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Biocompatible Dispersion Methods for Carbon Black

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The biological activity of particles is largely dependent on their size in biological systems. Dispersion in the aqueous phase has been both a critical impediment to and a prerequisite for particle studies. Carbon black has been used as a surrogate to investigate the biological effects of carbonaceous particles. Here, biocompatible methods were established to disperse carbon black into ultrafine and fine particles which are generally distinguished by the small size of 100 nm. Carbon black with a distinct particle size, N330 and N990 were suspended in blood plasma, cell culture media, Krebs-Ringer's solution (KR), or physiological salt solution (PSS). Large clumps were observed in all dispersion preparations; however, sonication improved dispersion - averaged particle sizes for N330 and N990 were 85.0 ± 42.9 and 112.4 ± 67.9 nm, respectively, in plasma; the corresponding sizes in culture media were 84.8 ± 38.4 and 164.1 ± 77.8 nm. However, sonication was not enough to disperse N330 less than 100 nm in either KR or PSS. Application of Tween 80 along with sonication reduced the size of N330 to less than 100 nm, and dispersed N990 larger than 100 nm (73.6 ± 28.8 and 80.1 ± 30.0 nm for N330 and 349.5 ± 161.8 and 399.8 ± 181.1 nm for N990 in KR and PSS, respectively). In contrast, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) exhibited little effect. Electron microscopy confirmed the typical aciniform structure of the carbon arrays; however, zeta potential measurement failed to explain the dispersibility of carbon black. The methods established in this study could disperse carbon black into ultrafine and fine particles, and may serve as a useful model for the study of particle toxicity, particularly size-related effects.

***Key words*:** Carbon black, Carbonaceous core, Particle dispersion, Biocompatible media, Ultrafine particles, Fine particles

INTRODUCTION

Engineered particles are receiving much attention with the rapid development of particle sciences. A growing number of engineered particles are being newly developed and used in the manufacturing industry (Castranova, 2011). Along with the application to biomedical sciences, safety issues surrounding their use is also attracting a great deal of attention (Ai *et al*., 2011). Health risk imposed by particle exposure has been widely investigated, including lung/pulmonary toxicity, cardiovascular effects, immunotoxicity, neurotoxicity and carcinogenicity (Xia *et al*., 2009; Boverhof and David, 2010). These are principally determined by diverse physicochemial characteristics of particles such as agglomeration/aggregation, particle size, specific surface area, surface charge, radical formation potential, and so on (Fubini *et al*., 2010; Madl and Pinkerton, 2009). Therefore, the characterization of particle properties is a prerequisite for toxicity studies.

Toxicity of particle is largely dependent on particle size (Brook *et al*., 2010; Kreyling *et al*., 2006; Sager and Castranova, 2009). Particles less than 10 mm are generally called fine particles, and particles with at least one dimension between 1 and 100 nm are called ultrafine particles or nanoparticles. Ultrafine particles exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials, and on the whole, 100 nm is regarded as a critical border to exhibit distinct properties overlapped between find and ultrafine particles (Stone *et al*., 2007; Nemmar *et al*., 2002). Ultrafine particles are known to impose higher health risks on living organisms than fine particles (Stone *et al*., 2007). This may be due to a characteristic high surface to volume ratio which makes the parti-

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cles very reactive or catalytic (Fubini *et al*., 2010). However, size dependency in particle toxicity remains largely unknown at this stage and is still controversial (Madl and Pinkerton, 2009).

Carbon black is a particulate form of pure elemental carbon that is produced by partial combustion or pyrolysis of gaseous or liquid hydrocarbons under controlled conditions (Valberg *et al*., 2006). Carbon black is an industrial chemical produced and consumed for diverse industrial purposes including reinforcement of rubber and coloring for dyestuffs (Baan, 2007). Because of its wide usage and high potential for exposure through inhalation, there is a high risk of occupational exposure in various industries (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 1996; Morfeld and McCunney, 2010). In addition, the daily use of toners and printing inks enhances the potential for carbon black exposure in an indoor homes or office environments (Morimoto *et al*., 2010; Yang *et al*., 2007). Most importantly, numerous studies have employed carbon black as a surrogate for the carbonaceous core of combustion particles or as a representative of particulate matter (PM) (Totlandsdal *et al*., 2010; Tankersley *et al*., 2004).

The nominal size of carbon black particles is typically in the range of 10~500 nm, and this wide range of size makes carbon black an attractive model compound to test the size-dependency of particle toxicity (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 1996; Totlandsdal *et al*., 2010; Tankersley *et al*., 2004). Carbon black is completely insoluble and tends to form clumps in aqueous solutions due to the structural development by aggregation and agglomeration (Gilmour *et al*., 2004; Porter *et al*., 2008). Thus, the actual size of carbon black may be larger in suspended conditions. Therefore, for its use as a model compound, it is important to establish the dispersion methods for dispersing carbon black to the intended size.

