Sporopollenin exine capsules modulate the function of microglial cells

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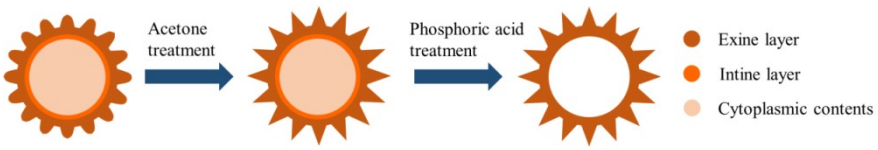
Table S1. The primer sequences for qRT-PCR of inflammatory or anti-inflammatory factors

Gene	Forward primer	Reverse
Actb	TCTTGGGTATGGAATCCTGTGGCA	TCTCCTTCTGCATCCTGTCAGCAA
TNF-α	CTCCAGGCGGTGCCTATG	GGGCCATAGAACTGATGAGAGG
IL-1β	GCACACCCACCCTGCA	ACCGCTTTTCCATCTTCTTCTT
IL-4	AACGTCCTCACAGCAACGAA	GCATCGAAAAGCCCGAAAGA
IL-10	CAGTACAGCCGGGAAGACAA	AGGCTTGGCAACCCAAGTAA
TGF-β	AAACGTCCTCACAGCAACGA	GAATCCAGGCATCGAAAAGCC



Scheme S1. Schematic diagram of sunflower pollen treatment process. Natural sunflower pollen is covered by pollenkitt, the surface lipids are removed after acetone treatment, the cell contents are removed after phosphoric acid treatment, and the three-dimensional morphology is maintained.

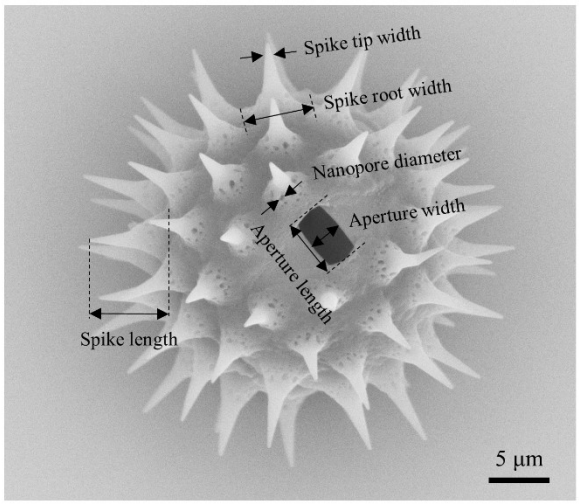


Figure S1. Image J software analysis of SEC morphology features.

Table S2. Morphological characteristics of SEC

Structure	Size
Length of spike	7.02 ± 0.53 μm
Tip width of spike	713.1 ± 0.14 nm
Root width of spike	4.02 ± 0.35 μm
Length of aperture	5.14 ± 0.96 μm
Width of aperture	3.24 ± 0.59 μm
Nanopore diameter	270.7 ± 79.4 nm

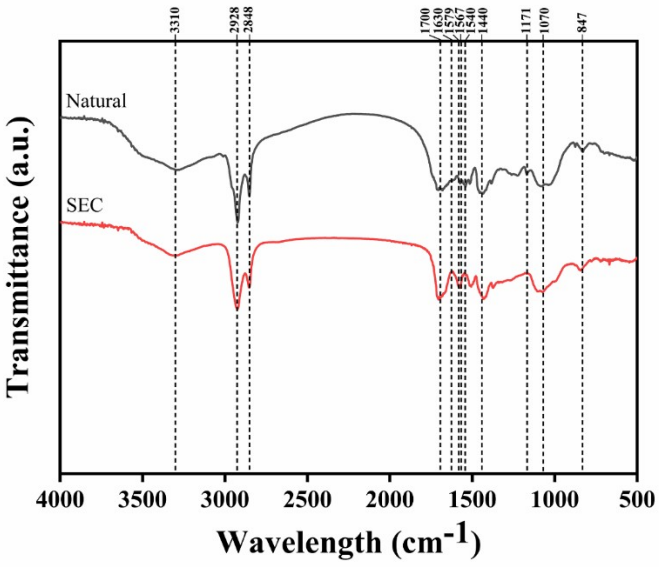


Figure S2. Infrared Spectrum of pollen before and after treatment. The strong vibrations at 2928 cm^{-1} and 2848 cm^{-1} belong to the C-H stretching of methylene, and the vibration at 1070 cm^{-1} belongs to the C-O stretching in carbohydrates.

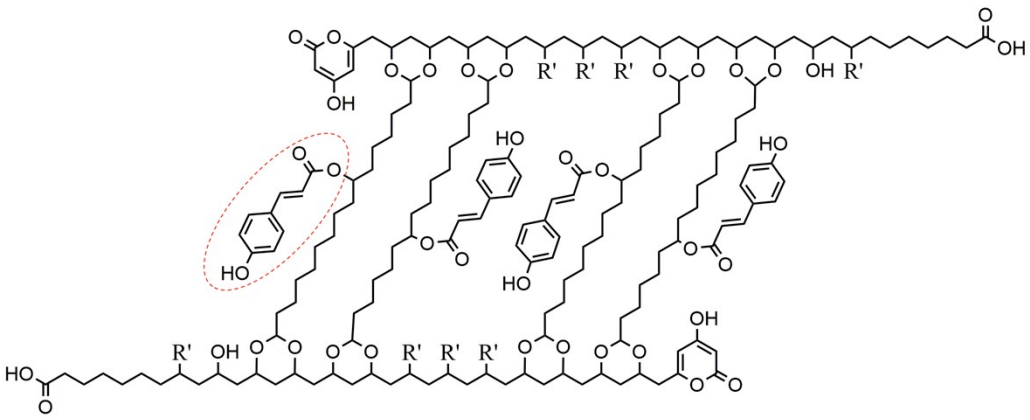


Figure S3. Average structure of sporopollenin[1]. The p-coumaric acid in the structure is marked with a red circle. Chemical structures were drawn using ChemDraw.

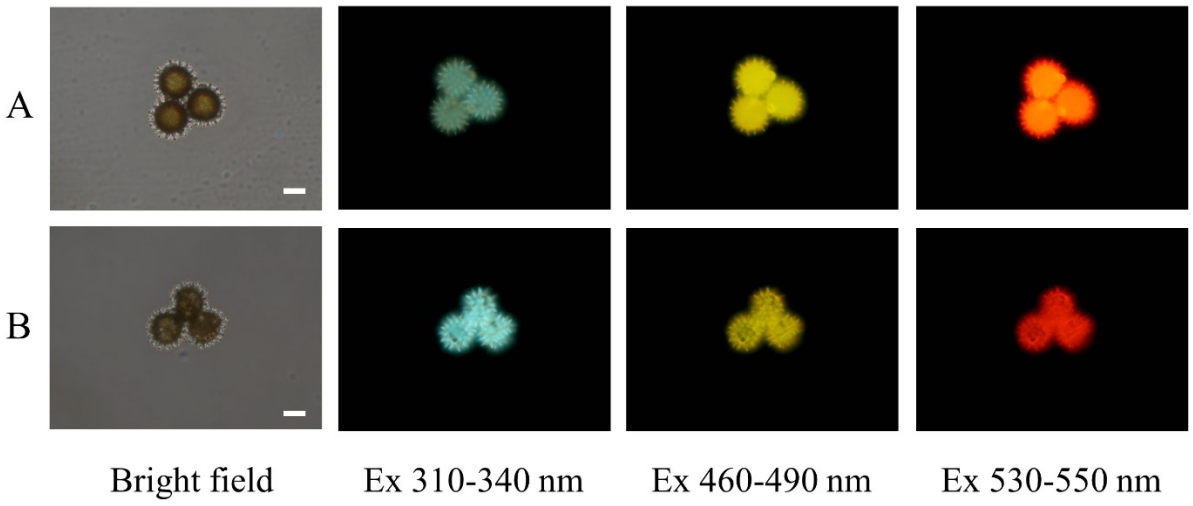


Figure S4. A and B represent the autofluorescence of pollen before and after treatment, respectively (scale bars are 20 μm).

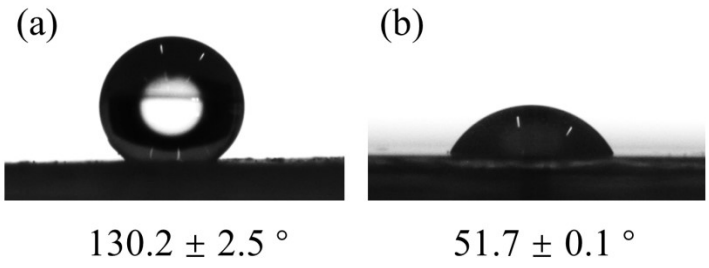


Figure S5. Optical microscope images of water droplets during contact angle measurements of pollen before and after treatment, (a) before treatment, (b) after treatment.

