Supporting Information for:

Titanium Dioxide (P25) Produces Reactive Oxygen Species in Immortalized Brain Microglia (BV2): Implications for Nanoparticle Neurotoxicity

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Table S1. Composition of HBSS*

Component	**Conc. (g/L)**	**M.W (g/mol)**	**Conc. (mM)**	**I (mM)**
HBSS				
INORGANIC SALTS				
$CaCl_2$ (anhyd)	0.14	111.0	1.26	*3.78*
$MgSO_4$ (anhyd)	0.098	120.4	0.81	*3.26*
KCl	0.4	74.6	5.37	5.37
KH_2PO_4 (anhyd)	0.06	136.1	0.44	0.44
$NaHCO_3$	0.35	84.0	4.17	4.17
NaCl	8.0	58.4	137	137
Na_2HPO_4 (anhydrous)	0.048	142.0	0.34	1.01
OTHER COMPONENTS				
D-Glucose	1.0	180.2	5.55	0.00
Total Ionic Strength				**155**

* Based on product literature from Mediatech, Inc.

Table S2. Composition of DMEM*

Component	Conc. (g/L)	M.W (g/mol)	Conc. (mM)	I (mM)
DMEM				
INORGANIC SALTS				
$CaCl_2$ (anhyd.)	0.20	111.0	1.8	*5.41*
$Fe(NO_3)3\text{-}9H_2O$	0.0001	403.9	0.0002	0.001
KCl	0.40	74.6	5.37	5.37
$MgSO_4$ (anhyd.)	0.01	120.4	0.08	*0.33*
NaCl	6.4	58.4	110	110
$NaHCO_3$	3.7	84.0	44.0	44.0
$NaH_2PO_4\text{-}H_2O$	0.125	138.0	0.91	0.91
OTHER COMPONENTS				
D-Glucose	4.5	180.2	25	0.00
Phenol Red•Na	0.015	376.4	0.04	0.04
Total Ionic Strength				**166**
AMINO ACIDS				
L-Arginine-HCl	0.084	210.7	0.4	
L-Cystine-2HCl	0.063	313.2	0.2	
L-Glutamine	0.584	146.2	4.0	
Glycine	0.030	75.1	0.4	
L-Histadine-HCl-H_2O	0.042	209.6	0.2	
L-Isoleucine	0.105	131.2	0.8	
L-Leucine	0.105	131.2	0.8	
L-Lysine-HCl	0.146	182.6	0.8	
L-Methionine	0.030	149.2	0.2	
L-Phenylalanine	0.066	165.2	0.4	
L-Serine	0.042	105.1	0.4	
L-Threonine	0.095	119.1	0.8	
L-Tryptophan	0.016	204.2	0.08	
L-Tyrosine-2Na-$2H_2O$	0.104	263.2	0.4	
L-Valine	0.094	117.2	0.8	
Total Amino Acids			**10.7**	
VITAMINS				
D-Ca pantothenate	0.004	476.5	0.008	
Choline Chloride	0.004	139.6	0.029	
Folic Acid	0.004	441.4	0.009	
i-Inositol	0.0072	180.2	0.040	
Niacinamide	0.004	122.1	0.033	
Pyridoxal-HCl	0.004	203.6	0.012	
Riboflavin	0.0004	376.4	0.001	
Thiamine-HCl	0.004	337.3	0.012	
Total Vitamins			**0.15**	

* Based on Product literature from Chemicon International.