This study was designed and performed to establish the dispersion methods for carbon black with an emphasis on biocompatibility. With regard to biocompatibility, dispersion in aqueous solutions is of great importance in testing and understanding its biological effects, otherwise it cannot defines the exposure, absorption, delivery, and the consequent biological activity of carbon black in *in vivo* as well as *in vitro*. Carbon black particles of distinct sizes were dispersed in dispersion media, including blood plasma, cell culture media, and widely used biological buffer solutions. Dispersion conditions were fine tuned to make the averaged size of carbon black less than 100 nm and larger than 100 nm which corresponds to the size of ultrafine and fine particles, respectively. Additionally, size distribution, surface charge and morphology were analyzed in each dispersion condition.

MATERIALS AND METHODS

Materials. Carbon black including Corax® N330 and Thermax® floform N990 were purchased from Evonic Carbon Black Korea (Incheon, Korea) and Cancarb Ltd. (Medicine Hat, Alberta, Canada), respectively. Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were from Invitrogen (Carlsbad, CA, USA). Polyoxyethylene (20) sorbitan monooleate (polysorbate 80, Tween 80), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the highest purity available and purchased from standard suppliers.

Animal. All animal experiments were conducted in accordance with protocols approved by the Ethics Committee of Animal Service Center at Dongguk University. Male Sprague-Dawley rats at 6-weeks of age were purchased from Daehan Biolink (Eumseong, Korea), and acclimated for one week before the experiments. The laboratory animal facility was maintained at constant temperature and humidity with a 12-hr light/dark cycle. Food and water were provided *ad libitum*.

Suspension of carbon black. Carbon black was dispersed in Krebs-Ringer's solution (KR), physiological salt solution (PSS), cell culture media, or rat blood plasma. Information regarding the components of each dispersion

Table 1. Components of dispersion solution tested

Solution	Components
Krebs-Ringer solution (KR solution)	115.5 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 11.1 mM glucose; pH 7.4
Physiological salt solution (PSS)	140 mM NaCl, 5.0 mM KCl, 1.4 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10.0 mM HEPES, 5.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 11.5 mM glucose; pH 7.4
Cell culture media	10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin in Dulbecco's modified Eagle Medium[a]
Blood plasma	Rat blood plasma containing 0.32% sodium citrate

[a]Gibco® Invitrogen, Cat. No. 11965 (media formulation is downloadable at http://www.invitrogen.com/site/us/en/home/support/Product-Technical-Resources/media_formulation.8.html).

medium is presented in Table 1. Blood plasma was prepared as previously described using 3.2% sodium citrate as an anticoagulant (1 : 9) (Lee *et al*., 2002). In all cases, carbon black suspension was prepared at a concentration of 100 μg/ml, and 10 ml of carbon black dispersion was sonicated at 100 W for 1 or 5 min with a VCX130 sonicator (Sonics & Materials, Newtown, CT, USA).

Light microscopy. After carbon black suspension was prepared, a 20 μl sample was placed onto a clean microslide and covered. The samples were then viewed at 10 × or 40 × on an Eclipse TS100-F photomicroscope (Nikon, Tokyo, Japan). Images were acquired and analyzed with a DS-Fi1 CCD camera (Nikon) and a Meta Imaging System (Molecular Devices, West Chester, PA, USA).

Measurement of size distribution and surface charge. The particle size was analyzed with a NICOMP 380 Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA, USA). The surface charge was measured using a Zeta-Plus zeta potential analyzer (Brookhaven Instrument, Holtsville, NY, USA).

Electron microscopy. Dispersed carbon black particles were mounted on a Formvar film coating Cu grid (Electron Microscopy Sciences, Hatfield, PA, USA) and dried. The dispersed particles were examined by a JEM1400 transmission electron microscope (JEOL, Tokyo, Japan) operated at 120 KV acceleration voltage. Images were acquired with a Veleta CCD camera (Olympus, Tokyo, Japan) and analyzed with a Meta Imaging System (Molecular Devices).

Statistical analysis. The mean and standard deviation (SD) of the mean were calculated and presented for all experimental groups.