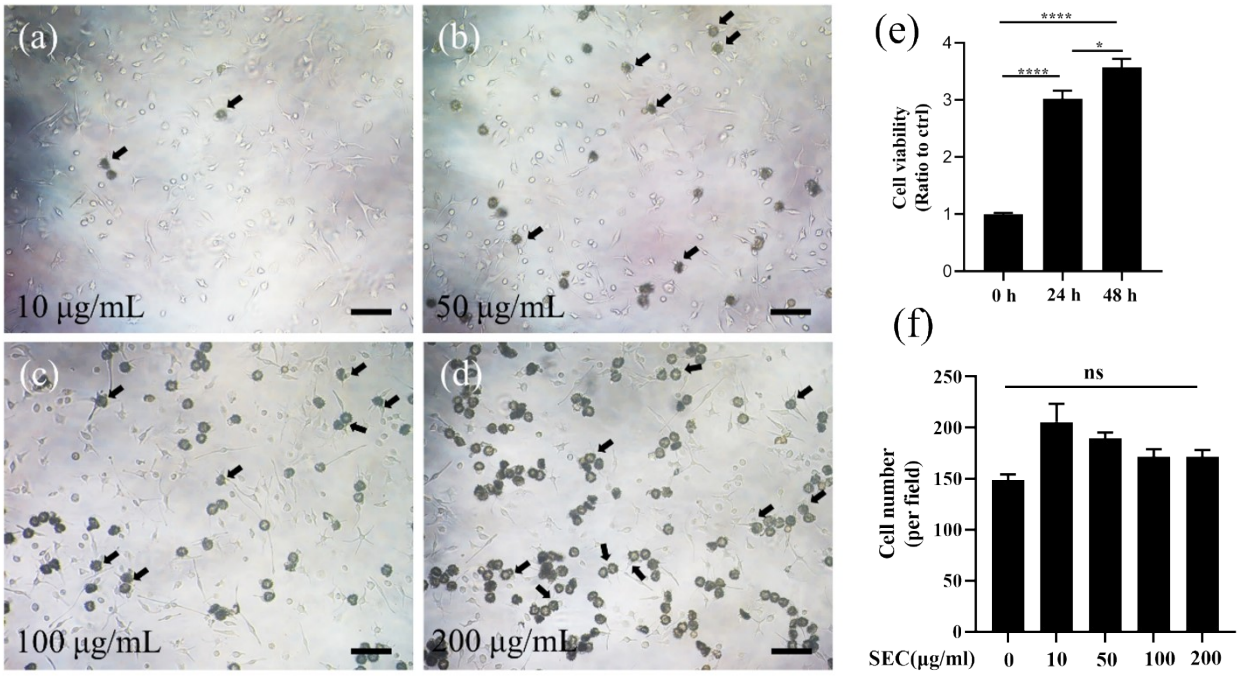


Figure S6. Optical images of primary microglia incubated with different concentrations of sunflower SEC for 48 h. The SEC concentrations of (a)-(d) are 10, 50, 100, and 200 μg/mL respectively. The black arrows indicate microglial cells aggregated to SEC (scale bars are 100 μm). (e) The cell viability increased with SEC (200 μg /ml) was time dependent. (f) The cell number after incubation with different concentrations of SEC for 48 h. ns = not significant, $P \leq 0.05$ [*], $P \leq 0.0001$ [****].

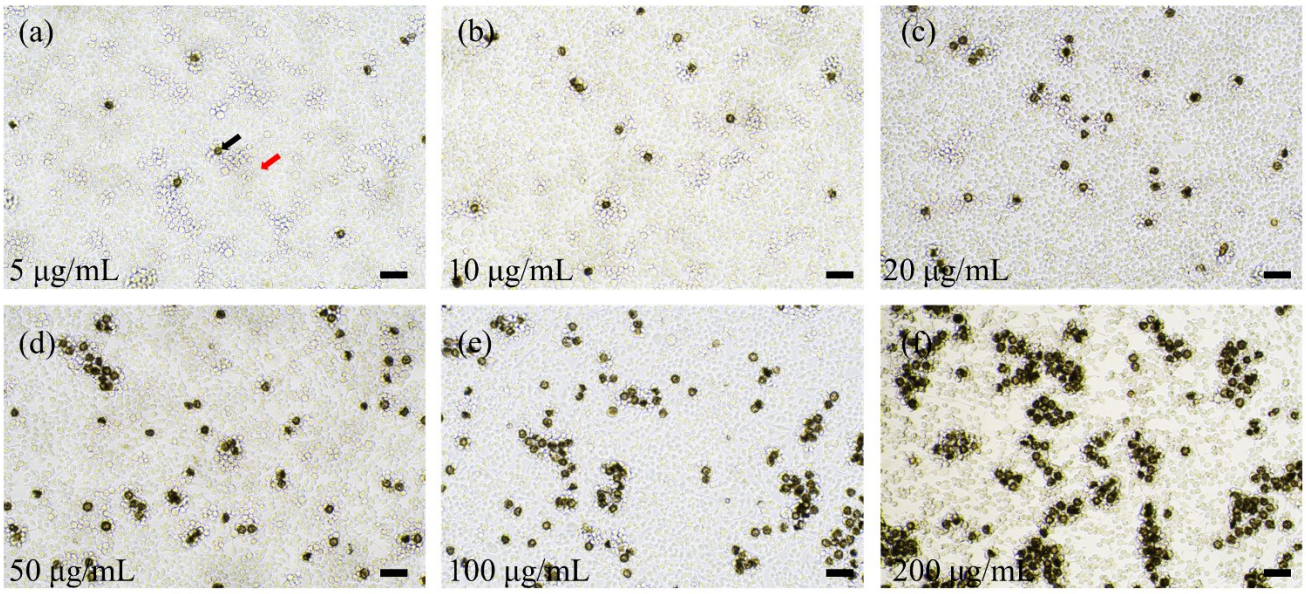


Figure S7. Light microscope images of different concentrations of SEC and BV2 co-incubated for 48 h, the concentrations of SEC in a-f graphs are 5, 10, 20, 50, 100, and 200 μg/mL, respectively. Black arrow indicates SEC, and red arrow indicates cells (scale bars are 50 μm).

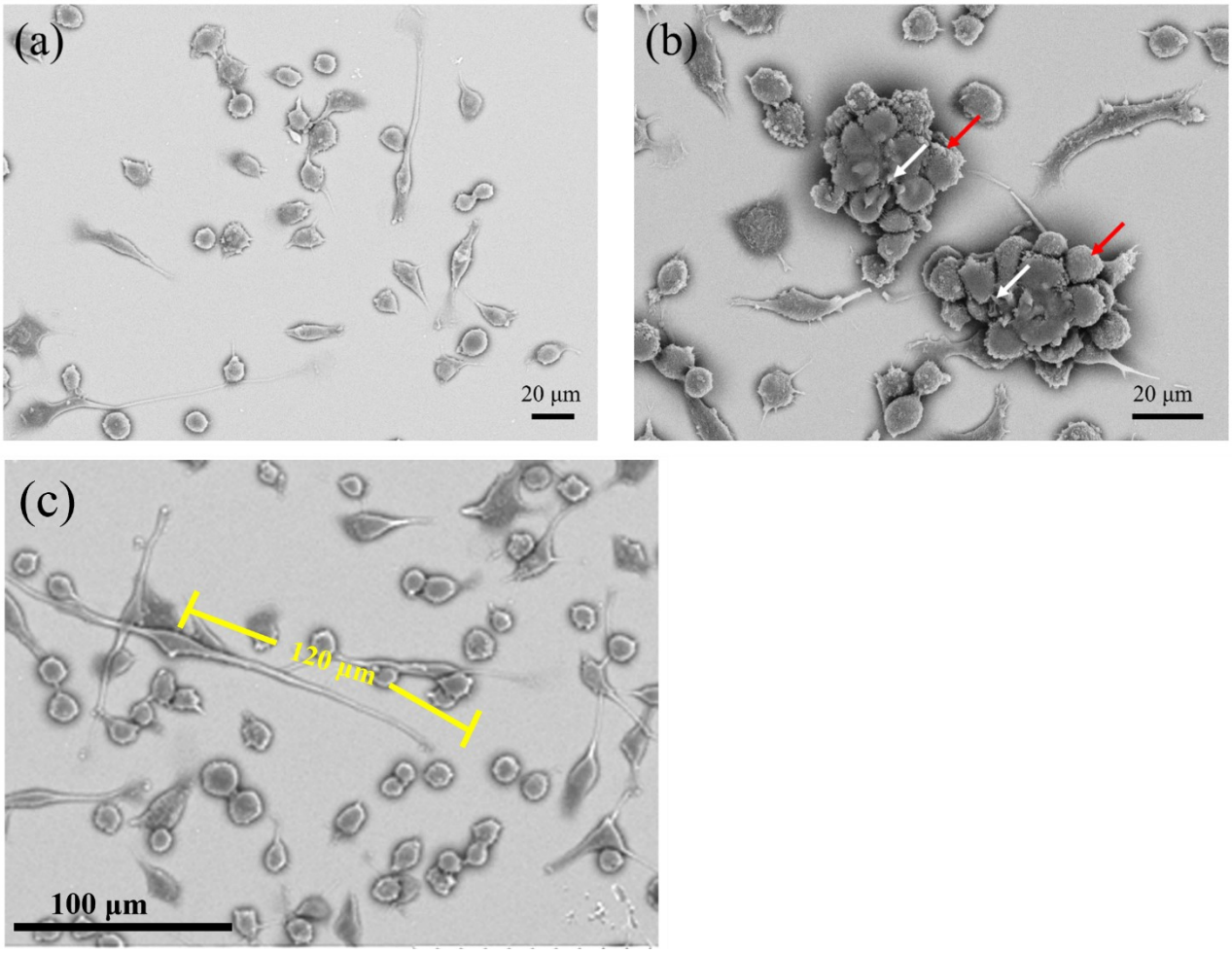


Figure S8. (a) BV2 under the scanning electron microscope. (b) SEC- BV2 cluster under the scanning electron microscope. The red arrows indicate the cells that crawled onto the SEC, and the white arrows indicate the SEC. (c) Length of filopodia of BV2 cell.

