Online Supplementary Materials

Novel Murine Model of Chronic Granulomatous Lung Inflammation Elicited by Carbon Nanotubes.

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Light Scattering: Specifically, the hydrodynamic size distributions of the MWCNT suspensions were measured using a Dynamic light scattering (DLS) device (Nanosizer S90, Malvern Instruments). The surface charges (and therefore stability) of the MWCNT suspension was determined using a zeta potential device (Zeta ZS, Malvern Instruments). The electrophoretic light scattering (ELS) method has been used to quantify the degree of dispersion of MWCNTs in various suspensions in terms of their Zeta potential (1, 2). Both DLS and ELS methods are complementary and thus when combined can provided a comprehensive evaluation of dispersivity through DLS and dispersion stability of nanotubes through ELS (3). We dispersed MWCNTs in a saline solution containing 35% surfactant (Infrasurf, a gift of ONY, Inc., Amherst, NY) at 2 µg/µl and the mixture was bath-sonicated (1510R-MTH, Branson Ultrasonics Corp.) for 45 min to obtain a suspension. DLS studies revealed an average hydrodynamic size of 850 ± 20 nm (Supplementary Figure 1). Further evaluation of the suspension showed a reduced MWCNT hydrodynamic size of 160 ± 20 nm after centrifuging the MWCNT suspension for 2 min (6,772g RCF) in the supernatant. The hydrodynamic size obtained using the DLS method was also found to concur with an optical microscope (Olympus IC 100x) images of the MWCNT suspension which also included larger aggregates greater than 10µm. (Supplementary Figure 2).

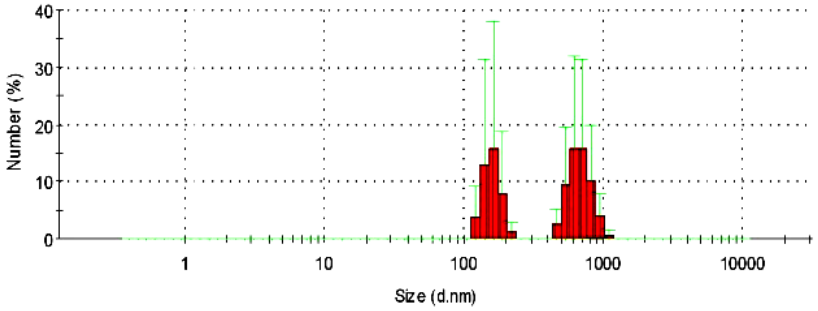
The surface charge of the MWCNT suspension was measured using a zeta potential device (Malvern Instruments). The zeta potential and isoelectric point (IEP) are primary indicators for describing the surface charge and stability of nanotube suspension. The MWCNT suspension displayed a zeta potential of -46.3 mV at neutral pH, suggesting a stable colloidal state of the MWCNT (left panel Supplementary Figure 4. The zeta potential of the MWCNT suspension remained negative across the range of neutral to acidic pH, with IEP of the sample identified at slightly below pH2 (arrow in right panel of Supplementray Figure 3). These results suggest that the suspension of the MWCNT is dispersed and relatively stable until solution pH fall below 4.

Reference List

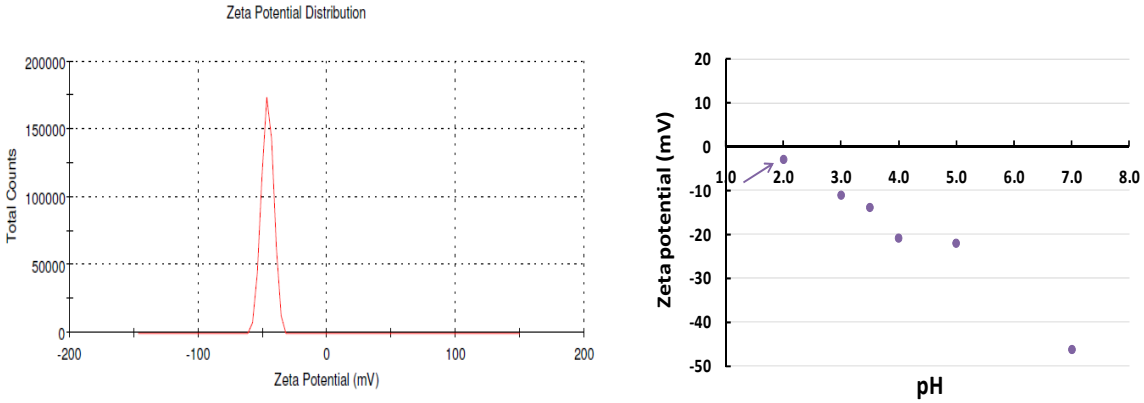
1. **Zhao L, Gao L. Stability of Multi-Walled Carbon Nanotubes Dispersion With Copolymer in Ethanol. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 2003;224:127-134.**

