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Electronic Supplementary Information

Matrix Stiffness-Dependent Microglia Activation in Response to Inflammatory Cues: In Situ Investigated by Scanning Electrochemical Microscopy

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Table of contents

1. Experimental section

1.1 Chemicals and reagents

Hexaammineruthenium (III) chloride ($[Ru(NH_3)_6]Cl_3$), acrylamide (Acr), N, N methylene-bis-acrylamide (MBA), lipopolysaccharide (LPS), ammonium persulfate (APS), N,N,N,N'-tetramethylethylenediamine (TEMED), chloroplatinic acid, sodium sulphate and poly-L-lysine were purchased from Sigma-Aldrich (U.S.A.). Advanced Tyrode's solution was bought from Procell Co., Ltd (China). High-glucose Dulbecco's modified Eagle's medium (HG-DMEM) was obtained from Gibco Life Technologies Corp (U.S.A.). Penicillin/streptomycin, trypsin and Radio immunoprecipitation assay (RIPA) lysis buffer were obtained from Solarbio Science and Technology Co., Ltd (China). The aqueous solutions used in this work were all made from a Milli-Q water purification system (Millipore, resistivity >18.2 MΩ, U.S.A.). Bicinchoninic acid (BCA) protein assay kit, mouse TNF-α, IL-1β, IL-6 ELISA Kit and Fluo-4 AM were obtained from Beyotime Biotechnology Co., Ltd. (China). Polyvinylidene difluoride (PVDF) membranes were purchased from Millipore Corp (U.S.A.). Fetal bovine serum (FBS), phosphate buffered saline (PBS), and goat anti-Rabbit/ Mouse IgG HRP conjugated antibody were purchased from InCellGene LLC (U.S.A.). DAPI was purchased from Thermo Fisher Scientific Inc (U.S.A.). The ROS/superoxide detection assay kit, Anti-PIEZO1 antibody (ab259949), anti-NOX2/gp91phox antibody (ab129068), and goat anti-Rabbit IgG H&L (Alexa Fluor® 488) were obtained from Abcam PLC (U.K.). Anti-Rac1 antibody (66122-1-Ig) was from Proteintech (China). All the solutions used in cell experiments were filtered with 0.22-μm membranes (Millex-GP, Germany) before use.

1.2 Western blotting analysis

BV2 cells were firstly cultured on the PA gels at a density of 5×10^4 cells cm^{-2} and incubated in an incubator (5% CO_2, 37 °C) for 24 h, and then lysed by RIPA lysis buffer. The total protein concentrations of the BV2 cells were determined using a BCA protein assay kit. Then the protein samples of the two cells were subjected to 6%, 8%, and 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to the PVDF membranes. Thereafter, the PVDF membranes were blocked with 1.5% BSA for 2 h and incubated with anti-PIEZO1, anti-RAC1 and anti-NOX2 primary antibodies. After 12 h, the membranes were stained with HRP-conjugated secondary antibody for 2 h at room temperature. Finally, the membranes were visualized and

measured using a chemiluminescence imaging system (Clinx, 3300 mini, China).

1.3 Measurements of TNF-α, IL-1β, IL-6, NO and ROS levels of BV2 cells after LPS treatment

For measurements of the extracellular inflammatory factors, including TNF-α, IL-1β, IL-6, NO and intracellular ROS levels, the BV2 cells were inoculated on the PA gels with different stiffness with density of 5×10^4 cells/well. Then, the cells were treated with 1 μM LPS for 24 h. The contents of TNF-α, IL-1β, IL-6, NO and intracellular ROS levels, were measured with TNF-α, IL-1β, IL-6 and NO assay kits and ROS/superoxide detection assay kit, respectively, following the manufacturer's instructions. A multifunctional microplate reader (TECAN SPARK 10 M) was used to record the absorbance and fluorescence intensities of the cells. The intracellular ROS levels of microglia were characterized with a confocal microscope (Olympus FV3000).

