Introduction to Societal Impacts of Nanotubes

-Japan's Activities on EHS & ELSI of Nanotechnology-

June 21st, 2006

Masahiro Takemura

National Institute for Materials Science

http://www.nims.go.jp

http://www.nanonet.go.jp

Nanotechnology Support Project

Advisory Board

Chairman: Hiroyuki Sakaki (Univ. of Tokyo)

Facility Support

National Network for Common Use Facility (14 Institutes)

Common use of cutting-edge facilities for fabrication & characterization

- High-voltage Electron Microscope
- Nano Foundry
- Synchrotron Radiation
- Molecular Synthesis & Analysis

Informational Support

Nanotechnology Researchers Network Center of Japan (Nanonet)

Director General: Teruo Kishi

- Dissemination of Information
- Support of Researcher Network
- Education and Public Relations
- Survey on policies, R&D accomplishments, and societal implications

Outline

- **Environmental, Health and Safety (EHS) Impacts and Ethical, Legal and Societal Issues (ELSI) of Nanotechnology**
- **Japan's Activities on Societal Implications of Nanotechnology**
  - **Third Science and Technology Basic Plan**
  - **Japan's National Projects**
  - **International Collaboration**
- **Recent Research Topics**
  - **Nanoscale Dispersion of C60 in Water for toxicological tests**
  - **Particle Measurement in Workplace**

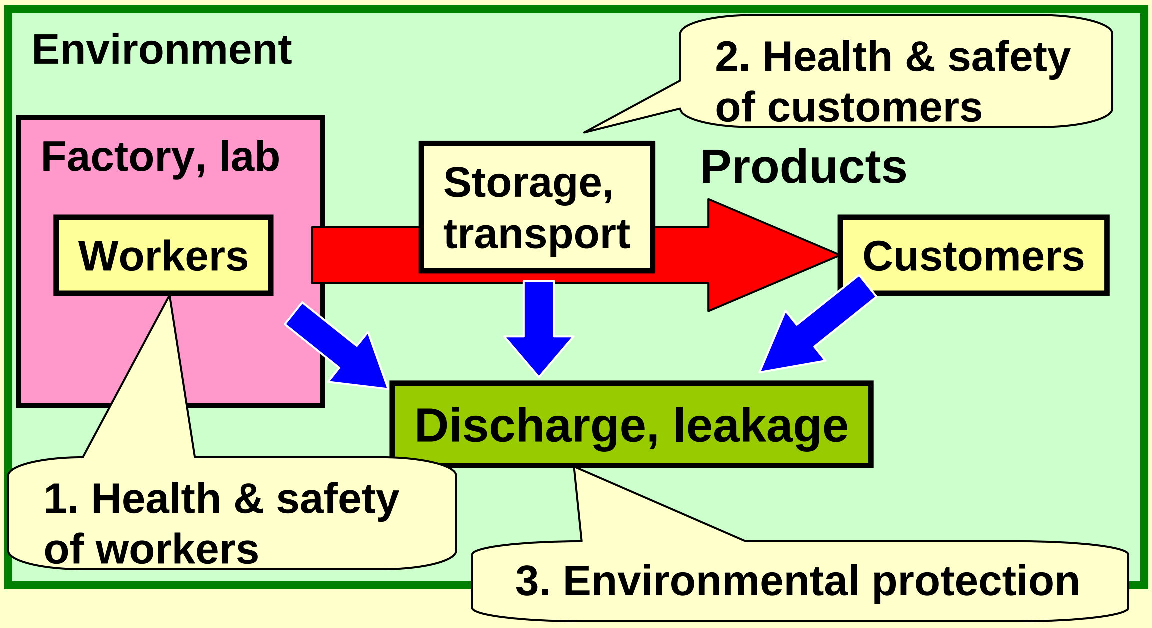
Nanotechnology and Society

- **Unidentified Risk in Emerging Technology**
  - **GMO experience in USA and Europe**
    - **Negative public perception of unidentified risk**
    - **Technical success but commercial failure**
- **Societal implications of Nanotechnology – short term and long term**
  - **Potential risks of nanomaterials**
    - **Health and safety of workers**
    - **Health and safety of consumers**
    - **Environmental Protection**
  - **Societal impacts of nanotechnologies**
    - **Life science**
    - **Information and communication technology**
    - **Energy and environment**
  - **Converging technology**
    - **Nano - Bio- Info - Cogno (NBIC) integration**