2. **Jiang L, Gao L, Sun J. Production of Aqueous Colloidal Dispersions of Carbon Nanotubes. *Journal of Colloid and Interface Science* 2003;260:89-94.**

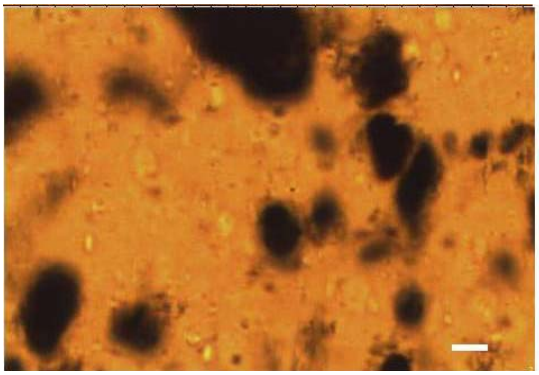
3. **Lee JY, Kim JS, An KH, Lee K, Kim DY, Bae DJ, Lee YH. Electrophoretic and Dynamic Light Scattering in Evaluating Dispersion and Size Distribution of Single-Walled Carbon Nanotubes. *J Nanosci Nanotechnol* 2005;5:1045-1049.**



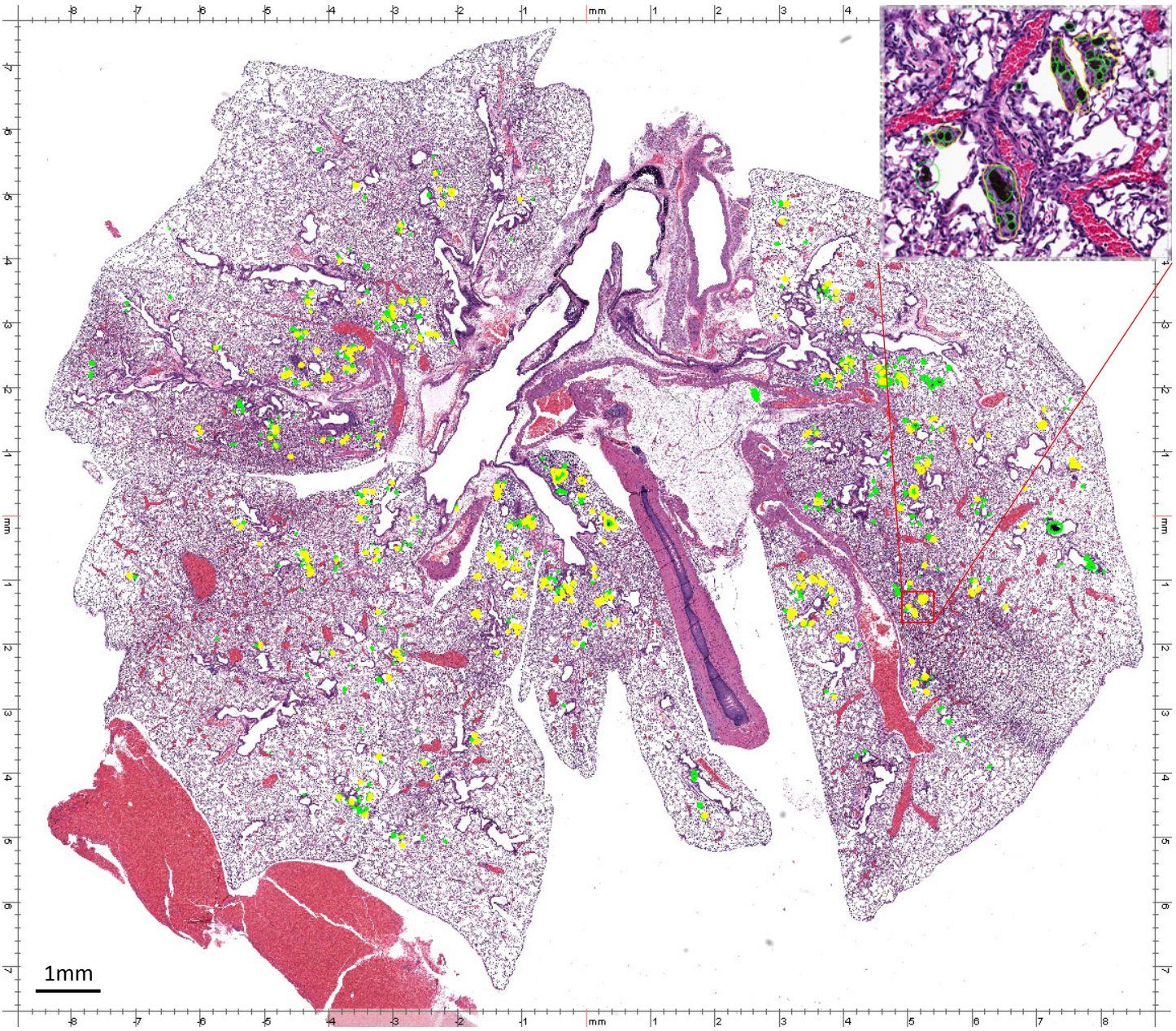
Supplementary Figure 1. Hydrodynamic size showing the bimodal distribution for the SES MWCNT agglomerates in 35% surfactant/ saline suspension.



