Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Material

S1 Collection of CBNs

CBNs from a tire manufacturing plant in central Taiwan were collected on Zefluor after-filter (Pall Life Sciences, 1.0 μm pore) using a micro-orifice uniform deposited impactor (MOUDI) (model-110 ; MSP Corporation, Minneapolis, MN, USA). The sampling flow rate for the MOUDI was 30 L/min. For each sample, the collected air volume approximated 43.2 m^3/day. Sampling was conducted for five working days per week during March and April (for a total of 20 days), 2016. Before and after each sampling, the filters were equilibrated in a dust-free desiccator (RH=60%; temperature=25°C) for 24h. An electrical balance with a sensitivity of 10 μg was used to weigh each filter.

S2 Calculation of the daily CBs alveolar deposition dose

The daily CBs alveolar deposition dose was determined as previously described with modification [1]. The human daily CBs alveolar deposition dose was calculated using the following equation:

$$Human\ \ alveolar\ \ deposition\ \ dose =$$

$$CB\ \ aerosol\ \ concentration \times V_E \times exposure\ \ duration \times alveolar\ \ deposition\ \ efficiency =$$

$$CB\ \ aerosol\ \ concentration \times (10\frac{\mathrm{L}}{\min}) \times (10^{-3}\frac{m^3}{L}) \times (8\frac{hr}{d}) \times (60\frac{\min}{h}) \times 50\%$$

where VE is the respiratory volume/min. For nanoparticles, the alveolar deposition efficiency was assumes as 50% [2]. The human daily CBs alveolar deposition dose was estimated as 112.83 μg/day. For nanoparticles, the alveolar retain efficiency was assumes as 60% [3]. The human daily CBs exposure dose in bloodstream was estimated as 45.13 μg/day.

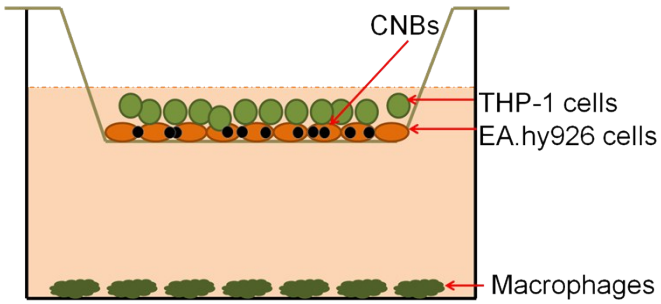

Fig. S1 Co-culture of EA.hy926 cells, THP-1 cells and macrophages to assay the trans-migration of THP-1 cells. EA.hy926 cells were firstly treated with CBNs and ROCK inhibitor Y-27632 for 24 h. Macrophages, which were seeded on the lower chamber, were used to secrete chemokines towards THP-1 cells. Finally, THP-1 cells were added to carry out the trans-migration assay.

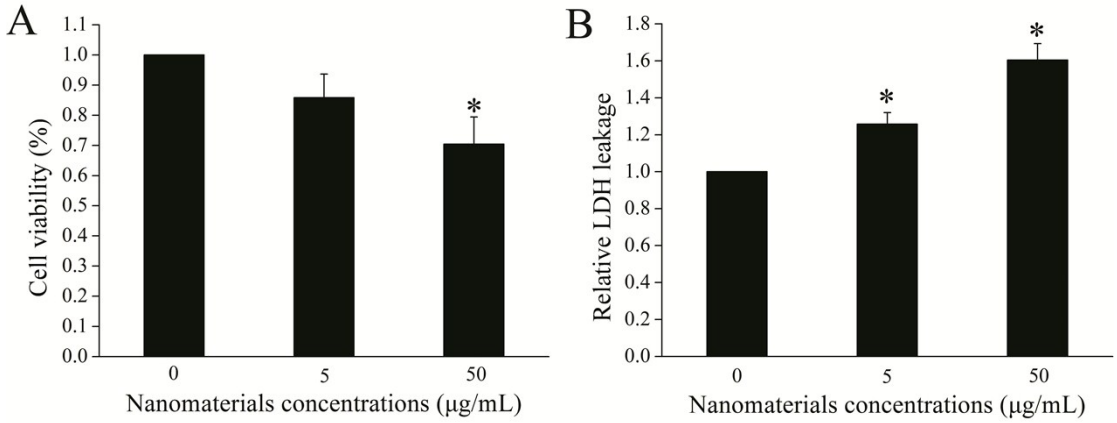

Fig. S2 Cell viability (A) and cell membrane integrity (B) of EA.hy926 cells after exposure to different concentrations of CBNs.*: $p < 0.05$ and **: $p < 0.01$ compared to the control (cells treated without CBNs).