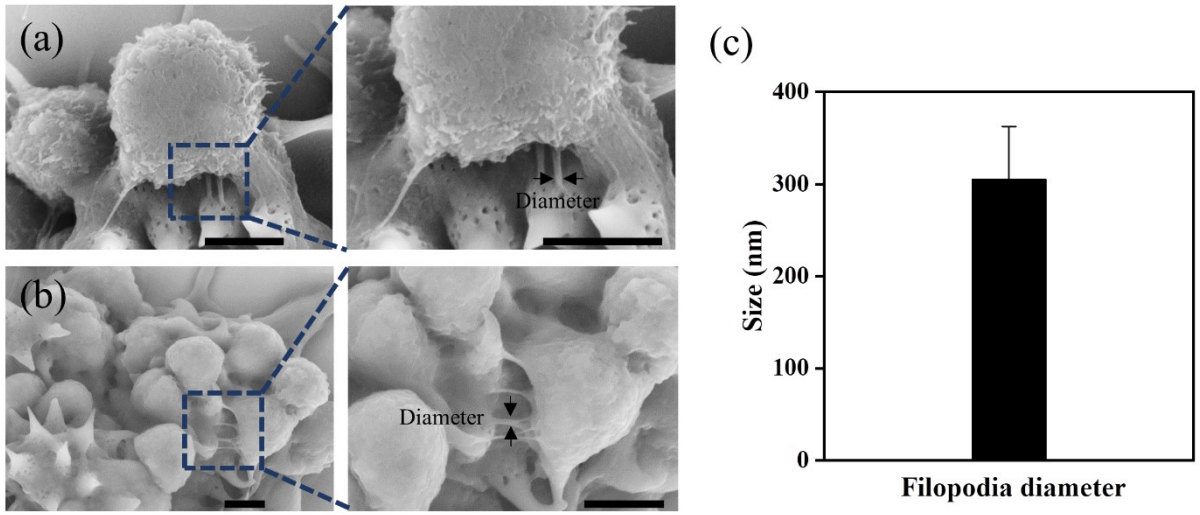


Figure S9. SEM images of BV2 filopodia extending into the nanopores of SEC. The zoomed images are magnified from the boxed areas of the images (a) and (b). Scale bars are 5 μm. (c) Filopodia diameter of BV2.

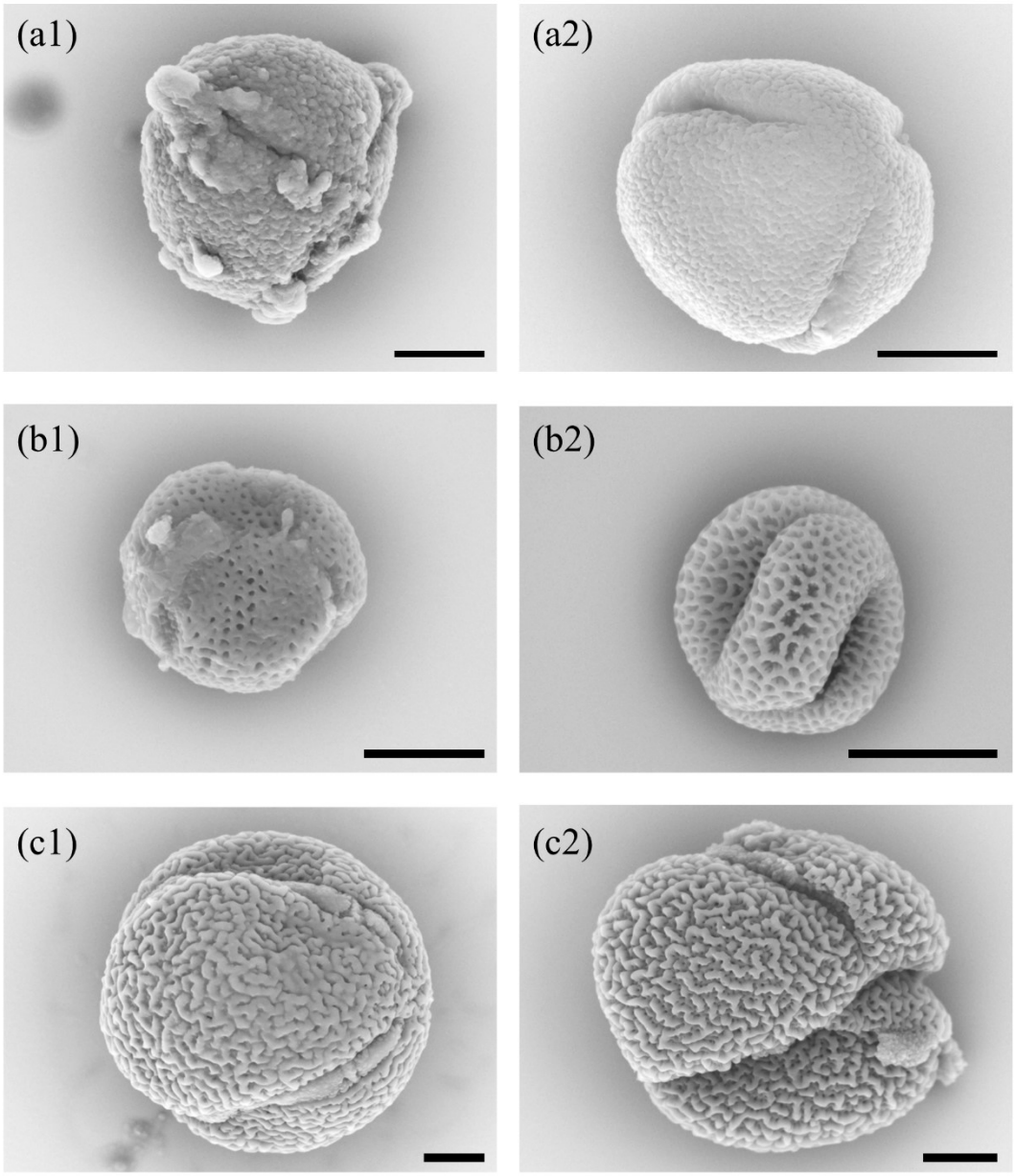


Figure S10. SEM characterization of different SEC before and after treatment. (a1)-(c1) Camellia, rape, and lotus pollen before treatment, (a2)-(c2) Camellia, rape, and lotus pollen after treatment (scale bars are 10 μm).

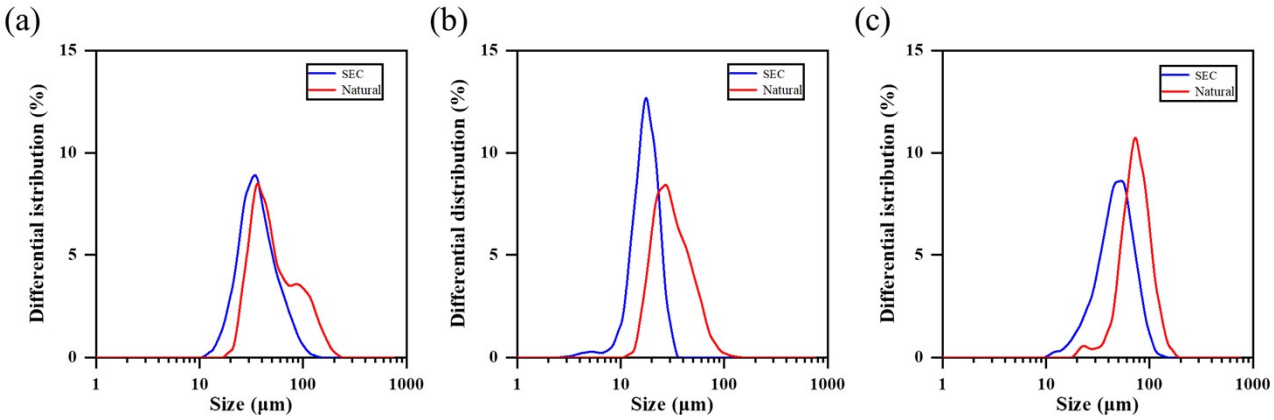


Figure S11. Particle size distribution of (a) camellia, (b) rape, and (c) lotus SEC.