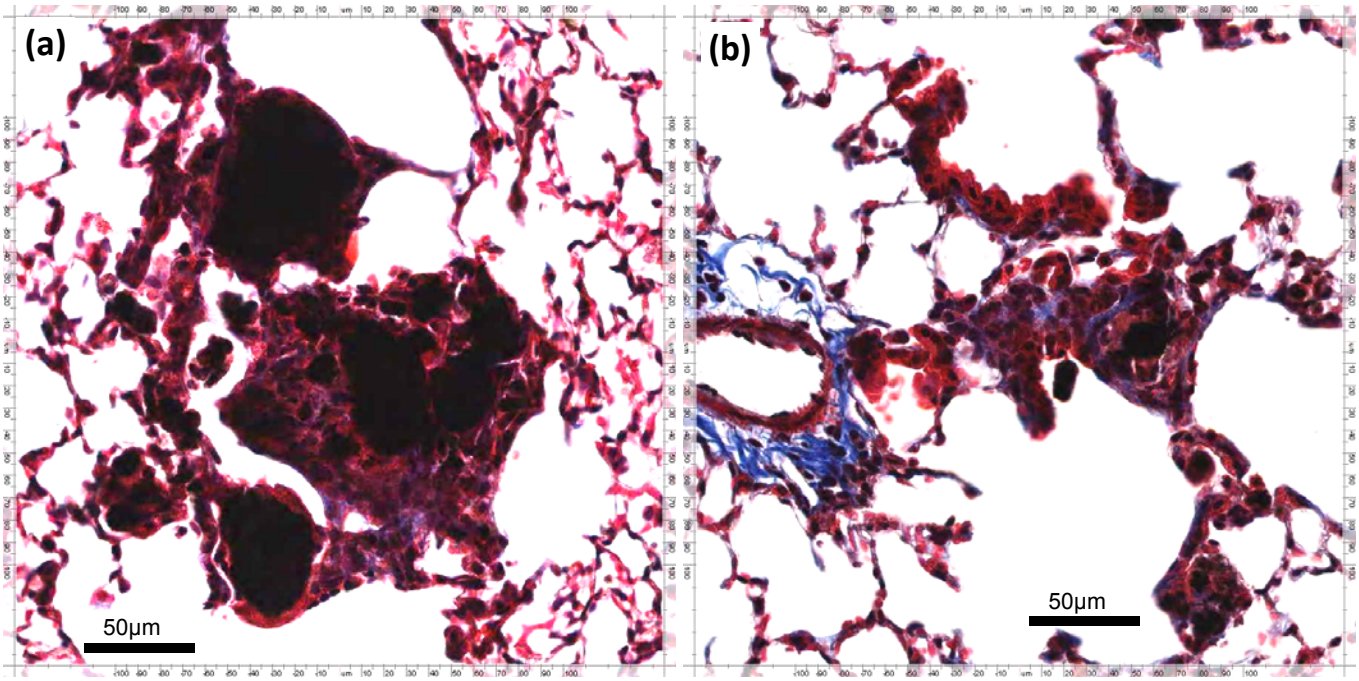
Supplementary Figure 2. Zeta potential and IEP value for the MWCNT suspension in 35% surfactant /saline. Left panel: Zeta potential in saline surfactant suspension reveal a single peak at -46.3 with a deviation of 4.6. Right panel plots the Zeta potential measurements made at pH values of 2, 3, 3.5, 4, 5, and 7 for determination of the suspensions stability. The arrow indicates the IEP of slightly less than a pH of 2 for the suspension.