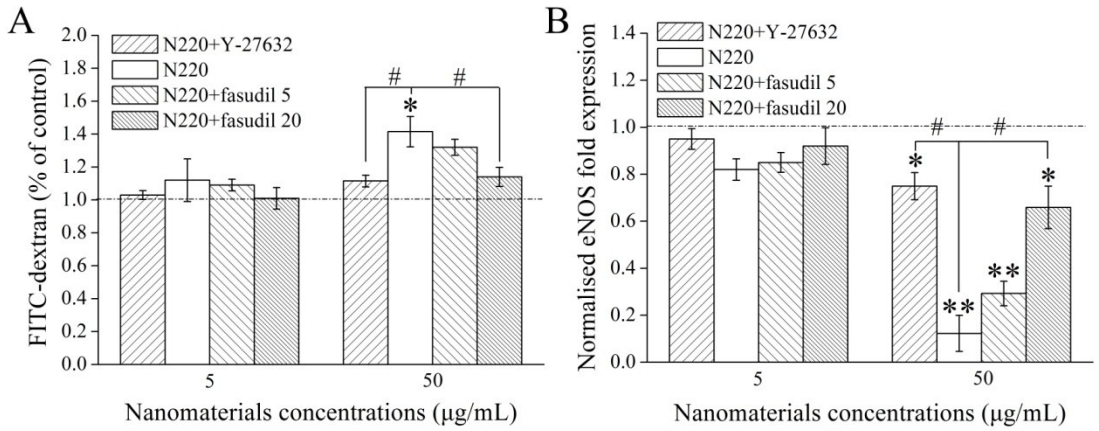

Fig. S3 Effects of Y-27632 (5 μM) and fasudil (5 and 20 μM) on CBNs treated endothelial cells. (A) Quantification of FITC-dextran in the lower chamber after treating EA.hy926 cells monolayer with CBNs and ROCK inhibitors. (B) The gene expression of eNOS in EA.hy926 cells treated with CBNs and/or ROCK inhibitors. *$p < 0.05$ and **$p < 0.01$ compared to the control. #$p < 0.05$ compared to cells treated with CBNs.

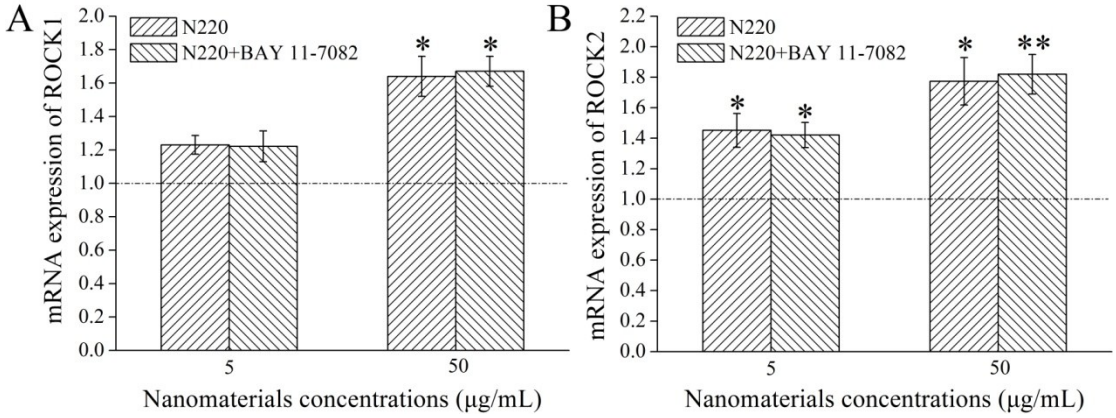

Fig. S4 Effects of NF-κB inhibitor (BAY 11-7082, 10 μM) on gene expression of ROCK1 (A) and ROCK2 (B) in CBNs treated endothelial cells. *$p < 0.05$ and **$p < 0.01$ compared to the control.

Table S1 Concentrations of elemental metals in N220 CBNs.

Metals	N220 (ng/mg)
Al	12.01±4.77
Cd	1.22±1.01
Co	0.25±0.09
Cr	2.13±1.33
Cu	0.57±0.19
Fe	5.52±0.73
Ga	4.05±1.88
In	1.01±0.19
Mn	1.81±0.07
Ni	9.92±3.05
Pb	8.47±1.02
Sr	7.14±1.79
Zn	229.22±41.31
Total	273.32

Table S2 Primers used for the targets amplification in this study.

Gene symbol	Forward sequence (5'-3')	Reverse sequence (5'-3')
GAPDH	GAGTCAACGGATTTGGTCGT	TTCATTTTGGAGGGATCTCG
IL-6	GAACTCCTTCTCCACAAGCG	GAATCCAGATTGGAAGCATCC
eNOS	CCAGCTAGCCAAAGTCACCAT	GTCTCGGAGCCATACAGGATT
ICAM-1	CGACTGGACGAGAGGGATTG	TTATGACTGCGGCTGCTACC
MCP-1	GCAATCAATGCCCCAGTCAC	GTGGTCCATGGAATCCTGAA
ZO-1	CAGGCAGCTTTCTATCCCCAGA	AAACTTGCGTTCAAATGGTCGG
Claudin-1	CTCACAGAGAGGGGTCGTTG	ACTGTTAGCGGCAGTTTGGT
ROCK1	TTACTGACAGGGAAGTGAGGTT	AGGTAGTTGATTGCCAACGAAA
ROCK2	GGATGAAACAGGCATGGTACA	GGAAAACACCTACAGACCACC

1. Erdely A, Dahm M, Chen BT, Zeidler-Erdely PC, Fernback JE, Birch ME, Evans DE, Kashon ML, Deddens JA, Hulderman T, Bilgesu SA, Battelli L, Schwegler-Berry D, Leonard HD, McKinney W, Frazer DG, Antonini JM, Porter DW, Castranova V, Schubauer-Berigan MK. Carbon nanotube dosimetry: From workplace exposure assessment to inhalation toxicology. *Particle and fibre toxicology*. 2013;10:53
2. Sturm R. Nanotubes in the human respiratory tract - deposition modeling. *Z Med Phys*. 2015;25:135-145
3. Chen J, Tan M, Nemmar A, Song W, Dong M, Zhang G, Li Y. Quantification of extrapulmonary translocation of intratracheal-instilled particles in vivo in rats: Effect of lipopolysaccharide. *Toxicology*. 2006;222:195-201