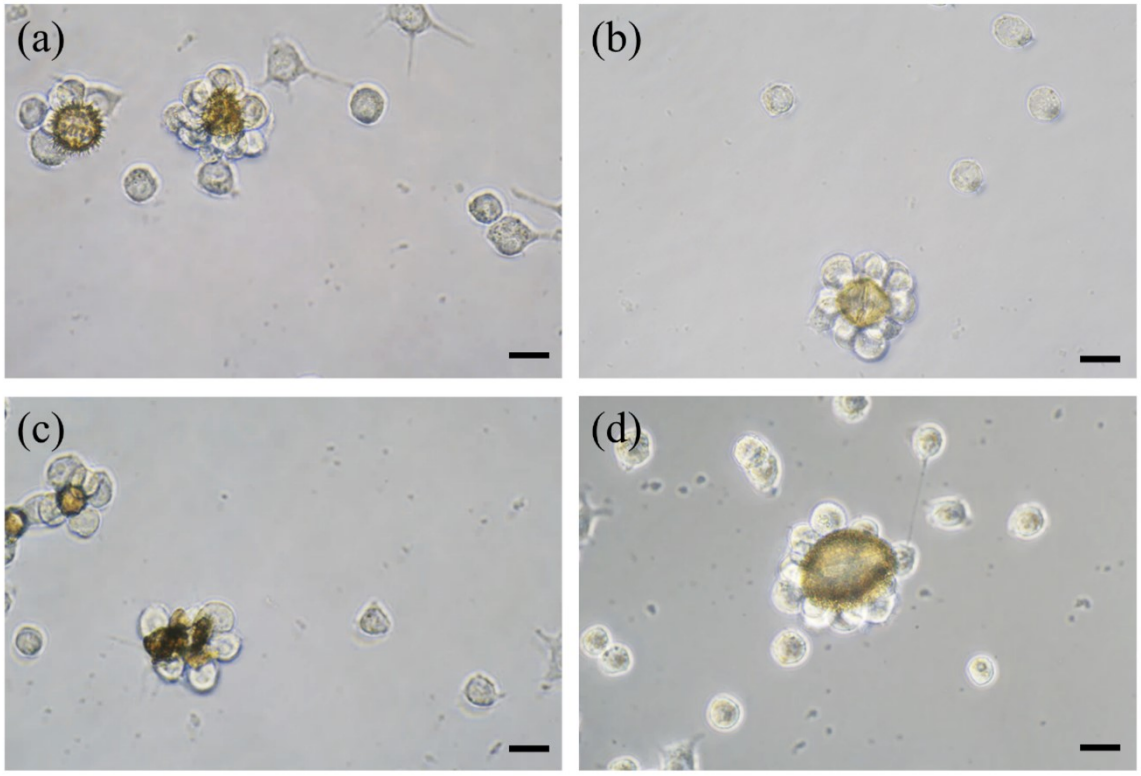


Figure S12. Aggregation of BV2 around different SEC. (a)-(d) were sunflower, camellia, rape, and lotus respectively. Image displayed that cells tended to aggregate near the SEC compared to the petri dish (scale bars are 20 μm).

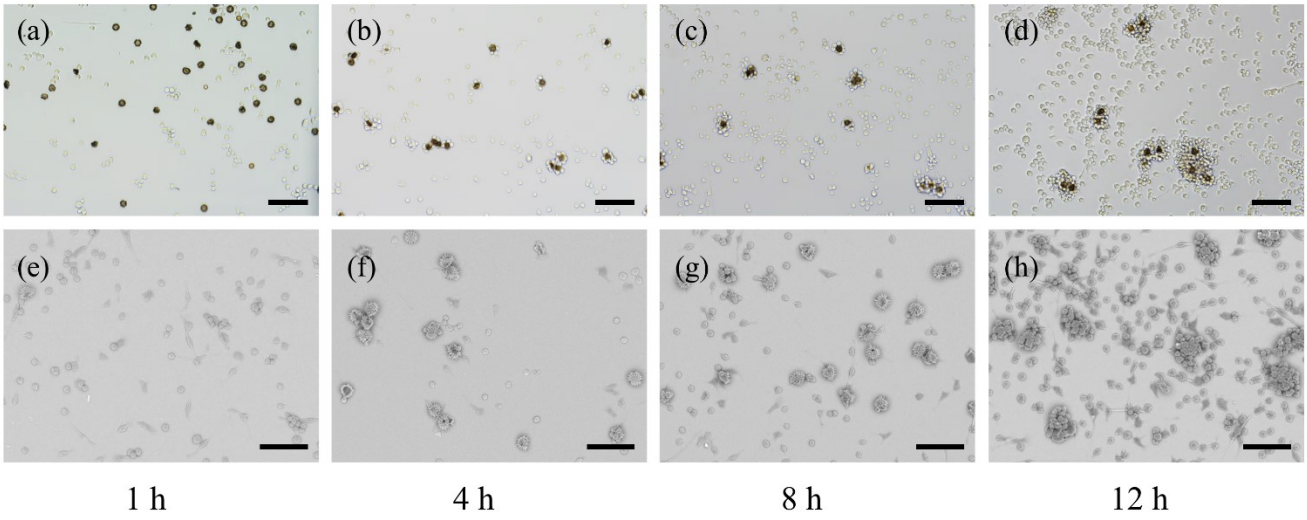


Figure S13. (a)-(d) Optical pictures of SEC incubated with BV2 for 1, 4, 8, and 12 hours. (e)-(h) Scanning electron micrographs of SEC incubated with BV2 for 1, 4, 8, and 12 hours (scale bars are 100 μm).

Video.mp4

Video 1. The dynamic process of BV2 clustering around the SEC. The original image was recorded every five minutes for a total of 135 minutes in the time-lapse mode of the IX73 Olympus. In the video, the one with a darker color and larger particles is SEC, and the one with lighter color is BV2.

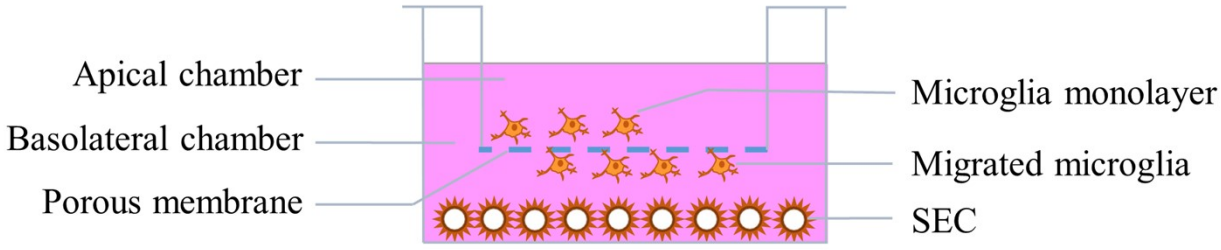


Figure S14. Schematic diagram of the transwell migration assay.

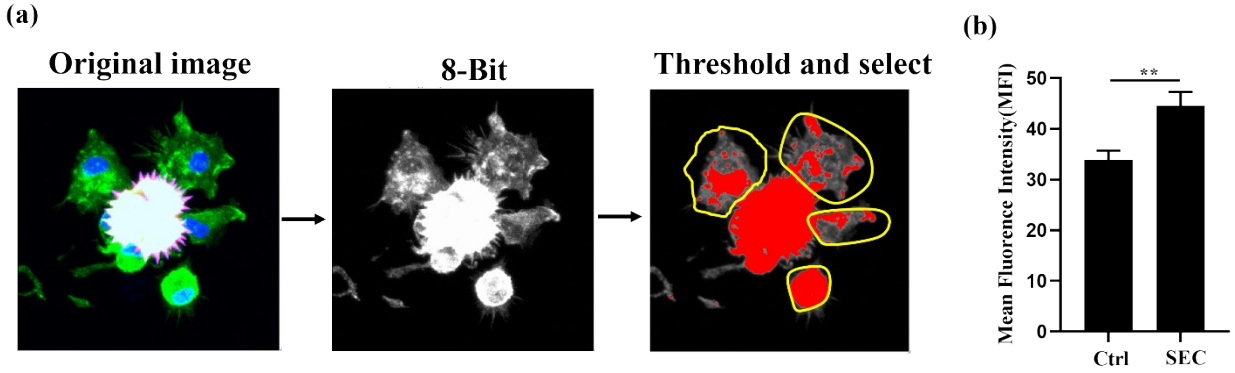


Figure S15. (a) Schematic diagram of quantitative image analysis by Image J software. (b)Comparison of the actin fluorescence intensity of microglia without SEC treatment (ctrl) and with SEC treatment. Image J software was used for quantitative fluorescence analysis.