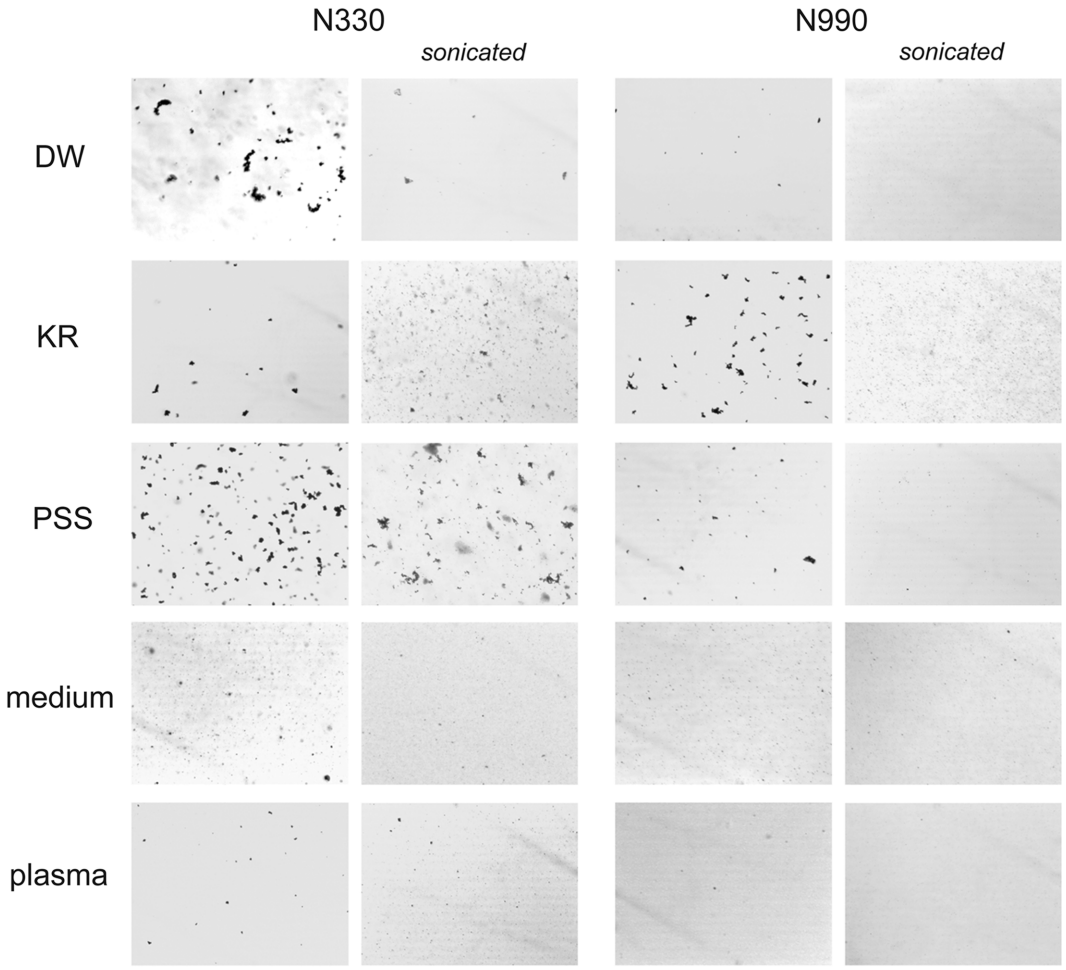

Fig. 1. Light microscopy evidence of improved dispersion of carbon black by sonication. Carbon black N330 and N990 were suspended in distilled water (DW) or dispersion media including Krebs-Ringer solution (KR), physiological salt solution (PSS), cell culture media, or blood plasma at a concentration of 100 μg/ml. Sonication was applied at 100 W for 1 min to enhance dispersibility (each right panel). Images were obtained using light microscopy at a magnification of 10 ×.

RESULTS

Carbon black particles were suspended in different media and were viewed using light microscopy (Fig. 1). Suspension of carbon black N330 had large agglomerates in all the media tested, including Krebs-Ringer solution (KR), physiological salt solution (PSS), cell culture media, and blood plasma, which was not too different from distilled water (DW) (Fig. 1, N330 left panels). To improve the dispersion of carbon black particles, physical ultrasound was applied to carbon black suspension. Sonication at 100 W for 1 min reduced clumps in all the suspensions, although large agglomerates were still observed in KR and PSS (Fig. 1, N330 right panels). Longer or stronger sonication than 1 min or 100 W did not result in seemingly better dispersion (data not shown). Similar results were obtained with carbon black N990 (Fig. 1, N990). Dispersion of N990 was more even in culture medium and blood plasma compared with N330, while it was not the case in KR and PSS. On the whole, sonication led to less agglomeration regardless of dispersion media. From these results, sonication appears to enhance the dispersibility of both carbon black N330 and N990, although it