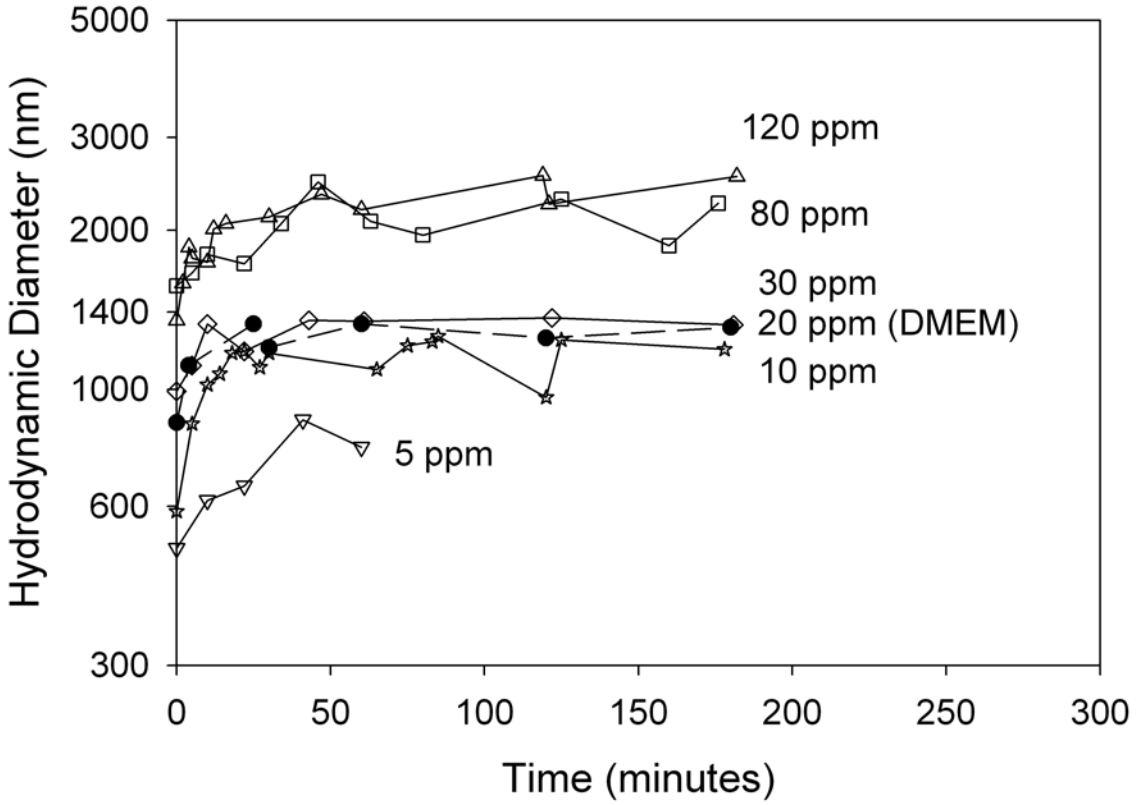

Figure S1. Particle aggregation over time at various concentrations of Degussa P25 TiO_2 in HBSS (open symbols) and in reduced serum DMEM (closed symbols). The steady state aggregate sizes are reported in Table 1.

