

Methods Collection

1. Characterization and preparation of MWCNTs and C60F

- MWCNTs (Mitsui-7) and C60F (Sigma Aldrich) were prepared in a dispersion medium (DM) following a two-step dispersion procedure.
- Transmission electron microscopy (TEM) was used to characterize MWCNTs and C60F.
- Field emission scanning electron microscopy (FESEM) was used to examine morphology of C60F.

2. Animals and treatment

- Six-week old male B6C3F1 mice (Jackson Laboratory), 10 mice per treatment at each timepoint, were treated with a single dose of 50 μ l of DM, MWCNTs (40 μ g/mouse), or C60F (640 or 1,280 μ g/mouse) in suspensions by pharyngeal aspiration.
- At day 1, 7, or 28 post-exposure, the mice were euthanized for molecular, immunologic, and pathological examinations.

3. Macrophage culture, polarization, and treatment

- J774A.1 murine monocyte/macrophage cells were grown in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum.
- M1 polarization was induced with interferon γ (20 ng/ml) plus lipopolysaccharides (100 ng/ml) and M2 polarization was induced with interleukin 4 (20 ng/ml) for indicated time (typically three days).
- Polarized M1 or M2-macrophages were treated with DM or MWCNTs (2.5 μ g/ml) for indicated time (i.e., 1, 2, or 3 days post-polarization).

4. Whole lung lavage (WLL) preparation

- WLL was performed through the cannula using ice cold Ca^{2+} - and Mg^{2+} -free phosphate buffered saline, pH 7.4.
- Total WLL cell counts were obtained using a Coulter Multisizer 3 and cell differentials were determined by LSR II flow cytometry.

5. Enhanced darkfield microscopy

- Enhanced darkfield microscope used to visualize MWCNTs and their deposition in lung tissue.
- Enhanced darkfield images were taken using an Olympus DP-73 digital camera.

6. Histopathology

- The lung was removed, fixed with 10 % neutral buffered formalin, and embedded in paraffin.
- Sections of 5 μ m thickness were subjected to hematoxylin and eosin (H&E) staining or Picrosirius red staining.

7. Flow cytometry for immune cell profiling

- Cells populations from WLL fluids were gated using LSR II flow cytometer.
- Data were analyzed using FlowJo software.

8. Cytokine measurement

- Measurement of cytokines (IFN- γ , IL-1 β , IL-6, TNF- α , IL-4, IL-13, and IL-10) in WLL fluids and lung tissue extract was performed by multiplex immunoassay using ProcartaPlex

mouse cytokine/chemokine 36-plex kit on a Luminex 200 instrument system equipped with xPONENT software.

9. Enzyme-linked immunosorbent assay (ELISA)

- Production of proinflammatory cytokines (LTB₄, PGE₂) or SPMs (RvD1, LXA₄, RvE1) in WLL fluids were measured using ELISA kits with a microplate reader equipped with SOFTmax PRO 4.0.

10. Immunofluorescent staining and confocal microscopy

- To detect cellular expression and localization of F4/80, CD68, CD206, ALOX5AP, or ALOX15, immunofluorescent staining was performed in formalin-fixed, paraffin-embedded lung tissue sections (5 µm).
- Images were taken using a Zeiss LSM 780 confocal microscope and captured microscopic images were analyzed using the ImageJ software.

11. Immunoblotting

- Detection and quantification of CD68, CD86, CD163, CD206, ALOX5, ALOX15, ALOX5AP, or β-actin.
- Images were scanned using HP scanjet and were used to quantify each band with ImageJ software.