Electronic Supplementary Information
for

Effect of water-soluble fullerenes on macrophage surface ultrastructure revealed by scanning ion conductance microscopy

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Supplementary Figure

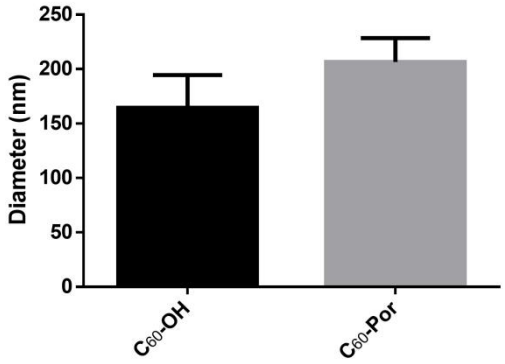

Figure S1 The size of C_{60}-OH and C_{60}-Por fullerenes characterized by dynamic light scattering. The average hydration diameter of C_{60}-OH is 164.5 ± 17.26 nm, and the average hydration diameter of C_{60}-Por is 206.6 ± 12.60 nm.

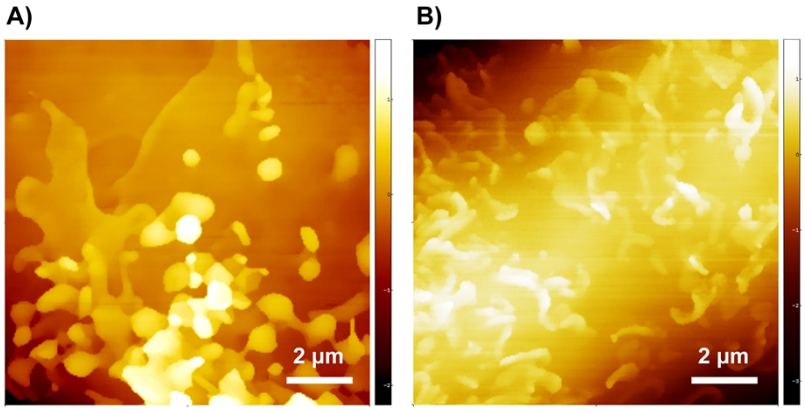

Figure S2 The surface ultrastructure of THP-1 macrophage treated with 0.1 mg/mL fullerenes. (A) SICM imaging of THP-1 macrophage treated with C_{60}-OH. (B) SICM imaging of THP-1 macrophage treated with C_{60}-Por.

Experimental section

Preparation and characterization of the water-soluble fullerenes

The water-soluble polyhydroxylated C_{60}-fullerenes (C_{60}-OH) and cationic porphyrin modified C_{60}-fullerenes (C_{60}-Por) were a gift from colleagues in our laboratory, they were prepared and characterized according to the previously reported method with minor modifications.[1, 2] In brief, C_{60}-OH was synthesized in liquid state, and the hydroxyl groups from sodium hydroxide are connected to C_{60}

fullerenes with tetrabutylammonium hydroxide (40% in water, TBAH, Sigma-Aldrich) serving as a catalyst.[3] The attachment of the hydroxyl groups was confirmed by Fourier-transform infrared spectroscopy (FTIR, Tensor 27, Bruker, Germany). C_{60}-Por, a cationic porphyrin modified C_{60} fullerene where three meso-positions of porphyrin are occupied by N-methylpyridinium-4-yl substituents, was synthesized by covalently coupling trismethylpyridylporphyrin to C_{60}. [4, 5] Their size was characterized by dynamic light scattering

Cell culture and PMA-induced differentiation

THP-1 monocyte cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and antibiotics (50 mg mL^{-1} streptomycin, 50 U mL^{-1} penicillin) at 37℃ in a 5% CO_2 atmosphere. To differentiate THP-1 cells, PMA (Sigma-Aldrich) was added to a final concentration of 100 ng mL^{-1} and cultured for 72 hours.[6, 7]

Sample preparation

For water-soluble fullerenes treatment experiments, the differentiated THP-1 macrophages were washed three times with PBS buffer before treated with 0.01 mg mL^{-1} of C_{60}-OH or C_{60}-Por for 24 hours. For the recovery experiments, the fullerene treated cells were continuing to culture in fresh medium for a further 48 hours after washed three times with PBS buffer. Before SICM imaging, the fullerene treated cells were fixed with 4% formaldehyde for 30 min at 4℃ and washed three times with PBS buffer.