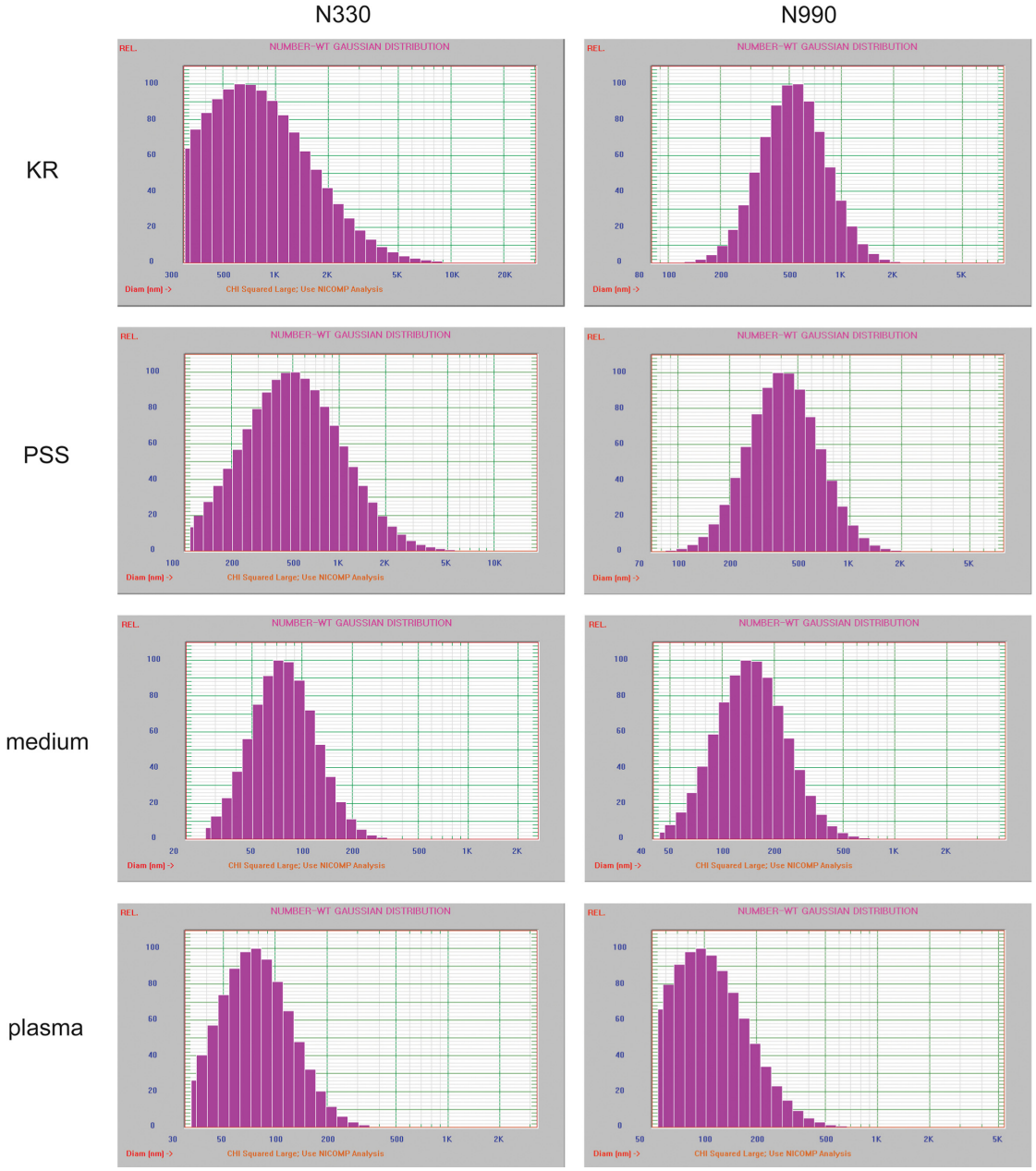

Fig. 2. The Gaussian distributions of particle size in carbon black dispersion. Carbon black N330 and N990 were suspended in dispersion media, and sonication was applied at 100 W for 1 min. The particle size was measured by dynamic light scattering.

is not clear if sonication is enough to disperse N330 in nano-size, less than 100 nm.

The particle size was measured by dynamic light scattering, and the gaussian distributions of particle size are presented in Fig. 2. The median values of N330 were lower than 100 nm in cell culture media and blood plasma, while those of N990 were higher than 100 nm. The averages of particle size were 84.8 ± 38.4 and 164.1 ± 77.8 nm for N330

Table 2. Actual particle sizes of N330 and N990 in each dispersed condition

Solution	Tween 80	N330 (nm)	N990 (nm)
Krebs-Ringer solution	–	936.9 ± 774.8	582.6 ± 254.6
(KR solution)[a]	+	73.6 ± 28.8	349.5 ± 161.8
Physiological salt solution	–	651.8 ± 490.1	460.4 ± 220.5
(PSS)[a]	+	80.1 ± 30.0	399.8 ± 181.1
Cell culture media[b]	–	84.8 ± 38.4	164.1 ± 77.8
Blood plasma[b]	–	85.0 ± 42.9	112.4 ± 67.9

[a]Tween 80 was added to carbon black suspension at a final concentration of 0.01%, and sonication was applied at 100 W for 5 min.
[b]The dispersion was sonicated at 100 W for 1 min.
Values are expressed as mean ± SD (n = 3).

