Supplementary Material (ESI) for Molecular BioSystems

Electronic Supplementary Information

Strict preparation and evaluation of water-soluble hat-stacked carbon nanofibers for biomedical application and their high biocompatibility: Influence of functional groups of nanofiber-surface on the cytotoxicity

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Chemicals

Mycoplasmal diacylated lipopeptide, referred to as FSL-1, S-(2,3-bispalmitoyloxypropyl)Cys- Gly-Asn-Asn-Asp-Glu-Ser-Asn-Ile-Ser-Phe-Lys-Glu-Lys, was synthesized according to a previously described method.[1] All other chemicals were obtained from commercial sources and were of analytical or reagent grade.

Activation of THP-1 cells

The human acute monocytic leukemia cell line THP-1[2] was obtained from the Health Science Research Resources Bank (Osaka, Japan). Cells were grown at 37^{o}C in a humidified 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% (v/v) FBS, penicillin G (100 units/ml) and streptomycin (100 μg/ml). Cells were incubated at 37°C for 24 h in the absence or presence of PMA. Following this, a 0.2-ml volume THP-1 cell suspension (5 x 10^5 cells) was placed into each well of a 96-well tissue culture plate and incubated at 37°C for 16 h in culture medium containing FSL-1 supplemented with 0.1% (v/v) human serum. Also, THP-1 cells were incubated for 16h with H-CNFs and CHAPS-H-CNFs in the same way. Culture supernatants were then collected by centrifugation at 400 x *g* for 10 min. The amount of TNF-α in the supernatants was determined using HU TNF-α Flexia (Biosource International, Inc., Camarillo, CA).

Activation of NF-κB reporter by Toll-like receptor 2 (TLR2)

Human embryonic kidney (HEK)293 cells obtained from ATCC (CRL-1573) were maintained in DMEM (Gibco BRL, Rockville, MD) containing 10% FBS. A human acute monocytic leukemia cell line, THP-1, obtained from Health Science Research Resources Bank (Osaka, Japan), was grown at 310 K in a humidified atmosphere of 5% CO2 in RPMI 1640 medium supplemented with 10% (vol/vol) FBS, penicillin G (100 units/ml) and streptomycin (100 μm/ml).

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Cloning of human TLR2 and TLR2 mutants

The cDNA of human TLR2 (2.35 kb) was obtained by RT-PCR of RNA isolated from THP-1 cells. The cDNA of TLR2 was cloned into a pEF6/V5-His TOPO vector (hereafter referred to as TLR2-TOPO) (Invitrogen Co., Carlsbad, CA, USA).

NF-κB reporter assay

HEK293 cells were plated at 1×10^5 cells per well in 24-well plates the day before transfection. Cells were transiently transfected using Fugene 6 Tranfection Reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) with 30 ng of an NF-κB reporter plasmid (pNF-κB-Luc, Stratagene) and 3.5 ng of a construct directing expression of *Renilla* luciferase under the control of a constitutively active thymidine kinase promoter (pRL-TK, Promega Co., Madison, WI, USA) together with 166.5 ng of each transfectant gene of TLR2-TOPO. Twenty-four hours after transfection, cells were stimulated for 6h with water-soluble H-CNFs and CHAPS-treated H-CNFs in the absence of FBS, and luciferase activity was measured using a Dual-Luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer's instructions.

Preparation of water-soluble H-CNFs coated with CHAPS

10 mg of highly purified H-CNFs and 370 mg of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) were added to 5.0 mL of glycerol and the resultant solution was mixed by rotation for 2 hours to prepare a well mixed colloidal solution. The colloidal solution was diluted with 50.0 mL of 0.1 M NaCl aqueous solution and then incubated at 50°C for approximately 20 hours. Under these conditions, adsorption of CHAPS, the typical zwitterionic surfactants, took place to form pseudo micelles with CNF being part of the active micelle kernel. Following this, the sample was washed several times with water to remove residual CHAPS in the dispersion. The resultant cake on the membrane filter was then placed in water and sonicated several times. No precipitation was

observed from this aqueous micelle solution during a two-week period of observation.

XRD pattern of acid-treated H-CNFs

Fig. S1 shows the XRD pattern of as-grown H-CNFs and annealed H-CNFs. Phase characterization was performed by means of X-ray diffraction using Cu-Kα radiation. As shown with the as-grown H-CNTs, the diffraction peaks of 44.4° and 51.7° could be indexed as a cubic crystal fcc structure Ni (111) and (200), respectively. The other diffraction peaks of 26.1° and 43.0° corresponded to (002) and (100) of graphite, and we also confirmed a weak peak of (004). The reason why the peak of (002) shifts to lower angle is considered to be due to the curvature of the graphene-hats.[3,4] Following purification, the H-CNFs possessed no Ni as shown in the XRD pattern. Furthermore, as the peak width at