For actin cytoskeleton staining, after treated with 0.01 mg mL^{-1} of C_{60}-OH or C_{60}-Por for 24 hours, PMA-differentiated THP-1 macrophages were fixed in PBS buffer containing 4% formaldehyde and permeabilized in PBS buffer containing 0.5% Triton X-100 and 3% formaldehyde at 4℃ for 30 min, and then incubated with rhodamine conjugated phalloidin (1:200, Invitrogen) for 30 min at 4℃. For nuclear staining, PMA-differentiated THP-1 macrophage was incubated with 100 ng mL^{-1} of 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) for 15 minutes.

For phagocytosis experiments, after treated with 0.01 mg mL-1 of C60-OH or C60-Por for 24 hours, PMA-differentiated THP-1 macrophages were exposed to fluorescent beads (1:200, yellow-green, Invitrogen) with a diameter of 2 μm overnight. The cells were then washed three times with PBS buffer to remove fluorescent beads that were not phagocytosed. Finally, the cells were fixed, and confocal imaging was performed to analyze the mean fluorescence intensity of THP-1 macrophages before and after fullerene treatment.

Cytotoxicity assay

THP-1cell suspension (100 μL) containing 100 ng mL^{-1} of PMA was dispensed into a 96-well plate (5000 cells/well). Incubate the plate for 72 hours at 37℃ in a 5% CO_2 atmosphere to differentiate the THP-1 cells. The differentiated THP-1 macrophages were washed three times with PBS and then incubated with 0.01 mg mL^{-1} of C_{60}-OH or C_{60}-Por for 24 hours. Then change to 100μL of fresh medium and add 10 μL of CCK-8 (Dojindo) solution to each well of the plate and continued to incubate for 3 hours. Then measure the absorbance at 450 nm using a microplate reader (Tecan Infinite M1000 PRO).

SICM imaging

Surface ultrastructural experiments were performed using a commercial scanning ion conductance microscope (Ionscope Ltd., Melbourn, U.K.), mounted on top of a Nikon TE2000 optical microscope (Nikon, Tokyo, Japan). Pipettes were pulled from borosilicate capillaries of 1.0 mm outside diameter and 0.58 mm inside diameter (BF100-58-15, Sutter Instrument, Novato, CA) using a CO2 laser powered

pipette puller (Model P2000, Sutter Instrument). Pulled pipettes should be stored in clean, covered containers until the time of use. Ideally they should be fresh before use, however we have experience using them up to 1-2 weeks after being pulled. Usually with time they become hydrophobic and more difficult to fill and as a result they may have to be discarded. And the pipettes were filled with PBS when performing topography imaging. For SICM imaging, we used hopping mode where the pipette is vertically approached to the sample surface until the ion current drops below a preset trigger value (The preset voltage is 200mV, and the corresponding preset current is about 0.2nA), thereby defining the height of the surface at this position. Repeating this procedure for many positions on the sample allows generating an image of sample topography. The ultrastructural images we obtained were either 256×256 pixels or 128×128 pixels. It should be noted that the resolution of the instrument has been confirmed by the standard substance. The lateral (x-y) resolution is about 60 nm and the vertical (z) direction resolution is about 10 nm.

Confocal imaging

The fluorescence imaging was performed by a commercial confocal microscope (FV1000-IX81, Olympus, Japan) equipped with a 100×1.40 NA objective. During the cytoskeleton and nuclear dual-color imaging, rhodamine was excited by a 559 nm laser (FV10-LD559, Olympus) and the emission was collected by using a 570-625 nm bandpass filter, DAPI was excited by a 405 nm laser (FV5-LD405-2, Olympus) and the emission was collected using a 425-475 nm bandpass filter. The fluorescent beads were excited by a 488 nm laser (FV5-LAMAR, Olympus) and the emission was collected by using a 500-545 nm bandpass filter.

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