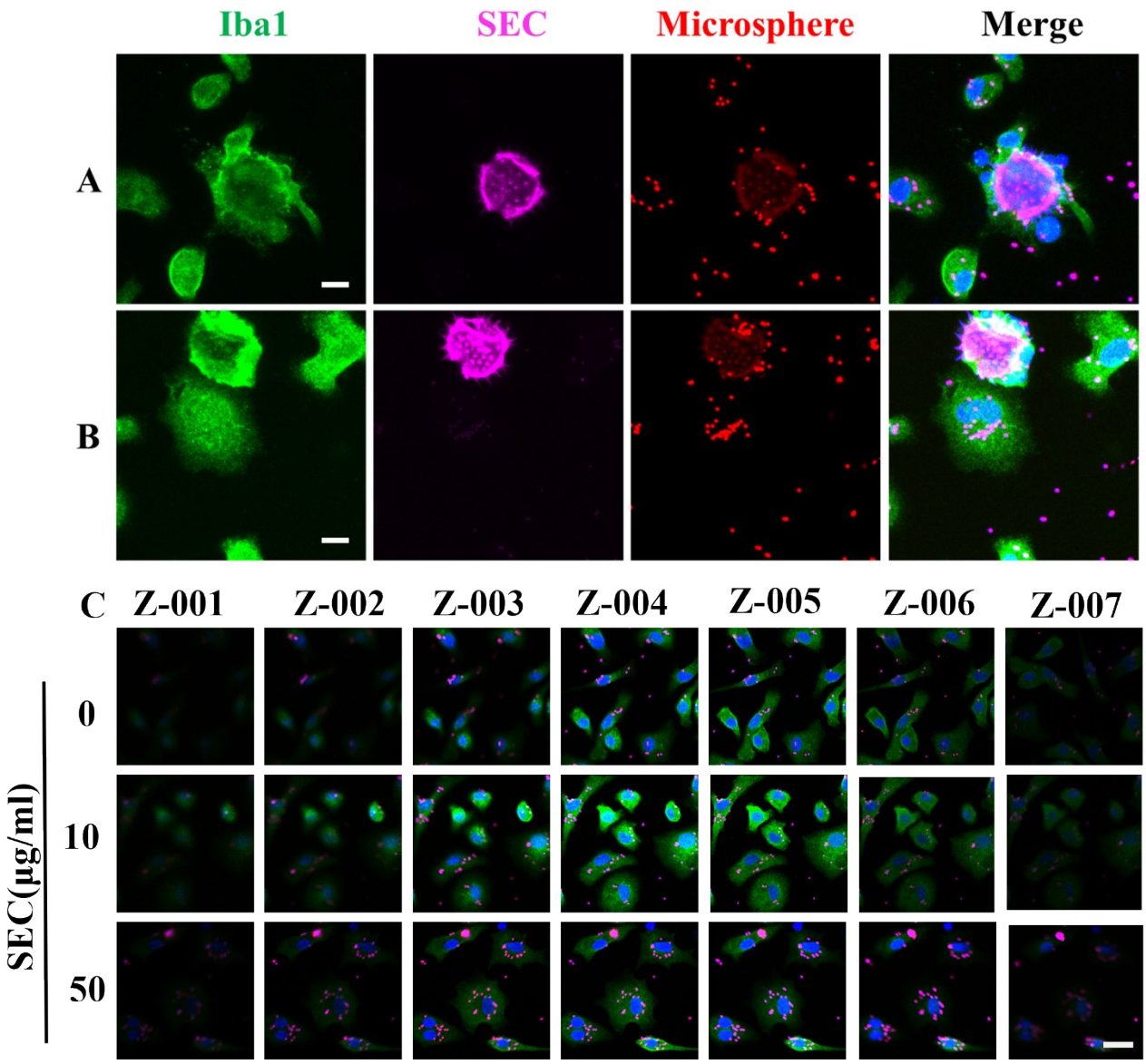


Figure S16. A and B represent the phagocytosis of fluorescent microspheres after cells were incubated with 10 μg/mL and 50 μg/mL SEC for 24h, respectively. Imaging was performed sequentially using four laser excitation channels: 559, 515, 405, and 488 nm, respectively. SEC exhibited autofluorescence in all channels (405 channel was not shown). Image colors were modified to distinguish SEC from microglia and microsoheres. The images showed that primary microglia clustered around SEC and engulfed more microspheres. Scale bars are 15μm. C. The Z-stack images after microglia were incubated with 10 μg/mL and 50 μg/mL SEC for 24h, respectively. 7 images were stacked with a thickness of 1μm.

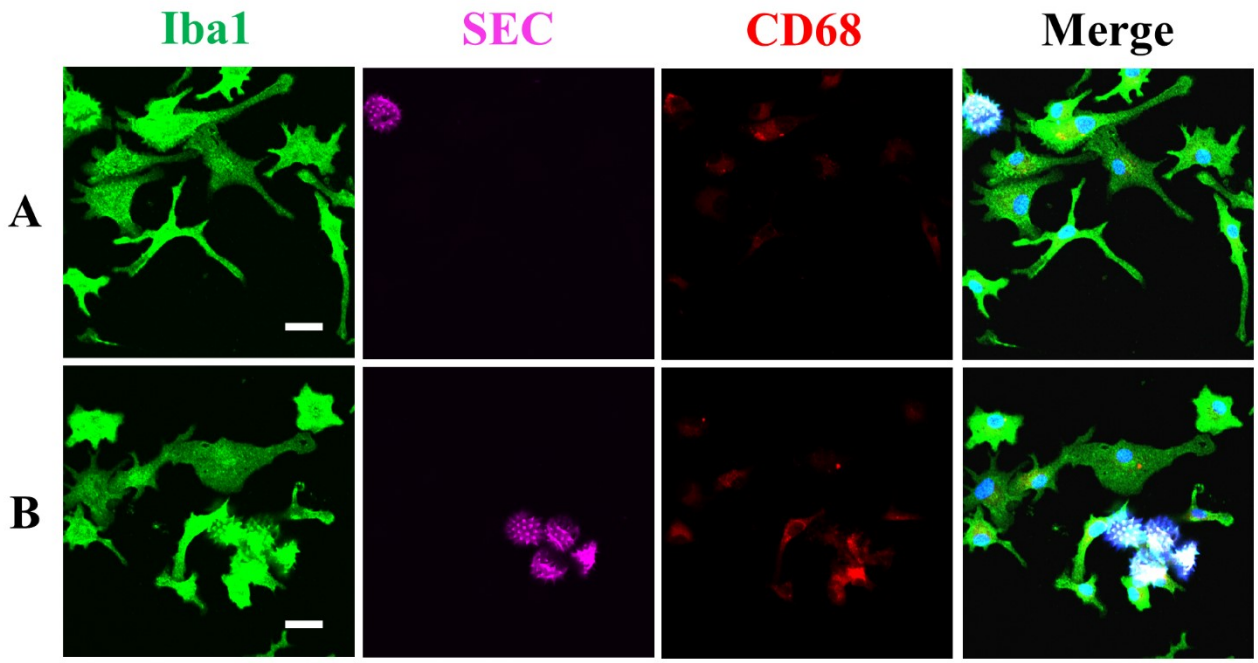


Figure S17. Morphology of primary microglia after incubation with different concentrations of SEC. The SEC concentration of A and B was 10 μg/mL and 50 μg/mL, respectively. Imaging was performed sequentially using four laser excitation channels: 488, 405, 559, and 635 nm, respectively. SEC exhibited autofluorescence in 488, 405 and 559 channels (the 405 channel was not shown). Image colors were modified to distinguish SEC from microglia. Scale bars are 30 μm.