1.4 Preparation of SECM probes

The fabrication processes of the 10-μm diameter Pt microelectrodes and the 1~2-μm diameter pyrolytic carbon electrodes followed the previous reports.[1] In brief, for the preparation of 10-μm diameter Pt microelectrodes, a borosilicate glass capillary (B100-58-10, Sutter Instruments) was pulled using a micropipette puller (PC-10, Narishige Instruments). A platinum wire (10-μm diameter, Goodfellow) was encapsulated in the pulled borosilicate glass capillary, which was then heated under vacuum to produce a tapered end and then polished to create a smooth and flat surface with a desired RG ratio of the electrode surface (Pt) to the glass sheath. For the preparation of the 1~2-μm diameter pyrolytic carbon electrodes, a quartz glass capillary (Q100-70-10, Sutter Instruments) was pulled using a laser puller (Model P-2000, Sutter Instruments) using the parameters (Heat: 565; Fil: 4; Vel: 60; Del: 145; Pull: 175). Then, the pyrolytic carbon was deposited into the inner of the pulled quartz pipette filled with methylbenzene through heating with a Bunsen burner for 5 s. Last, the pulled quartz pipette filled with pyrolytic carbon was milled for controlling the disk size and geometry to obtain the 1~2-μm diameter pyrolytic carbon electrodes. Then, the Pt-modified carbon microelectrodes were fabricated by electrochemical deposition on the exposed carbon disk surfaces through applying a potential range of 0.1 V - −0.6 V (vs. Ag/AgCl RE) in an aqueous solution containing 1 mM H_2PtCl_6 and 0.2 mmol L^{-1} sodium sulfate.[2]

1.5 Characterization of SECM probes

A SEM microscope (TM4000puls, HITACHI) was used to characterize the radius of carbon microelectrodes and Pt-modified carbon microelectrodes. All samples were coated by a sputtered gold layer to enhance the electrode tip's conductivity in SEM measurements. The electrochemical performances of the as-prepared carbon microelectrodes and Pt-modified carbon microelectrodes were tested by cyclic voltammetry (CV) in 0.1 M KCl solution containing 1 mM $[Ru(NH_3)_6]Cl_3$.

1.6 Cyclic voltammograms of dissolved oxygen in advanced Tyrode's solution

The reduction potential of dissolved oxygen was measured through cyclic voltammetry (CV) in advanced Tyrode's solution and advanced Tyrode's solution containing 1mM $[Ru(NH_3)_6]Cl_3$ using the carbon microelectrode as the working electrode.

2. Parameters of SECM theoretical model

Table S1. Main experimental parameters used in the SECM 2D simulation model

Parameter	Unit	Value
a	μm	0.5-1.25
RG	-	1.13-1.50
C_{R1}	mmol	1
C_{R2}	mmol	0.25
T	K	310.0
$D_{Ru(NH_3)_6Cl_3}$	$cm^2\ s^{-1}$	1.30×10^{-5}
D_{oxygen}	$cm^2\ s^{-1}$	2.30×10^{-5}
h_{cell}	μm	8.0-13.0
r_{cell}	μm	8.0-13.0

3. Supporting experimental data

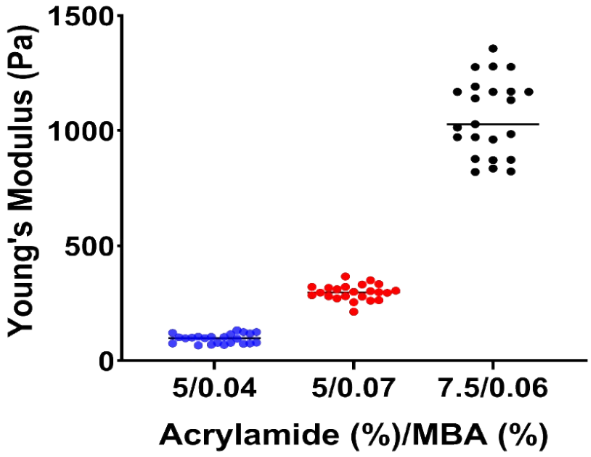

Fig. S1 The Young's modulus of the as-prepared PA gels characterized by a nanoindenter.

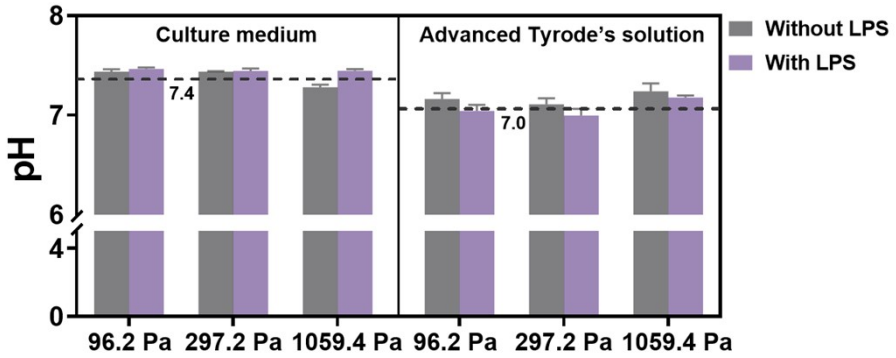

Fig. S2 pH values of the cell culture medium and the advanced Tyrode's solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ without and with LPS treatment.

