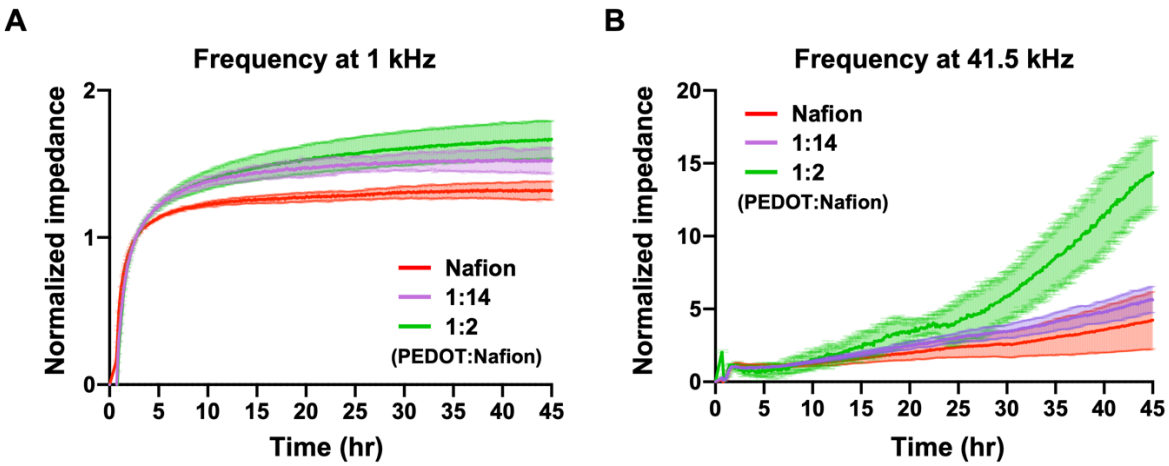

Fig. S7. Impedance behaviors of PEDOT:Nafion composites and its application to monitor cellular proliferation for 45 hours. Normalized impedance curves for cells (hCMEC/D3, a human cerebral endothelial cell line) seeded on PEDOT:Nafion composite as they proliferate for 45 hours, measured at frequencies of (A) 1 kHz and (B) 41.5 kHz.

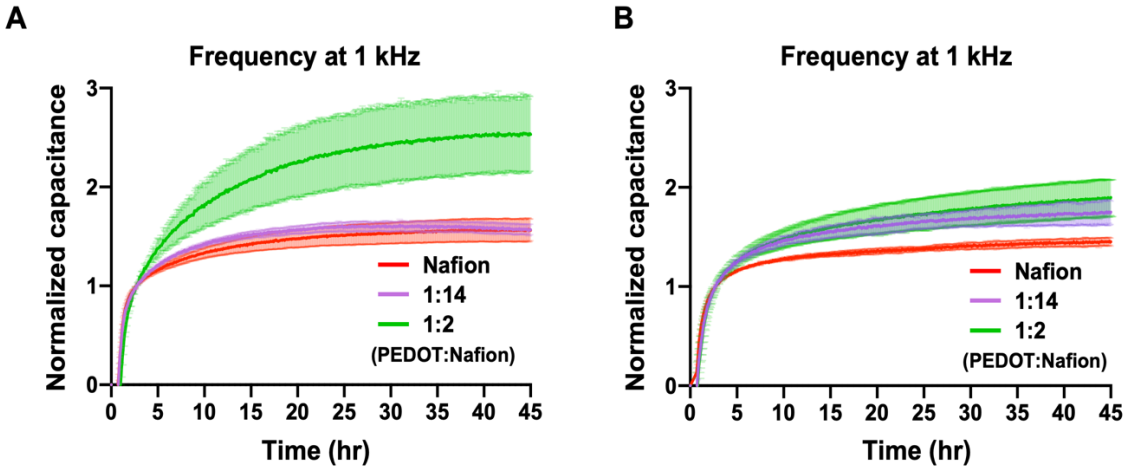

Fig. S8. Total capacitance behaviors, measured at the frequency of 1kHz, of PEDOT:Nafion composites to monitor cellular proliferation for 45 hours. (A) T47D-human breast cancer cell line, (B) hCMEC/D3-human cerebral endothelial cell line.