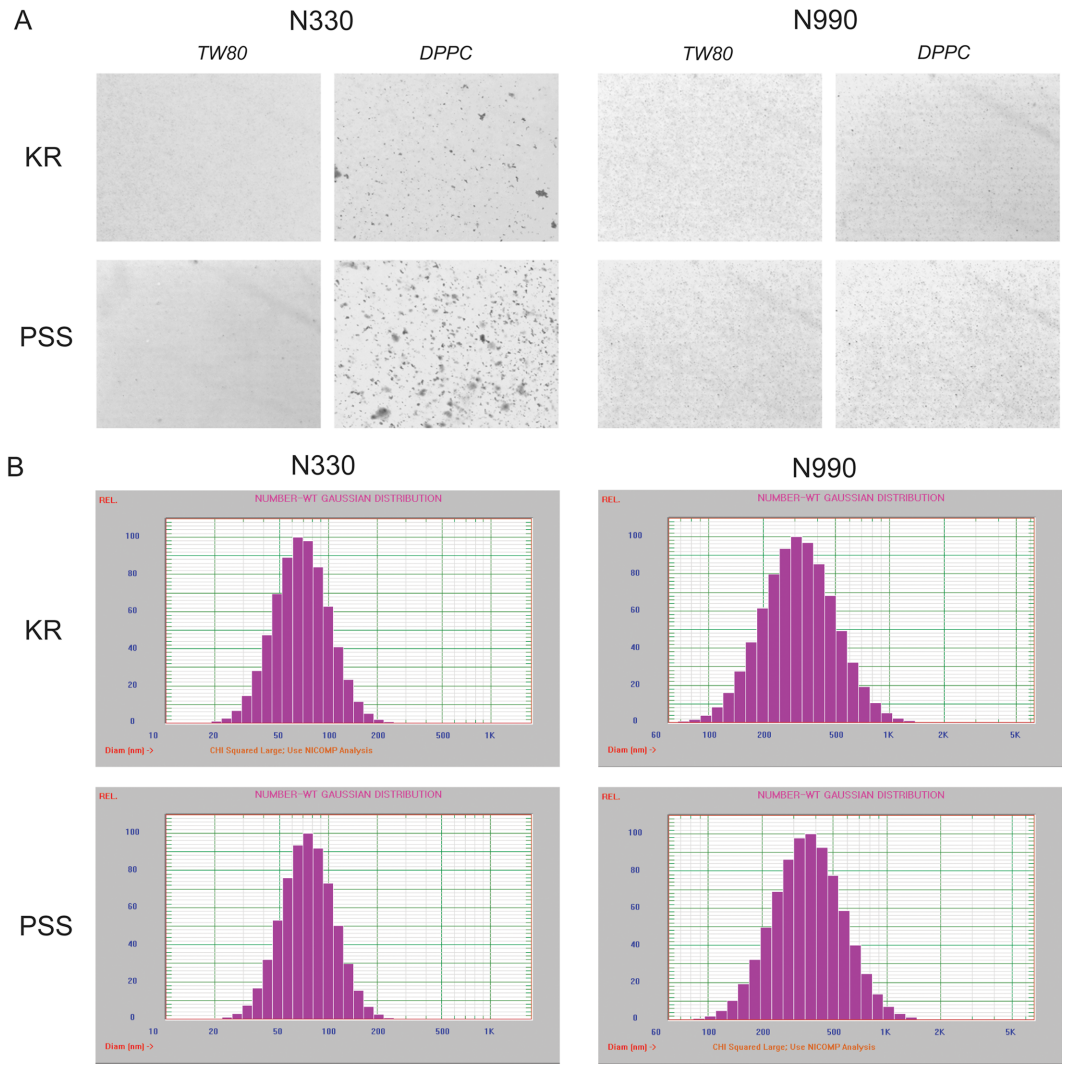

Fig. 3. Improvement of carbon black dispersion by surfactants. (A) Carbon black was suspended in KR or PSS containing 0.01% Tween 80 (TW80) or 10 μg/ml DPPC, and sonication was applied at 100 W for 5 min. Images were obtained using a light microscope at a magnification of 10 ×. (B) Distributions of the particle size are presented.

and N990 in culture media, respectively. In blood plasma, the averages were 85.0 ± 42.9 and 112.4 ± 67.9 nm for N330 and N990, respectively (Fig. 2 and Table 2). These results indicate that N330 and N990 can be dispersed to the size ranges of ultrafine and fine particles, respectively. However, both N330 and N990 exhibit sizes larger than 400 nm in KR and PSS. In KR, carbon black N330 and N990 measured 936.9 ± 774.8 and 582.6 ± 254.6 nm in diameter, respectively and they were 651.8 ± 490.1 nm and 460.4 ± 220.5 nm in PSS. These values are extremely large considering the nominal size of N330, which suggest that dispersion was not complete in KR and PSS, even compared with the dispersion in blood plasma or culture medium.