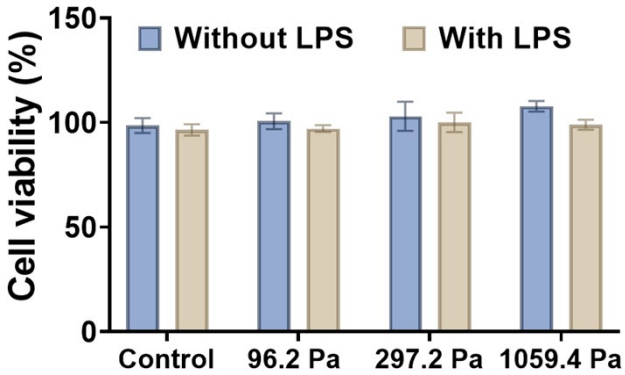

Fig. S3 Cell viability of BV2 cells on the PA gels with stiffness of 96.2, 297.2 and 1,059.4 Pa, respectively, and LPS treatment after culturing in advanced Tyrode's solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ for 6 h using CCK-8 assay kit (n = 3).

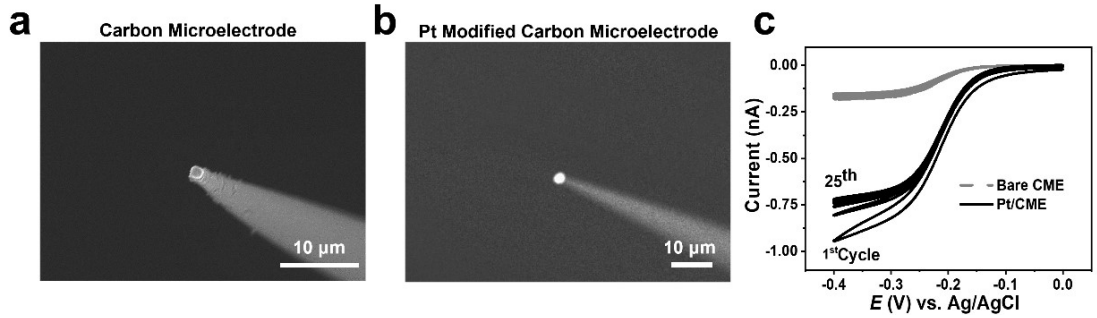

Fig. S4 Characterization results of the as-prepared carbon and Pt modified carbon microelectrodes. SEM images of (a) a carbon microelectrode and (b) a Pt-modified carbon microelectrode. (c) Cyclic voltammograms in 0.1 M KCl solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ with using the carbon microelectrode or Pt-modified carbon microelectrode as the working electrode, respectively (scan rates: 10 mV s^{-1}).

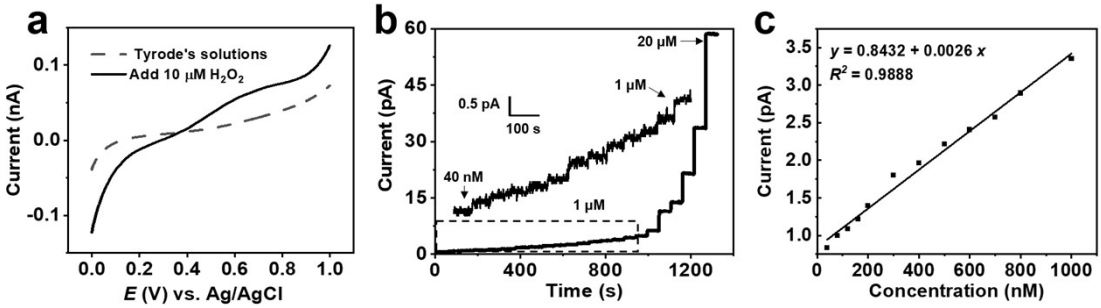

Fig. S5 Calibration plots of H_2O_2 oxidation currents versus H_2O_2 concentrations. (a) Linear sweep voltammograms of advanced Tyrode's solution without (dotted curve) and with adding 10 μM H_2O_2 (black curve) using the Pt-modified carbon microelectrode as the working electrode. (b) Amperometric responses of Pt-modified carbon microelectrode at 0.65 V (vs. Ag/AgCl RE) with successive additions of H_2O_2 (indicated by arrows with marked concentrations) in advanced Tyrode's solution. (c) Calibration plot of oxidation currents and concentrations of H_2O_2. The experiments were performed in Tyrode's solutions containing 40-1000 nM H_2O_2 with applying 0.65 V (vs. Ag/AgCl RE) at the working electrode.