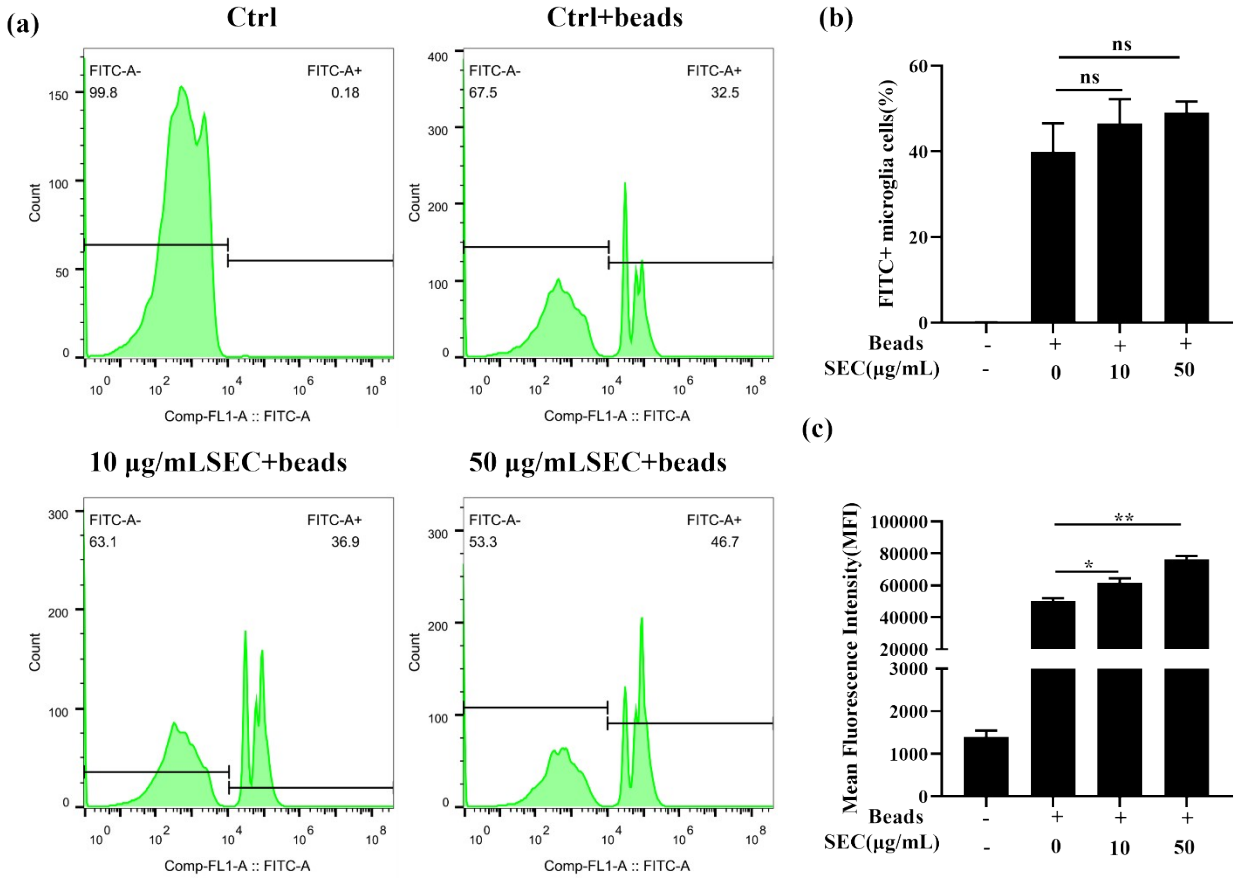


Figure S18. Identification phagocytic ability of primary microglia by flow cytometry.(a) After primary microglia were treated by SEC for 24h, the cells were treated with phagocytosis beads prepared with serum-free DMEM for 3h, then test the phagocytic ability of primary microglia by flow cytometry. (b) Quantified $FITC^+$ cells after phagocytosis beads treatment. (c) Quantified the mean fluorescence intensity of phagocytic microglia.

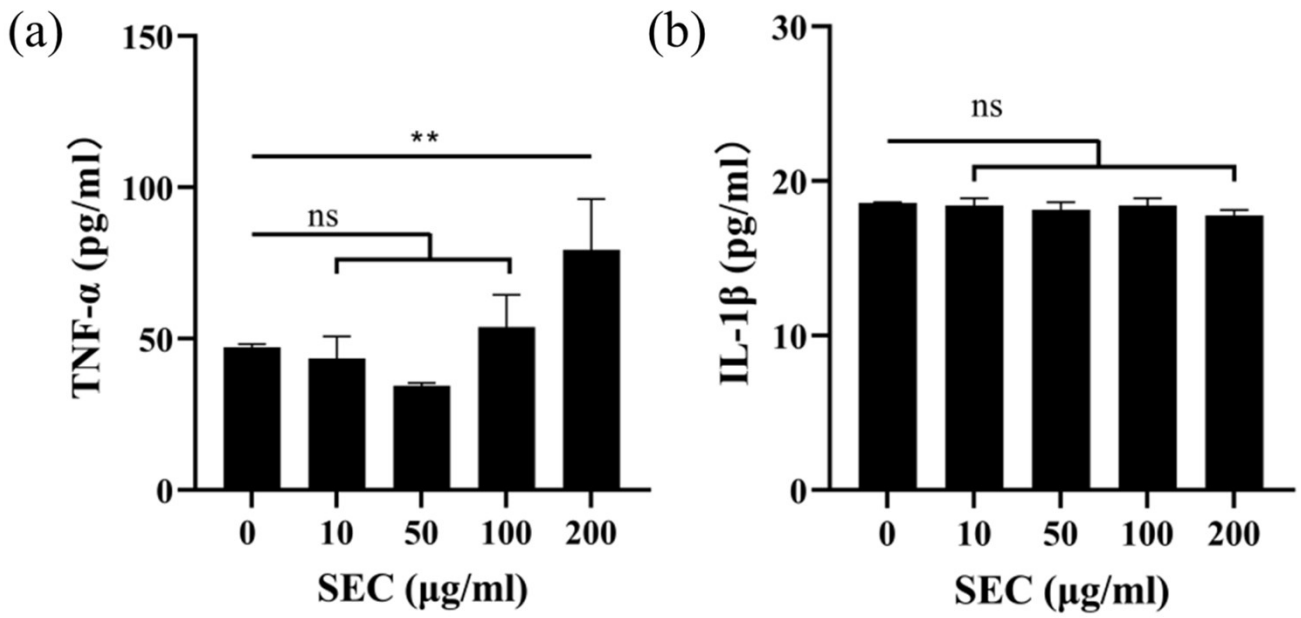


Figure S19. Cytokines secreted by primary microglia at different SEC concentrations. (a) TNF-α, (b) IL-1β. ns = not significant, P ≤ 0.05 [*], P ≤ 0.01 [**], P ≤ 0.001 [***], P ≤ 0.0001 [****].

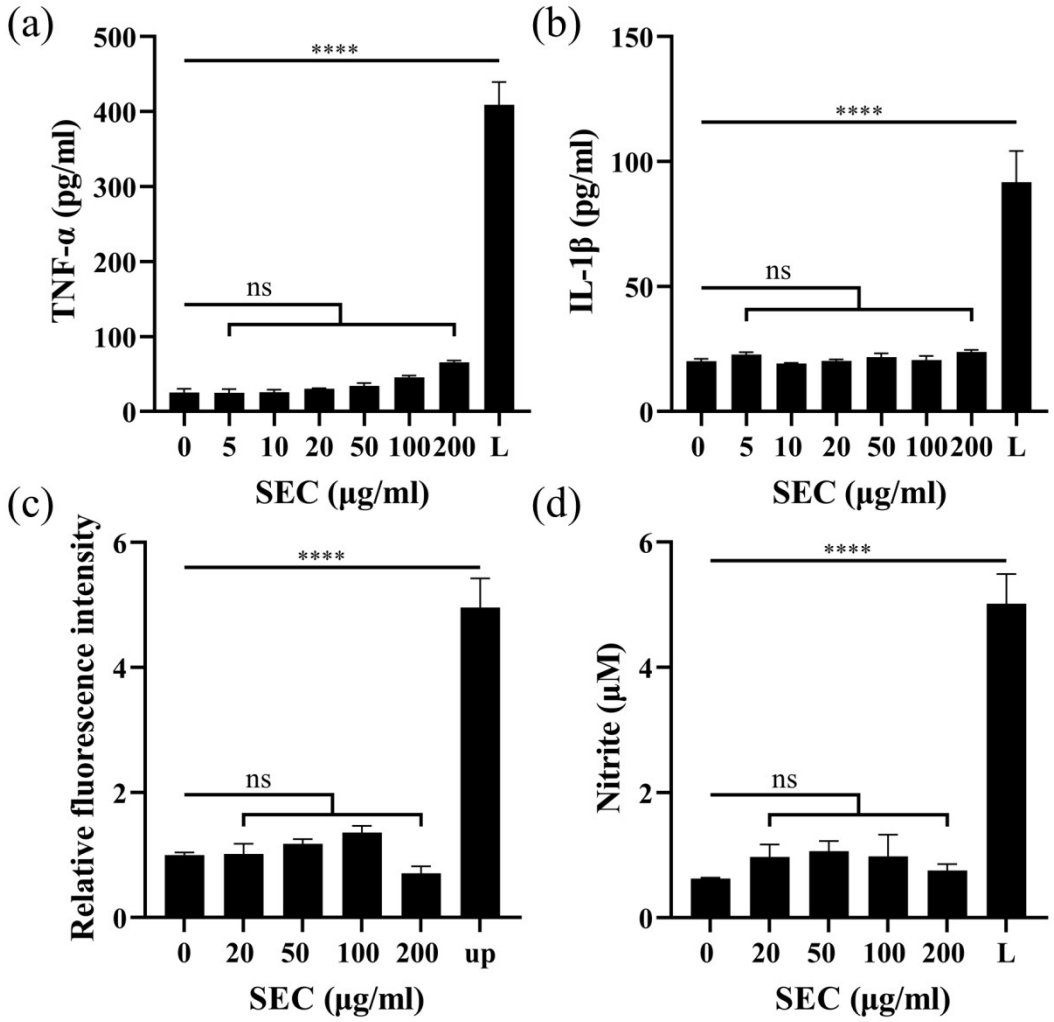


Figure S20. Cytokines secreted by BV2 at different SEC concentrations. (a) TNF-α, (b) IL-1β, (c) ROS, (d) NO. The L in Figures a and b means that the cells are stimulated by 100 ng/mL LPS. The up in figure c represents cells stimulated by an active oxygen-positive control reagent (Beyotime, China). The L in figure d means that the cells are stimulated by 1 μg/mL LPS.