To disperse carbon black N300 in nano-size with KR and PSS, surfactants were employed in dispersion media in addition to sonication. Tween 80, a nonionic surfactant and an emulsifier, is one of the most frequently used detergent in nanoparticle dispersion. DPPC is a lipid surfactant and a component of bronchoalveola lavage (BAL) fluid, and thus is known as an endogenous surfactant. Carbon black was suspended in KR or PSS with or without 0.01% Tween 80 and 10 μg/ml DPPC, and then sonication was applied at 100 W for 5 min. As shown in Fig. 3A, the application of Tween 80 noticeably improved the dispersibility of both carbon black N330 and N990. However, DPPC failed to decrease large agglomerates. These results suggest that Tween 80 is efficient for dispersion of carbon black, while DPPC exhibited little, if any, effect on dispersion. Therefore, the actual size distribution was analyzed with Tween 80 by dynamic light scattering (Fig. 3B). The particle size of carbon black N330 was reduced from 936.9 ± 774.8 to 73.6 ± 28.8 nm in KR and from 651.8 ± 490.1 to 80.1 ± 30.0 nm in PSS by the application of Tween 80 (Fig. 3B and Table 2). The particle size of N990 was decreased from 582.6 ± 254.6 to 349.5 ± 161.8 nm and from 460.4 ± 220.5 to 399.8 ± 181.1 nm in KR and PSS, respectively. These results suggest that the dispersion of carbon black could be improved by the application of Tween 80.

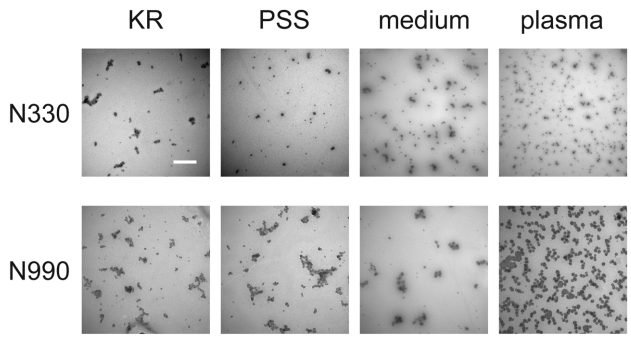

Fig. 4. Electron microscopic images of dispersed carbon black. Carbon black was suspended in each dispersion medium with (KR and PSS) or without (cell culture medium and blood plasma) 0.01% Tween 80. Each particle suspension was sonicated at 100 W for 1 (cell culture medium and blood plasma) or 5 min (KR and PSS). Individual carbon black was viewed using a transmission electron microscope (TEM) at 120 KV acceleration voltage. TEM micrographs were taken at a magnification of 5,000 × with a CCD camera. Scale bar = 300 nm.

Table 3. Surface charges of N330 and N990 in each dispersed condition

Solution	Carbon black type	Tween 80	Surface charge (mV)
Krebs-Ringer solution[a]	N330	–	–9.49 ± 2.30
		+	2.17 ± 0.77
	N990	–	–8.58 ± 3.87
		+	4.92 ± 0.98
Physiological salt solution[a]	N330	–	–10.32 ± 3.64
		+	1.50 ± 4.28
	N990	–	–9.30 ± 3.92
		+	1.88 ± 1.32
Cell culture media[b]	N330	–	–7.62 ± 4.65
	N990	–	1.27 ± 2.29
Blood plasma[b]	N330	–	34.24 ± 6.64
	N990	–	21.05 ± 4.60

[a,b]Sonication was applied at 100 W for 1 or 5 min, respectively.

To examine the appearance of carbon black, particles were viewed with a transmission electron microscope (TEM). Particles exhibited the typical aciniform of the carbon array (Fig. 4) (International Carbon Black Association, 2010). Relatively large particles were observed in N990, which were well correlated with the results of size analysis (Table 2).

As a major determinant of particle dispersibility, surface charge was analyzed. The zeta potentials of dispersed carbon black were 34.24 ± 6.64 and 21.05 ± 4.60 mV in blood plasma for N330 and N990, respectively, which are relatively high charges and are consistent with the high dispersibility in N330 and N990 (Table 3). The absolute value of zeta potential was not large enough to interpret the high dispersibility in culture media, and Tween 80 reduced the absolute value of zeta potential in both KR and PSS, as expected. Taken together, the results show that explaining the dispersibility of carbon black solely with surface charge is difficult.