The oxidation potential of H_2O_2 was measured through running linear sweep voltammetry (LSV) in Tyrode's solutions containing H_2O_2 with different concentrations using the Pt-modified carbon microelectrode as the working electrode. To quantitatively monitor the H_2O_2 levels released from microglia, we recorded the amperometric responses in the Tyrode's solutions with different H_2O_2 concentrations using the Pt-modified carbon microelectrode applied with 0.65V (vs. Ag/AgCl RE) as the working electrode. As shown in **Fig. S5b and c**, the current responses of H_2O_2 oxidation showed a linear correlation with the H_2O_2 concentration in the range of 40 to 1000 nM, and the limit of detection of H_2O_2 was 16.7 nM (based on three times the

standard deviation of the noise) (R^2 = 0.9888).

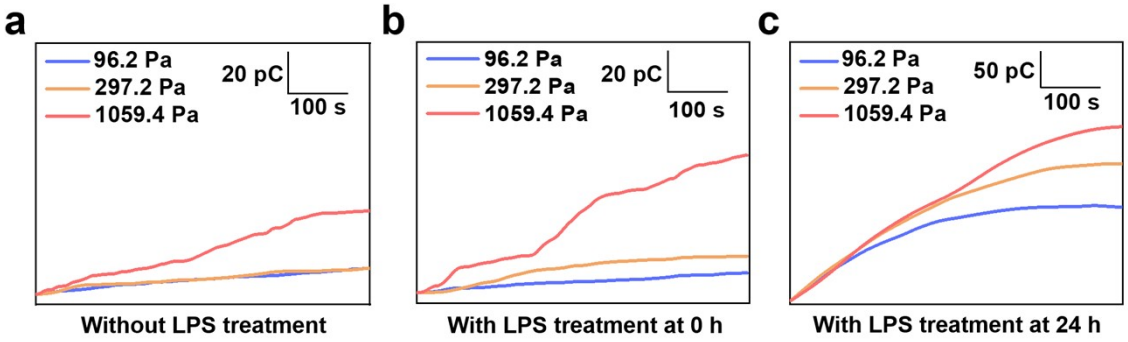

Fig. S6 Charges of H_2O_2 of BV2 cells on the PA gels with stiffness of 96.2, 297.2 and 1,059.4 Pa, respectively, and with LPS treatment at 0 h and 24 h through time-integration of amperometric currents in Fig. 3c (n = 3).

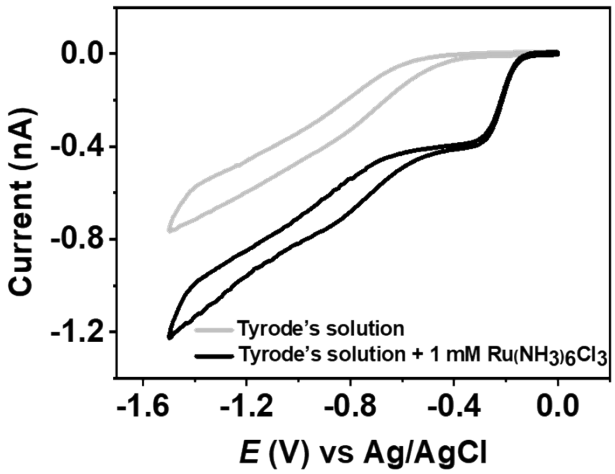

Fig. S7 Cyclic voltammogram in advanced Tyrode's solution (grey curve) and advanced Tyrode's solution containing 1mM $[Ru(NH_3)_6]Cl_3$ (black curve) using a 2.8-μm diameter carbon microelectrode as the working electrode (scan rates: 20 mV s^{-1}).