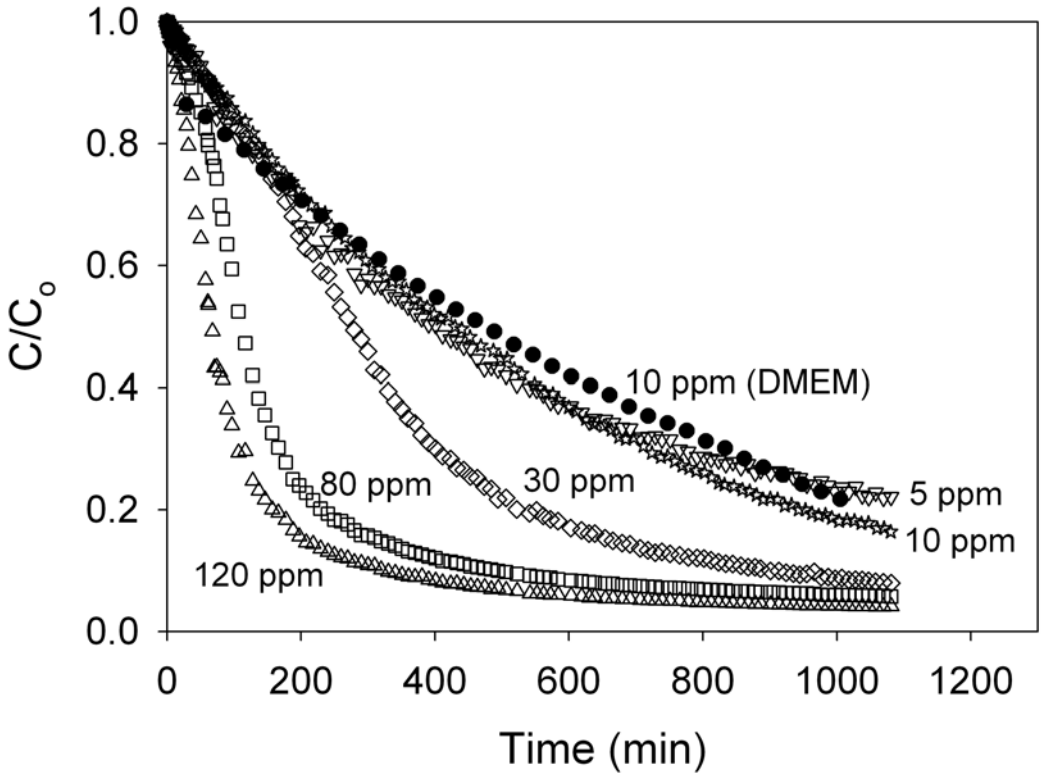

Figure S2. Degussa P25 sedimentation over 18 hours in HBSS or reduced serum DMEM at various concentrations. Suspensions were sonicated for 1 minute prior to introduction into a cuvette. Absorbance was linear over the range of concentrations at the wavelengths used (λ=508 nm or 450 nm).

