Supporting information

Neuroinflammatory response on a Newly Combinatorial Cell-cell Interactions Chip

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(1) Optimization of Coating Conditions for Adherent Cell Culture

To create a more suitable microenvironment on the chip to support the growth of adjacent cells, we systematically explored optimal coating conditions. Various coatings, including Matrigel (0-100 μg/mL), type I collagen (0-100 μg/mL), fibronectin (0-400 μg/mL), poly-L-lysine (0.1 mg/mL), and Laminin-521 (100 μg/mL), were individually selected. Additionally, glass surfaces were subjected to specialized treatments. The selection of optimal coating conditions for fibronectin, Matrigel, and type I collagen (COLL I) was based on assessing cell viability through the cultivation of SH-SY5Y cells. For the remaining coating conditions, the recommended concentrations were adopted based on information available on the official website. With the exception of poly-lysine coated culture plates, which were incubated for 15 min, the other coated reagents were allowed to incubate overnight to ensure proper coating. Cells were seeded at a concentration of 2×10^4 cells/mL. Subsequently, the viability of the cells was determined using the CCK-8 assay.

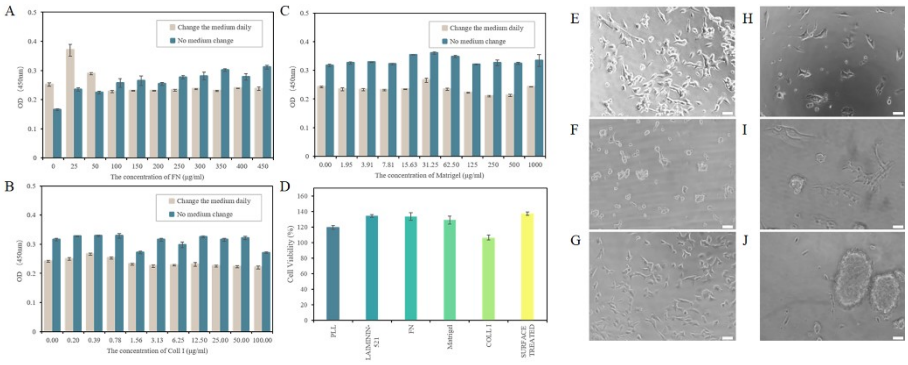

Figure S1 Optimization of coating conditions for walled cell culture (scale bar 50 μm). Cell activity CCK-8 test with different concentrations of FN coating conditions. (B)

Cell activity CCK-8 test with different concentrations of type I collagen coating. (C) Cellular activity CCK-8 test under different Matrigel coating conditions. (D) Cellular activity CCK-8 test with different coating conditions and optimal concentrations. (E~J) are hydrophilic treatment of the chip, GelMA, FN, Matrigel, Laminin-521, and PEGDA.

(2) Optimization of Staining Conditions

To accurately assess cellular activity while maintaining the integrity of the cellular membrane, it was essential to employ a staining approach that retained the intracellular CFDA without extracellular release. Thus, CFDA was chosen as the staining agent for THP-1 cells, allowing the assessment of cellular activity through its staining effect. After a comprehensive investigation, CFDA-SE emerged as a suitable cell staining reagent, commonly employed for fluorescently labeling live immune cells. To facilitate the visualization of THP-1 cell migration, we meticulously optimized the staining conditions using CFDA-SE (Figure 3-C). Ultimately, a staining concentration of 5 μM, a single wash with PBS, and a staining duration of 1 hour were identified as the optimal conditions. This specific set of conditions was selected due to its minimal impact on the cellular morphology of THP-1 cells while delivering efficient and distinct staining outcomes. It is important to highlight the significance of thorough liquid removal during the PBS washing step to ensure the validity of results.

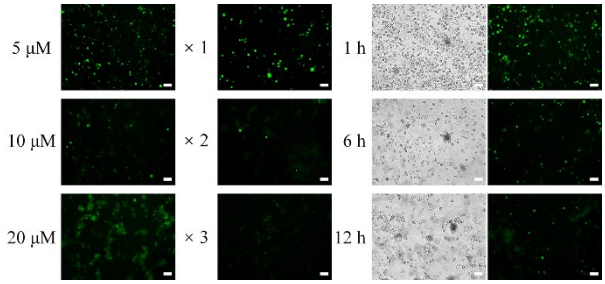

Figure S2 Optimization of CFDA-SE staining conditions for THP-1 live cell labeling (scale bar 100 μm).

Video 1 Video of cell migration near the chamber end at the microfence structure of the SH-SY5Y cell culture chamber (+) in the LPS-Go group (Video compressed from 36 min to 1 min) (scale bar: 20 μm).

Video 2 Video of cell migration away from the chamber end at the microfence structure of the SH-SY5Y cell culture chamber (++) in the LPS-Go group (Video compressed from 36 min to 1 min) (scale bar: 20 μm).