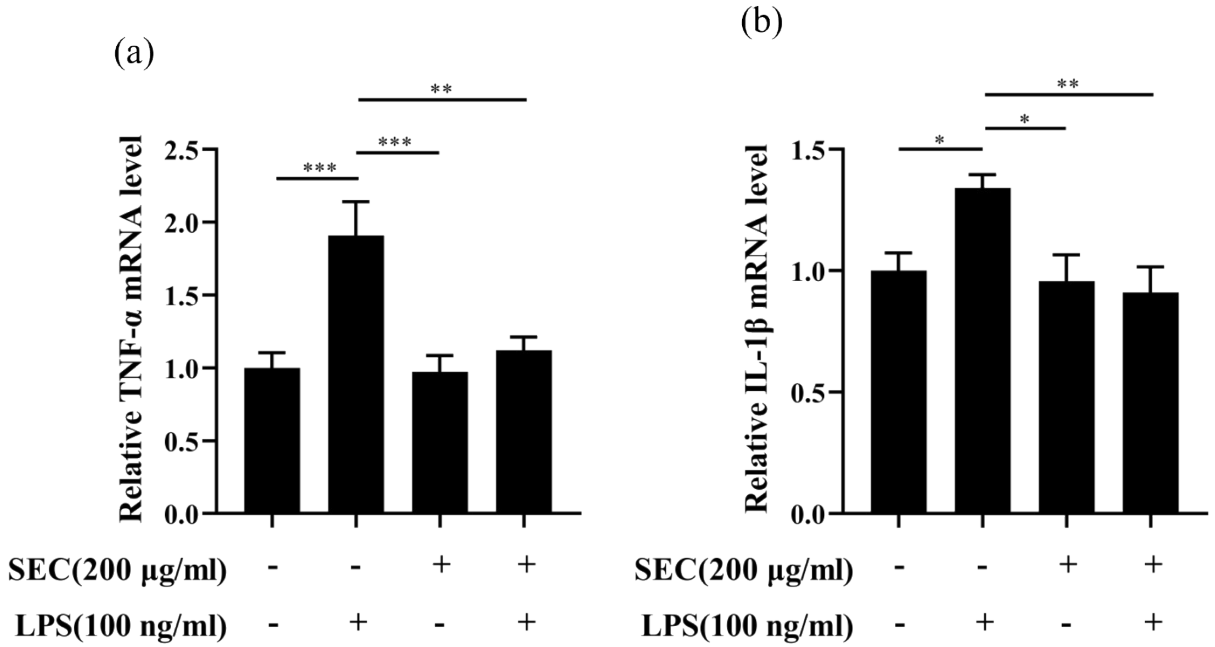


Figure S21. Transcription level of inflammatory factors TNF-α and IL-1β by RT-PCR. (a) TNF-α, (b) IL-1β. After BV2 cells were treated with different concentrations of SEC for 24 hours, LPS (100 ng/ml) prepared with free-FBS DMEM was incubated with BV2 cells, the transcription level was detected by RT-PCR.

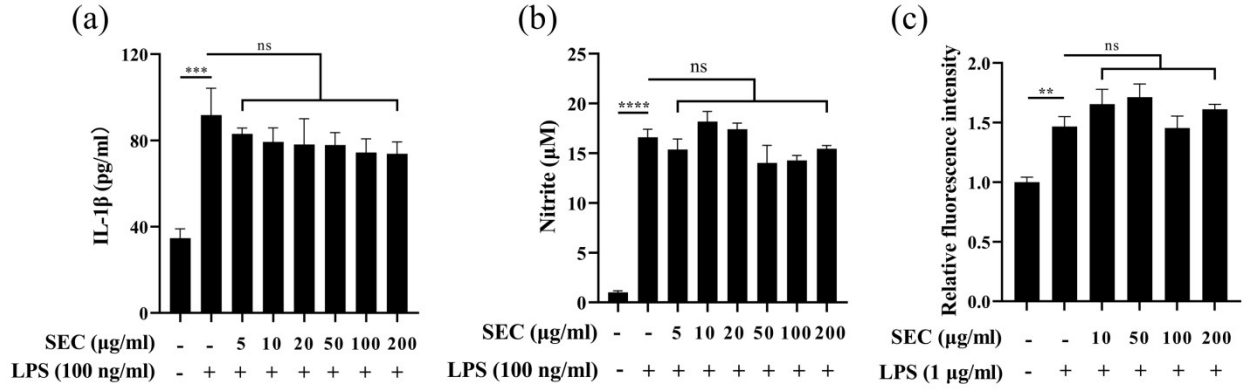


Figure S22. Cytokines secreted by BV2 cells in the presence of SEC and LPS. (a) IL-1β, (b) NO, (c) ROS.

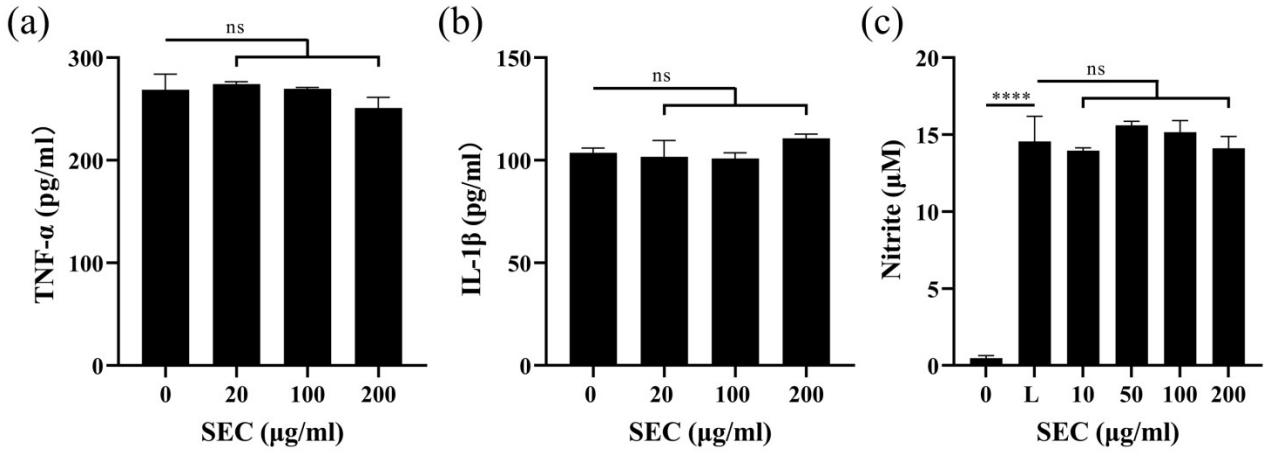


Figure S23. Adsorption of cytokines by SEC. After the TNF-α and IL-1β standards were incubated with different concentrations of SEC for 24 hours, the concentration changes were detected by ELISA (a) TNF-α, (b) IL-1β. (c) After co-incubating 1 μg/mL LPS and different concentrations of SEC for 24h, the supernatant was used to stimulate the cells for 24h, and then the concentrations of NO were detected by Griess Reagent. L stands for LPS, and 10, 50, 100, and 200 represent that LPS was incubated with different concentrations of SEC for 24 h before detection.

Note: The procedure of NO assay is as follows. We aspirated 1 μL, 5 μL, 10 μL, and 20 μL 10 mg/mL SEC (dispersed in serum-free DMEM), respectively. Then we mixed SEC with 1 mL of 1 μg/mL LPS and incubated for 24 h (The concentrations of SEC were 10 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, respectively). After centrifugation at 3000 rpm for 5 min, SEC was removed and the supernatant was taken to stimulate BV2 for 24 h. Finally, NO content in the supernatants of the cells in different groups was detected to indirectly determine whether SEC absorbed LPS. The amount of NO decreases if it's adsorbed by SEC.