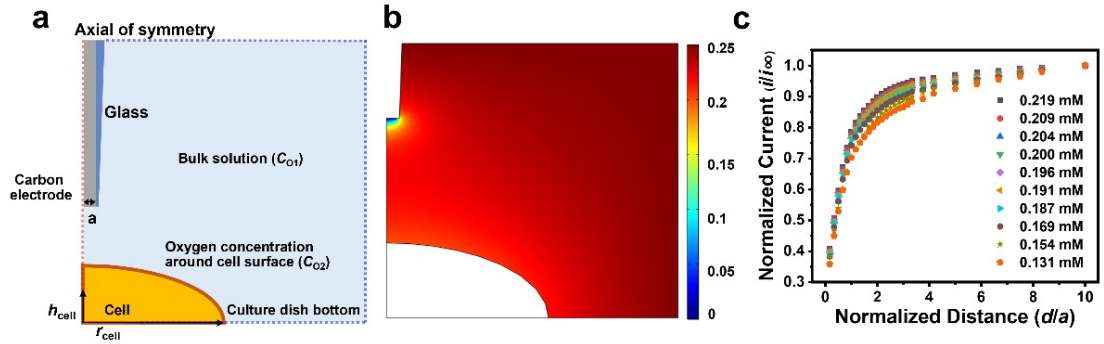

Fig. S8 (a) Schematic diagram of SECM theoretical model in the 2D axial symmetry and (b) the coordinate simulated oxygen concentration map under SECM probe positioned 10 μm above the BV2 cells. (c) Theoretical retract curves of the concentration gradient of oxygen around BV2 cell.

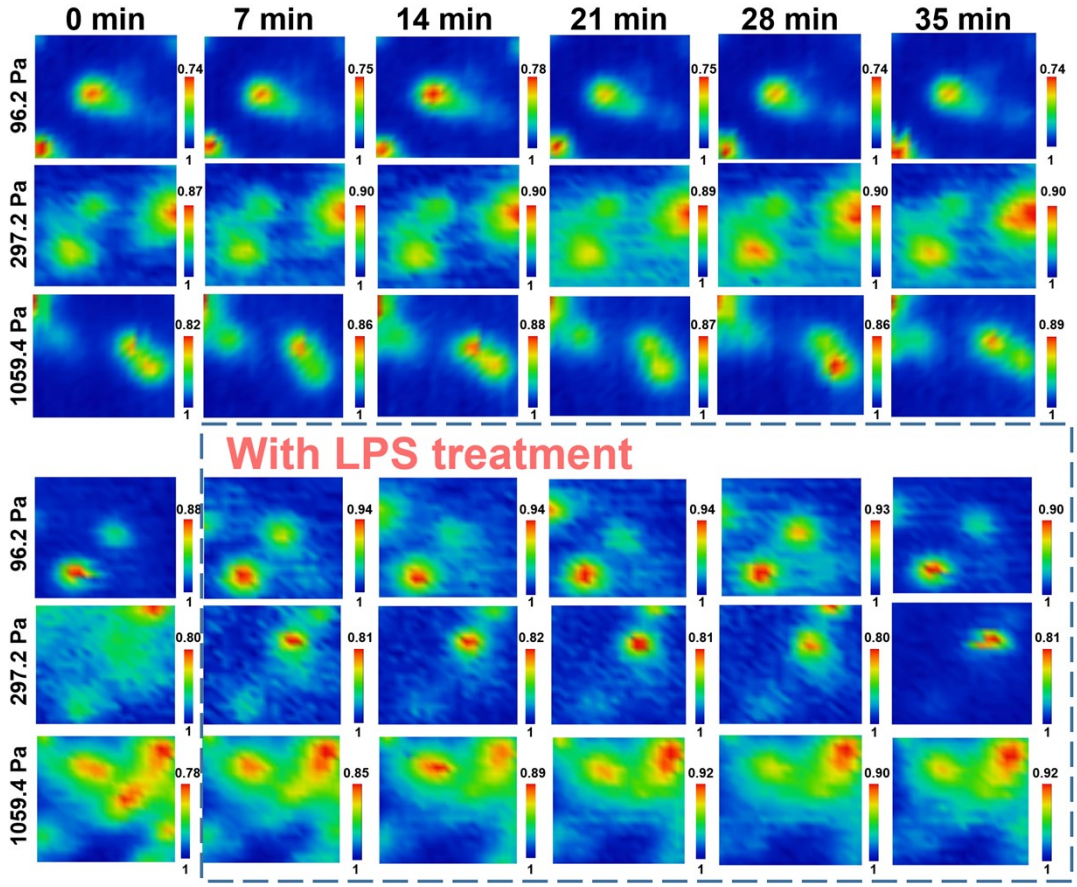

Fig. S9 SECM images of BV2 cells on the 96.2, 297.2 and 1,059.4 Pa PA gels without and with adding 1 μM LPS. The SECM experiments were performed in advanced Tyrode's solution containing 1 mM $[Ru(NH_3)_6]Cl_3$ (scan range: 100 × 100 μm^2, scan rates: 5 μm s^{-1}, E_{probe} = –0.35 V (vs. Ag/AgCl RE)).

References

1. A. J. Bard and M. V. Mirkin, Scanning Electrochemical Microscopy. CRC Press: 2012.
2. F. Zhang, Q. Qiu, X. Yang and W. Huang, Real-time electrochemical detection of retrograde messengers, *Chinese J. Anal. Chem.* 2019, ***47***, 6.