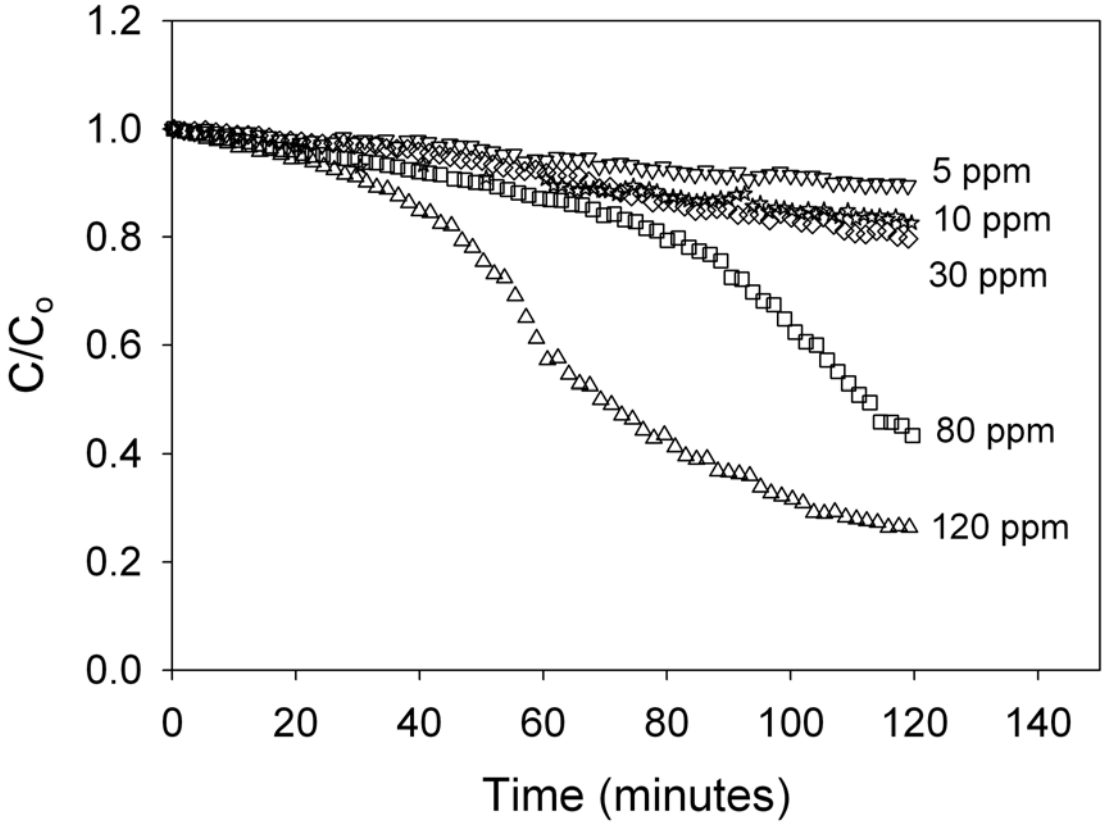

Figure S3. Degussa P25 sedimentation over 2 hours in HBSS at various concentrations. Suspensions were sonicated for 1 minute prior to introduction into a cuvette. Absorbance was linear over the range of concentrations at the wavelengths used (λ=508 nm or 450 nm).

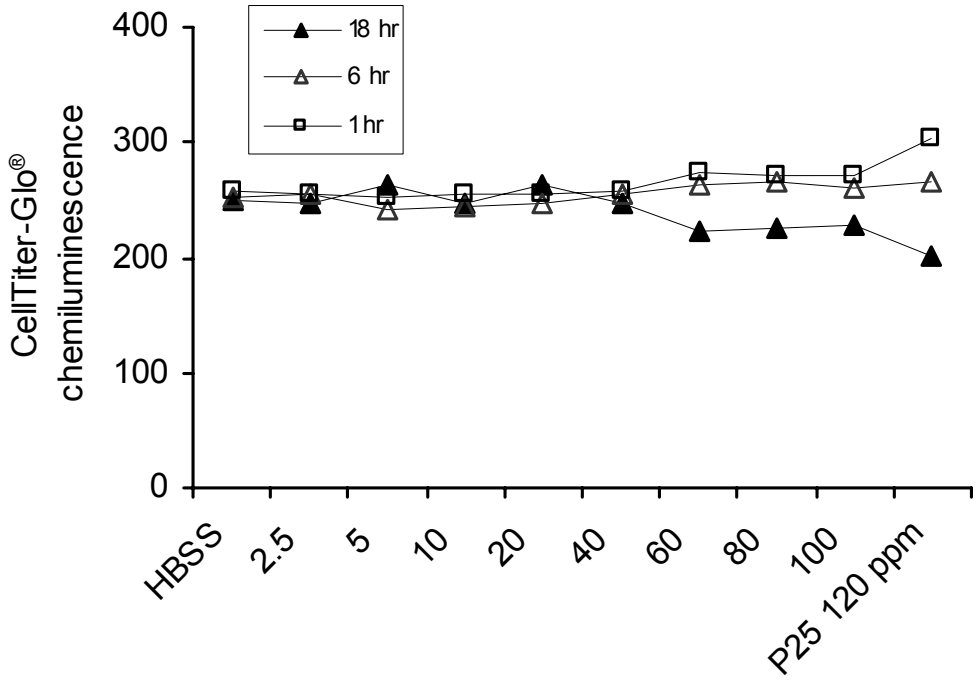

Figure S4, CellTiter-Glo® Chemiluminescence. Semi-confluent cultures of BV2 cells (~75%) were exposed to P25 (2.5-120ppm) for 18 hr. Intracellular levels of adenosine triphosphate (ATP) were measured as an index of cell viability using CellTiter-Glo® at 1, 6, and 18 hr post-exposure. ATP levels remained stable at all measured time points, indicating that all ROS and TEM measures were collected on viable microglia.