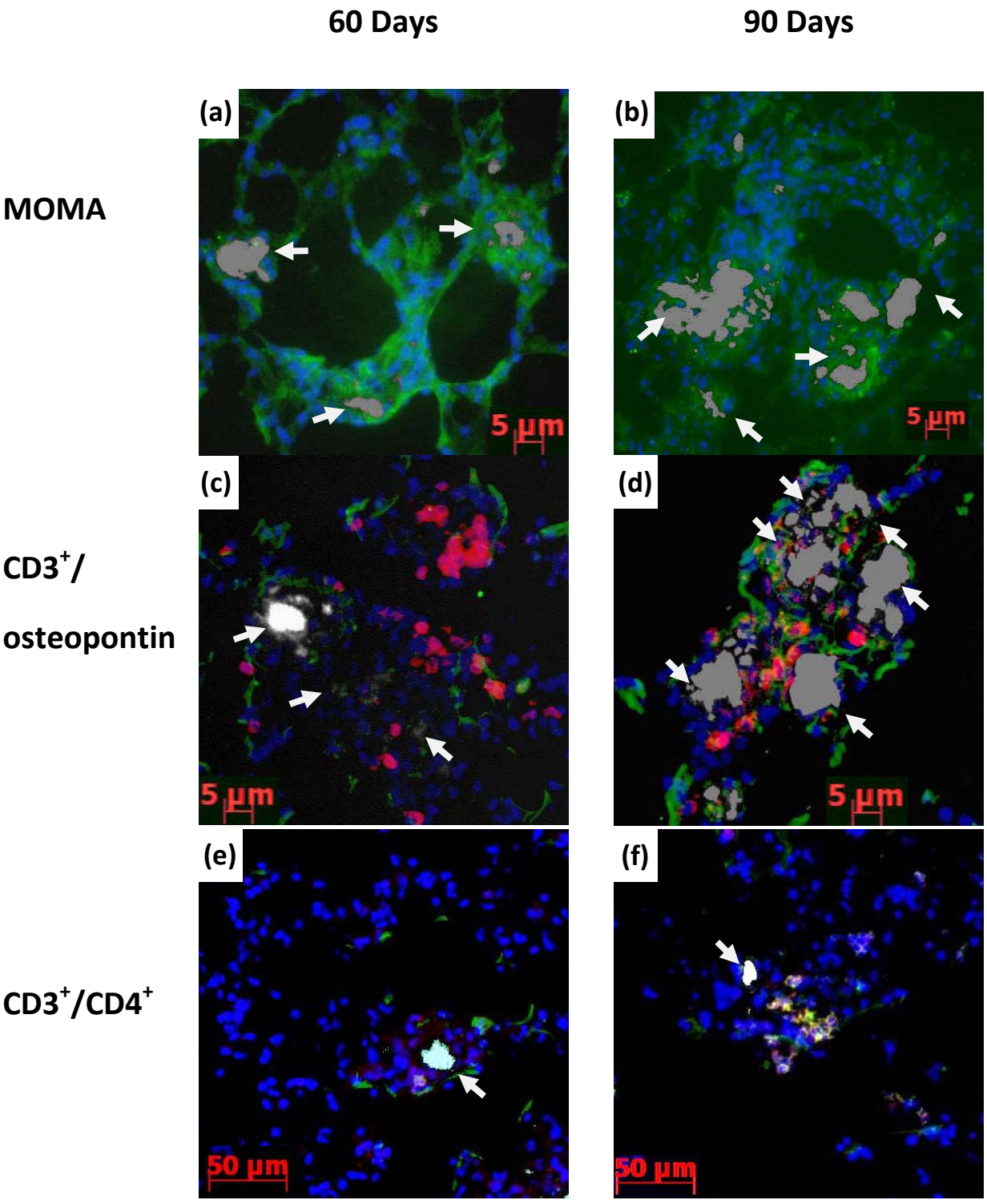
Supplementary Figure 3: Typical optical microscope image of SES MWCNT suspension in 35% surfactant /saline. Scale bars = 10 μm.



Supplementary Figure 4. Representative section of lung tissue at 60 days post MWCNT exposure. Low magnification(0.5x H&E stained). Multiple dispersed MWCNT aggregates (green)can be appreciated with surrounding granulomas (yellow). Right upper corner reveals multiple MWCNT aggregates surrounded by granulomatous reaction (25x).



Supplementary Figure 5. Low magnification(1x) of trichrome stained lungs at 60 and 90 days post MWCNT exposure. (a) 60 days (b) 90 days post MWCNT exposure



Supplementary Figure 6. . Immunohistochemistry with inverted images of brightfield over imposed to facilitate the visualization of MWCNT. Macrophages (MOMA-green) demonstrating surrounding MWCNT aggregates. (a) 60 days and (b) 90 days post MWCNT exposure. Immunohistochemistry with inverted images of brightfield over imposed to facilitate the visualization of MWCNT. $CD3^+$ T cells (red) and osteopontin (green) found within granulomatous lesions of murine lung post MWCNT exposure; (c) at 60 days and (d) 90 days post MWCNT exposure. $CD3^+$ T cells (red) and $CD4^+$ (green) in murine lung post MWCNT exposure; (e) at 60 days and (f) 90 days post MWCNT exposure. White arrows indicate location of MWCNT aggregates.