Supplementary information

A microfluidic dermal fibroblast–macrophage co-culture on a chip linking inflammatory signalling to barrier-associated function

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Table S1. Photomask Design Parameters of the Dermal Fibroblast–Macrophage-On-A-Chip Platform Device

Detailed geometric specifications of the photomask layout used for SU-8 master fabrication, including channel widths, micropillar dimensions, pillar spacing, and reservoir geometry. Dimensions are provided in micrometers (μm) and represent design values prior to PDMS replication.

Microchanel	**Size (μm)**
Medium	497.05 ± 3.61
Hydrogel	250.28 ± 4.48
Pillar gap	93.43 ± 3.04

Table S2. Measured Microchannel Dimensions of Fabricated PDMS Devices

Quantitative measurements of channel width, height, hydrogel lane width, and micropillar gap obtained from inverted microscopy and ImageJ analysis. Data are presented as mean ± SD (N = 3 devices), confirming fabrication fidelity relative to photomask specifications.

Microchanel	**Size (µm)**
Medium	500.40 ± 6.90
Hydrogel	250.21 ± 4.99
Pillar gap	105.08 ± 12.22
Height	217.34 ± 7.89

Table S3. Composition and Concentration of Plai–Black Ginger Nanostructured Lipid Carrier (Plai-BG NP) Formulations

Formulation details of nanostructured lipid carriers used in anti-inflammatory screening, including concentration of Plai oil, Black Ginger extract, lipid components, surfactants, and final working concentrations (NP-1x, NP-2x, NP-3x) applied in dermal fibroblast–macrophage co-culture on-chip experiments.

Formulation	Concentration of NP (mg/mL)
NP-1x	0.2
NP-2x	0.4
NP-3x	0.6

Table S4. Nitric Oxide Inhibition in 2D RAW 264.7 Monoculture

Quantitative comparison of nitric oxide (NO_2^-) production in conventional 2D macrophage monoculture following LPS stimulation and treatment with Plai-BG NP or dexamethasone. Data are presented as mean ± SD (N = 3), with calculated IC_{50} values.

Conditions	**%NO inhibition** **24h**
DEX 40 µg/mL	77.95 ±2.19
Plai-BG NP-1x	33.87±0.47
Plai-BG NP-2x	71.41±0.84
Plai-BG NP-3x	100±1.25

Table S5. Nitric Oxide Inhibition in 3D Transwell Co-culture Model

Nitric oxide inhibition measured in a 3D transwell-based macrophage–fibroblast co-culture system at 6 h and 24 h post-treatment. Results are expressed as percentage inhibition relative to LPS control (mean ± SD, N = 3).

Conditions	%NO inhibition 6h	%NO inhibition 24h
DEX 40 µg/mL	3.07 ±7.31	12.86 ± 3.62
Plai-BG NP-2x	7.12 ± 6.25	12.06 ± 2.06

First, transwell inserts (0.4 µm pore size) were coated with 0.2 mg/mL Matrigel to promote gel adhesion. CRL-2522 fibroblasts were trypsinized and seeded at 1×10^5 cells/well into a neutralized collagen matrix composed of 2 mg/mL bovine type I collagen, 10X PBS, distilled water, and 1N NaOH. The constructs were allowed to polymerize and maintained with daily medium refreshment. On day 5, RAW 264.7 macrophages were seeded into the lower compartment at 4×10^5 cells/well to establish dermal–immune interaction. On day 6, inflammation was induced by adding 1 µg/mL LPS (in 1,000 µL DMEM) to the lower well. Plai-BG NP (NP-2x concentration) was administered into the upper (gel) compartment at a final volume of 200 µL. Treated and control groups were incubated for 6–24 hours, and conditioned media were collected from the basal well at 6 h, and 24 h post-treatment for nitric oxide quantification via the Griess assay.

Table S6. Mono-culture control analysis of TNF-α inflammatory response in RAW 264.7 macrophages and dermal fibroblasts in the microfluidic dermal-on-chip system. Data are presented as mean ± SD (N = 3)

Condition	TNF alpha (pg/mL) 6h	SD
MQ mono-culute	139.52	0.75
Fibroblast mono-culute	32.36	1.63

Figure S1. Negative control for fibronectin immunostaining. (A) Top-view image of CRL-2522 fibroblasts in the reservoir processed without the primary anti-fibronectin antibody. (B) Cross-sectional view of the fibroblast layer under identical conditions. Scale bars: 50 μm (top view), 20 μm (cross-section).

Samples were processed without the primary anti-fibronectin antibody but incubated with Alexa Fluor 488-conjugated secondary antibody under identical imaging conditions. Minimal green fluorescence was observed, confirming low nonspecific background signal in the fibronectin detection channel.