DISCUSSION

Carbon black has been frequently used as a surrogate to investigate the biological effects of the carbon core of particles (Totlandsdal *et al*., 2010; Tankersley *et al*., 2004). It has advantages as a model compound for toxicity studies of particles, especially for studies on size dependent effects of particle toxicity. Carbon black is a pure carbon material, and thus has a relatively inert nature, which minimizes artifacts in toxicity caused by chemical composition of parti-

cles. The size of carbon black varies depending on the structural arrangement of carbon elements, which enables carbon black to be dispersed into various particle sizes. However, carbon black particles tend to readily agglomerate and form clumps in aqueous solutions. Accordingly, dispersion has been a critical impediment and the control of dispersion has become a prerequisite for studies on carbon black. Currently, it is widely accepted that toxicity of particles is largely dependent on particle size, although underlying mechanisms are not clear (Sager and Castranova, 2009). Therefore, we tried to disperse carbon black to both smaller and larger than 100 nm to develop an experimental model for the comparison between ultrafine and fine particles. The data from the present study indicate that cell culture media and blood plasma are excellent media for dispersion of carbon black but only if sonication is applied. However, physical disruption by sonication was not enough to disperse carbon black N330 to less than 100 nm in KR and PSS. Thus, surfactants were employed, and Tween 80 along with sonication, was able to disperse N330 successfully (Fig. 2 and Table 2).

Agglomeration is a collection of primary particles, which involves diverse chemical and electrostatic forces such as van der Waals forces, magnetic forces and sintered bonds (Fubini *et al*., 2010). Sonication is commonly and most frequently used in nanotechnology to disrupt such inter-particle interactions and thereby to disperse nanoparticles evenly in liquids (Wang *et al*., 2010). In addition, sonication could avoid potential artifacts caused by chemical dispersants in biological studies. As expected, sonication disrupted agglomerates and improved dispersion in our study (Fig. 1). However, it was not enough to make dispersion of carbon N330 less than 100 nm in KR and PSS. There are many differences in the composition of dispersion media tested in this study, and there are various factors that determine agglomeration such as ionic strength, temperature, fluid density and dynamic viscosity (Fubini *et al*., 2010). Among them, the presence of proteins appears to make the most striking difference (Table 1). Proteins are well known to improve dispersion of nanoparticles by forming particle-protein corona, thereby diminishing particle-particle interactions. Indeed, albumin or globulin proteins have been widely employed to improve dispersion of nanoparticles (Wang *et al*., 2011). Although proteins may enhance the dispersibility of carbon black, the use was avoided in KR and PSS because they affect various analytical methods and limit the uses of these salt solutions. Hence, surfactants were employed in this study. DPPC is an endogenous lipid and a component of BAL fluid, which indicates that it is a completely biocompatible surfactant (Zhang *et al*., 2011). Additionally, DPPC has been successfully employed for dispersion of various nanoparticles such as multi-walled carbon nanotubes (MWCNT) and TiO_2 (Porter *et al*., 2008; Sager *et al*., 2007). However, it was not apparently effective in carbon black (Fig. 3). Instead, Tween 80 resulted in better dispersion for N330 showing less than 100 nm at a concentration of 0.01%. According to our experience, 0.01% Tween 80 has little effect on most physiological functions of any cells or tissues, thus being useful as a biocompatible vehicle.

In summary, blood plasma and cell culture media with FBS were effective and biocompatible dispersion media for carbon black. Carbon black was also dispersed successfully in inorganic salt solutions such as KR and PSS by addition of a surfactant, Tween 80. With the methods confirmed in this study, carbon black can be dispersed into both ultrafine and fine particles. Carbon black can be used for studies that investigate size related differences in toxicity by the developed dispersion methods, which will serve as valuable model systems for particle toxicity.

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REFERENCES

Ai, J., Biazar, E., Jafarpour, M., Montazeri, M., Majdi, A., Aminifard, S., Zafari, M., Akbari, H.R. and Rad, H.G. (2011). Nanotoxicology and nanoparticle safety in biomedical designs. *Int. J. Nanomed*, **6**, 1117-1127.

Baan, R.A. (2007). Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. *Inhal. Toxicol.*, **19 Suppl 1**, 213-228.

Boverhof, D.R. and David, R.M. (2010). Nanomaterial characterization: considerations and needs for hazard assessment and safety evaluation. *Anal. Bioanal. Chem.*, **396**, 953-961.

Brook, R.D., Rajagopalan, S., Pope, C.A. 3rd., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A., Peters, A., Siscovick, D., Smith, S.C. Jr., Whitsel, L. and Kaufman, J.D. (2010). Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation*, **121**, 2331-2378.

Castranova, V. (2011). Overview of current toxicological knowledge of engineered nanoparticles. *J. Occup. Environ. Med.*, **53**, S14-S17.

Fubini, B., Ghiazza, M. and Fenoglio, I. (2010). Physico-chemical features of engineered nanoparticles relevant to their toxicity. *Nanotoxicology*, **4**, 347-363.