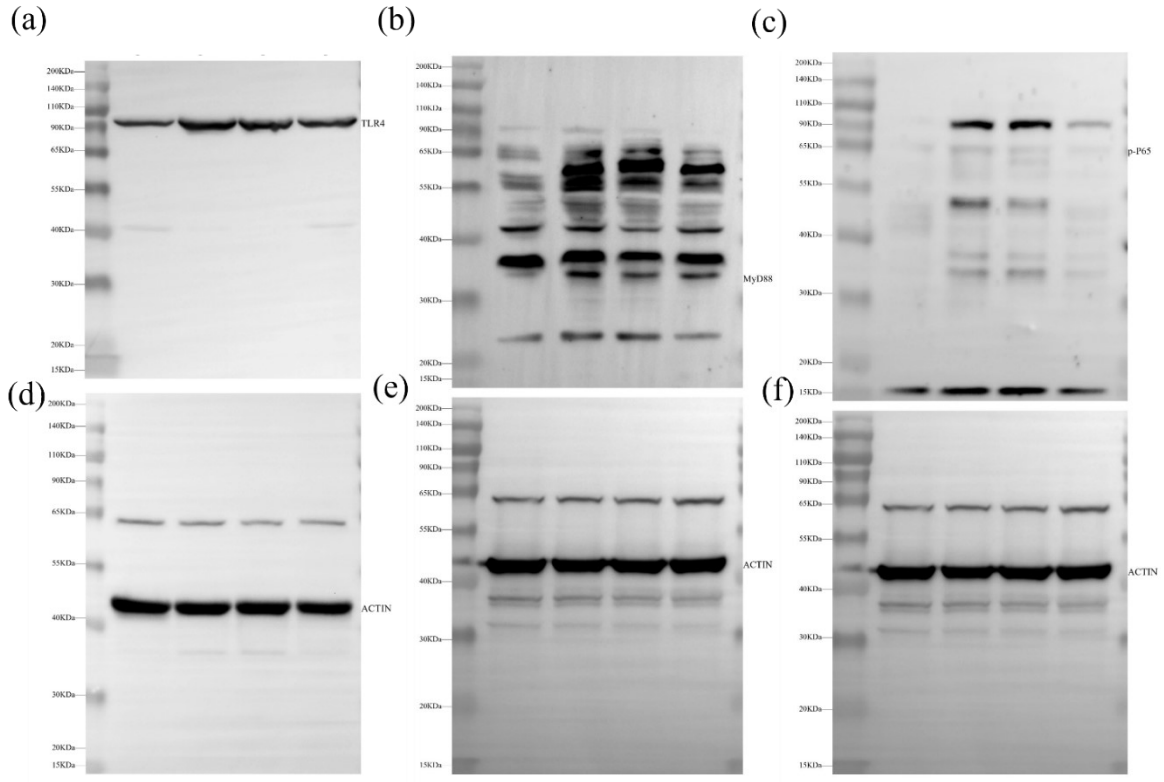


Figure S24. The original Western Blot images of TLR4, MyD88, p-p65 and ACTIN. (a) The TLR4 original image. (b) The MyD88 original image. (c) The p-p65 original image. (d-f) The ACTIN original image.

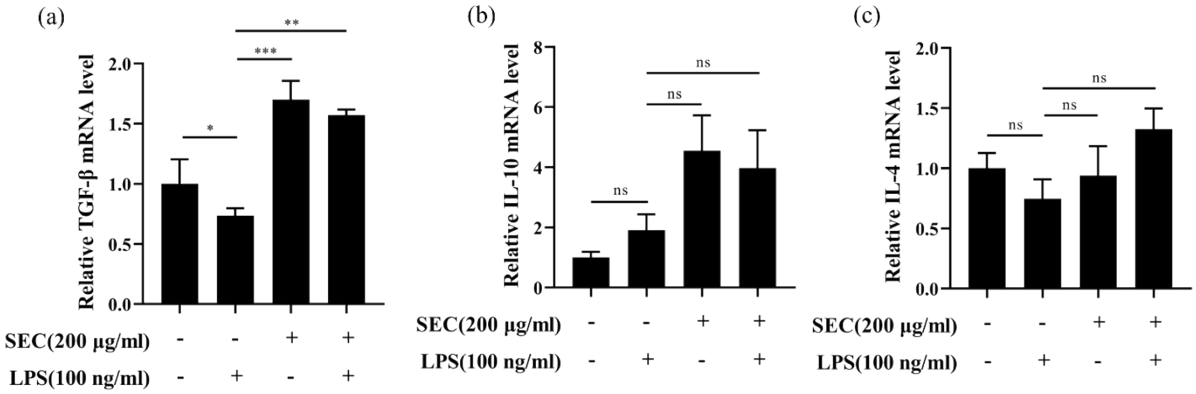


Figure S25. Transcription level of anti-inflammatory factors TGF-β, IL10, and IL4 by RT-PCR. After BV2 cells were treated with different concentrations of SEC for 24 hours, LPS(100 ng/ml) prepared with free-FBS DMEM was incubated with BV2 cells, the transcription level were detected by RT-PCR (a) TGF-β, (b) IL-10 and (c) IL-4.

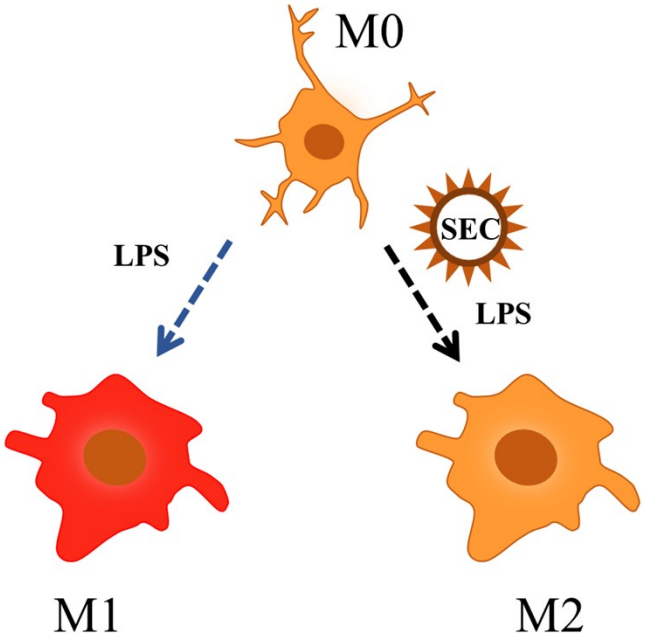


Figure S26. Schematic representation of the effect of SEC and LPS on the phenotype of microglia.

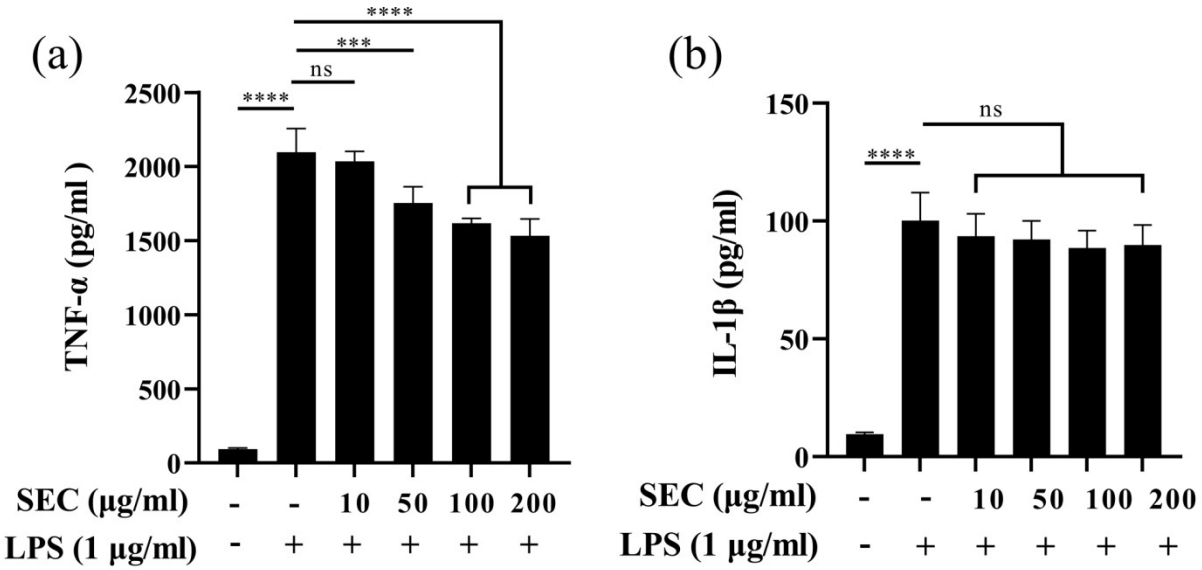


Figure S27. Cytokines secreted by RAW264.7 cells in the presence of SEC and LPS. (a) TNF-α, (b) IL-1β.

References

1. F. S. Li, P. Phyo, J. Jacobowitz, M. Hong and J. K. Weng, Nature Plants, 2019, 5, 41-46.

Author Contributions

Mengwei Li: Data curation, Writing of original draft. Banglian Hu: Data curation. Zhaojie Wu**:** Validation. Ziwei Wang: Data curation. Jian Weng: Conceptualization, Methodology. Honghua Zheng: Funding acquisition, Supervision. Liping Sun: Project administration, Funding acquisition, Supervision, Writing - review & editing.