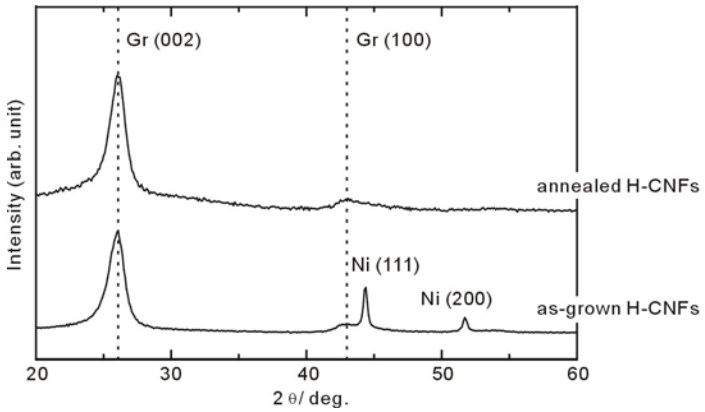

Fig. 1S XRD pattern of as-grown H-CNFs and annealed H-CNFs.

half height of (002) of purified H-CNFs was narrower than that of as-grown H-CNFs, crystalline H-CNFs grew well due to the annealing effect at high temperature *in vacuo*.

Length Distribution of H-CNFs following sonication for various times

Fig. S2 shows the SEM photographs of acid-treated H-CNFs following 2h (a), 4h (b) and 6h (c) of sonication in a mixture of concentrated H_2SO_4 and HNO_3. The average length was 1500 nm, 800 nm and 400 nm for sonication times of 2h, 4h and 6h, respectively. The length of H-CNFs decreased with increasing sonication time (Inset in figure S2(c) represents the high magnification). It is thought that two effects affect cutting of the H-CNFs. Firstly, acid ions such as SO_4^{2-} and NO_3^- were intercalated into graphene-hat layers.[5] The intercalated layers become weaker due to expansion of the graphene-hat layers. Following this, when the force acts on H-CNFs, the layers come off from the intercalated layer

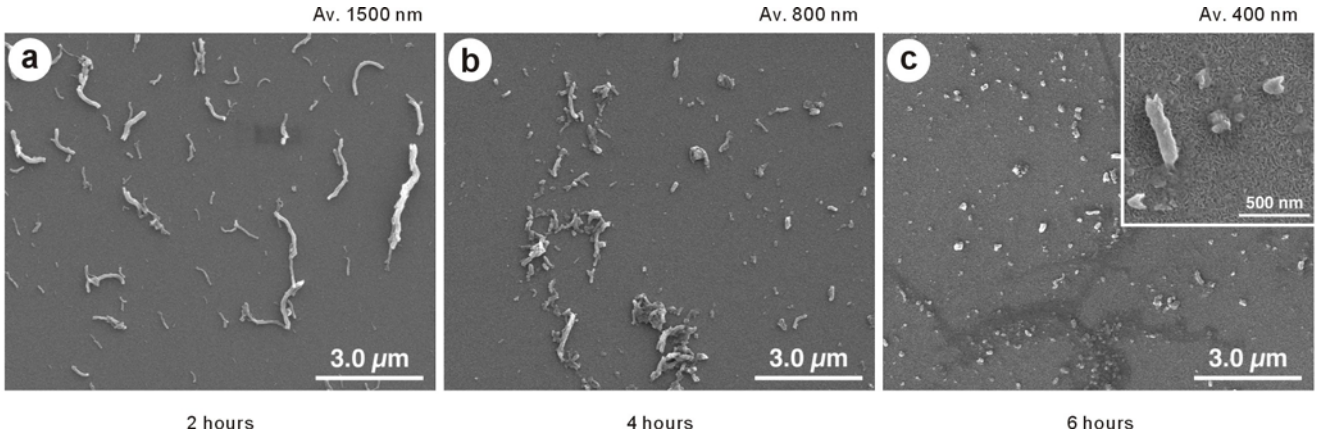

Fig. 2S SEM photographs of acid-sonicated H-CNFs following 2h (a), 4h (b) and 6h (c) sonication in a mixture of concentrated H_2SO_4 and HNO_3.

parts. This phenomenon is referred to as the "delamination effect"[6], observed in layered materials such as organo-layer silicate[6] and graphite.[5]

Reference

1. K-I. Shibata, A. Hasebe, T. Into, M. Yamada and T. Watanabe, *J. Immunol.*, 2000, **165**, 6538.

2. S. Tsuchiya, M. Yamabe, Y. Yamaguchi, Y. Kobayashi, T. Konno and K. Tada, *Int. J. Can.*, 1980, **26**, 171.

3. C. R. Houska and B. E. Warren, *J. Appl. Phys.*, 1954, **25**, 1503.

4. Y. Saito, T. Yoshikawa, S. Bando, M. Tomita and T. Hayashi, *Phys. Rev. B*, 1993, **48**, 1907.

5. S. F. McKay, *J. Appl. Phys.*, 1964, **35**, 1992.

6. J. Bujdak, E. Hackett and E. P. Giannelis, *Chem. Mater.*, 2000, **12**, 2168.