Gilmour, P.S., Ziesenis, A., Morrison, E.R., Vickers, M.A., Drost, E.M., Ford, I., Karg, E., Mossa, C., Schroeppel, A., Ferron, G.A., Heyder, J., Greaves, M., Macnee, W. and Donaldson, K. (2004). Pulmonary and systemic effects of short-term inhalation exposure to ultrafine carbon black particles. *Toxicol. Appl. Pharmacol.*, **195**, 35-44.

IARC Monographs on the Evaluation of Carcinogenic Risks to

Humans. (1996). Printing processes and printing inks, carbon black and some nitro compounds, *International Agency for Research on Cancer*, Geneva, pp. 1-35.

Kreyling, W.G., Semmler-Behnke, M. and Möller, W. (2006). Ultrafine particle-lung interactions: does size matter? *J. Aerosol Med.*, **19**, 74-83.

Lee, M.Y., Bae, O.N., Chung, S.M., Kang, K.T., Lee, J.Y. and Chung, J.H. (2002). Enhancement of platelet aggregation and thrombus formation by arsenic in drinking water: a contributing factor to cardiovascular disease. *Toxicol. Appl. Pharmacol.*, **179**, 83-88.

Madl, A.K. and Pinkerton, K.E. (2009). Health effects of inhaled engineered and incidental nanoparticles. *Crit. Rev. Toxicol.*, **39**, 629-658.

Morfeld, P. and Mccunney, R.J. (2010). Bayesian bias adjustments of the lung cancer SMR in a cohort of German carbon black production workers. *J. Occup. Med. Toxicol.*, **5**, 23.

Morimoto, Y., Ogami, A., Kochi, I., Uchiyama, T., Ide, R., Myojo, T. and Higashi, T. (2010). Continuing investigation of effect of toner and its by-product on human health and occupational health management of toner. *Sangyo Eiseigaku Zasshi*, **52**, 201-208.

Nemmar, A., Hoet, P.H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L. and Nemery, B. (2002). Passage of inhaled particles into the blood circulation in humans. *Circulation*, **105**, 411-414.

Porter, D., Sriram, K., Wolfarth, M., Jefferson, A., Schwegler-Berry, D., Andrew, M.E. and Castranova, V. (2008). A biocompatible medium for nanoparticle dispersion. *Nanotoxicology*, **2**, 144-154.

Sager, T.M. and Castranova, V. (2009). Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine titanium dioxide. *Part. Fibre Toxicol.*, **6**, 15.

Sager, T.M., Porter, D.W., Robinson, V.A., Lindsley, W.G., Schwegler-Berry, D.E. and Castranova, V. (2007). Improved method to disperse nanoparticles for in vitro and in vivo investigation of toxicity. *Nanotoxicology*, **1**, 118-129.

Stone, V., Johnston, H. and Clift, M.J. (2007). Air pollution, ultrafine and nanoparticle toxicology: cellular and molecular interactions. *IEEE Trans. Nanobiosci*, **6**, 331-340.

Tankersley, C.G., Campen, M., Bierman, A., Flanders, S.E., Broman, K.W. and Rabold, R. (2004). Particle effects on heart-rate regulation in senescent mice. *Inhal. Toxicol.*, **16**, 381-390.

Totlandsdal, A.I., Refsnes, M. and Låg, M. (2010). Mechanisms involved in ultrafine carbon black-induced release of IL-6 from primary rat epithelial lung cells. *Toxicol. In Vitro*, **24**, 10-20.

Valberg, P.A., Long, C.M. and Sax, S.N. (2006). Integrating studies on carcinogenic risk of carbon black: epidemiology, animal exposures, and mechanism of action. *J. Occup. Environ. Med.*, **48**, 1291-1307.

Wang, J., Jensen, U.B., Jensen, G.V., Shipovskov, S., Balakrishnan, V.S., Otzen, D., Pedersen, J.S., Besenbacher, F. and Sutherland, D.S. (2011). Soft interactions at nanoparticles alter protein function and conformation in a size dependent manner. *Nano Lett.*, **11**, 4985-4991.

Wang, L., Castranova, V., Mishra, A., Chen, B., Mercer, R.R., Schwegler-Berry, D. and Rojanasakul, Y. (2010). Dispersion of single-walled carbon nanotubes by a natural lung surfactant for pulmonary *in vitro* and *in vivo* toxicity studies. *Part. Fibre Toxicol.*, **7**, 31.

Xia, T., Li, N. and Nel, A.E. (2009). Potential health impact of nanoparticles. *Annu. Rev. Public Health*, **30**, 137-150.

Yang, H.M., Nam, W.K. and Park, D.W. (2007). Production of nanosized carbon black from hydrocarbon by a thermal plasma. *J. Nanosci. Nanotechnol.*, **7**, 3744-3749.

Zhang, H., Wang, Y.E., Fan, Q. and Zuo, Y.Y. (2011). On the low surface tension of lung surfactant. *Langmuir*, **27**, 8351-8358.