Risk Assessment and Management of Nanomaterials

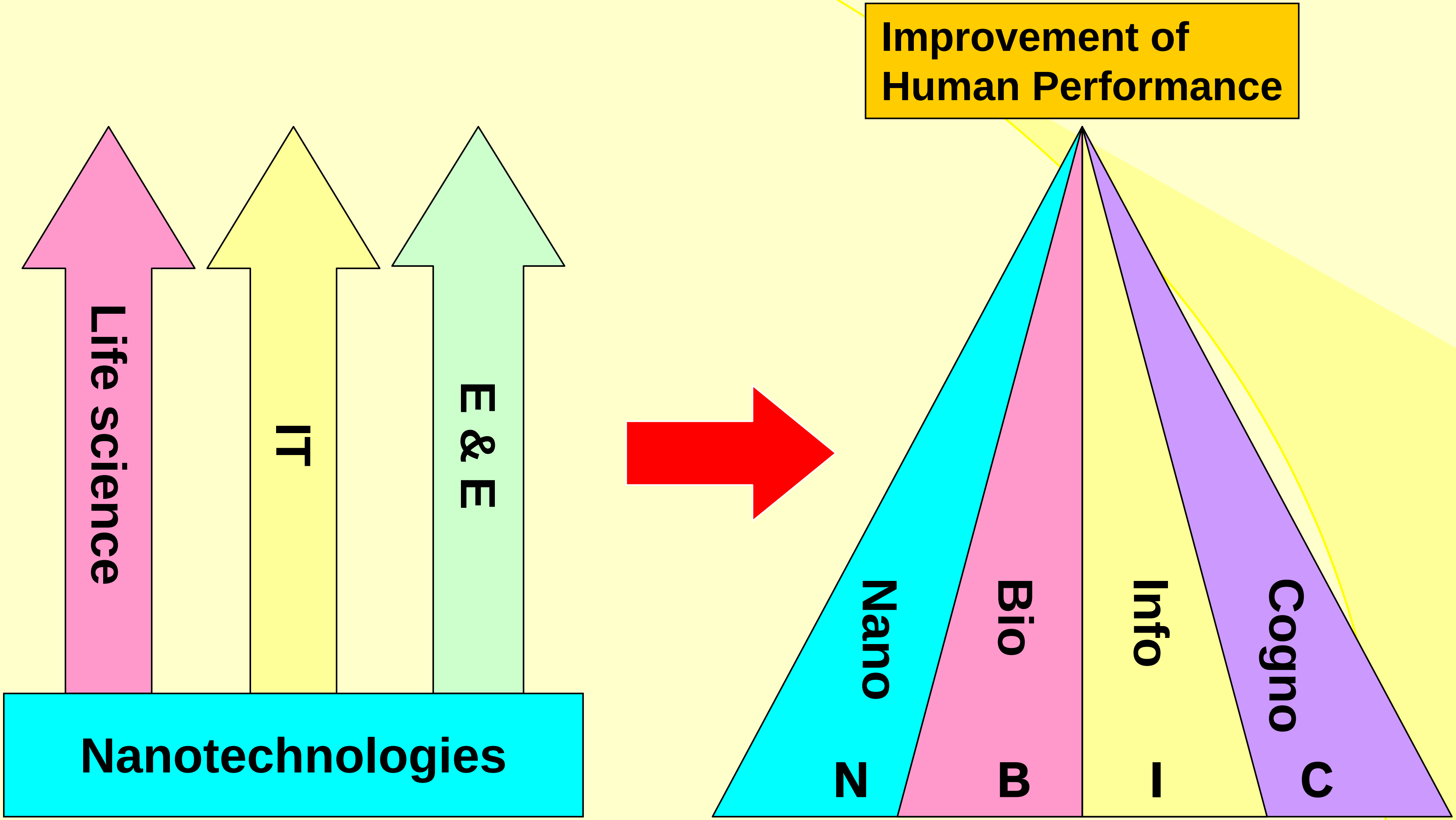
Classification of Nanomaterials

- Diameter < 100 nm, properties different from bulks
- Intentionally/unintentionally produced
  - Engineered nanoparticles/diesel exhaust particles
- Intentionally/unintentionally introduced into body/environment
  - Medicine, environmental remediation/nanoparticles inhaled at workplace



The Royal Society & The Royal Academy of Engineering, Nanoscience and nanotechnologies: opportunities and uncertainties, 2004

Converging Technology -
NBIC (Nano-Bio-Info-Cogno) Integration



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Third Science & Technology Basic Plan (2006-2010) by CSTP

1. Basic Ideas
2. Strategic Priority Setting in S&T
   - Promotion of basic researches
   - Prioritization of R&D for policy-oriented subjects
     - Priority promotion areas; Life science, IT, Environmental sciences, Nanotechnology & materials
     - Promotion areas; Energy, MONODZUKURI tech., Infrastructure, Frontier (outer space & oceans)
   - Promotion strategy for prioritized areas
3. S&T system reforms
4. Public Confidence and Engagement
   - Responsible actions regarding ELSI
   - Reinforcement of accountability and public relations of S&T activities
   - Promotion of public understanding of S&T
   - Facilitation of public engagement with S&T-related issues
5. Missions of CSTP

National Institutes for Nano-R&D & EHS

CSTP
NTPT

METI
Flagship type R&D
NEDO (FA)
AIST (RI)

Industries
NBCI

MEXT
Basic research
JSPS (FA)
Universities

Long term challenge for industrial use
JST (FA)

Generic technology
NIMS (RI)
RIKEN (RI)

MHLW
Safety of foods & drugs
NIHS (RI)

Occupational health
NIIH (RI)

MOE
Environmental Protection
NIES (RI)

FA: Funding Agency
RI: National Research Institute

CSTP: Council for Science and Technology Policy, MEXT: Ministry of Education, Culture, Sports, Science and Technology,
METI: Ministry of Economy, Trade and Industries, MHLW: Ministry of Health, Labor and Welfare,
MOE: Ministry of the Environment, NTPT: Nanotechnology Project Team, JSPS: Japan Society for the Promotion of Science,
JST: Japan Science and Technology Agency, NIMS: National Institute for Materials Science,
NEDO: New Energy and Industrial technology Development Organization,
AIST: National Institute of Advanced Industrial Science and Technology, NIHS: National Institute of Health Sciences,
NIIH: National Institute of Industrial Health, NIES: National Institute for Environmental Studies

Japan's Open Discussion on Societal Implications of Nanotechnology Kicked off in 2004

- **Open Forum "Nanotechnology and Society"**
  - **Organized by AIST, August 2004 – March 2005**
- **Symposium "Nanotechnology and Society"**
  - **Organized by AIST, NIMS, NIES and NIHS, Feb. 1st, 2005**

Symposium "Nanotechnology and Society"

National Projects for EHS Implications and ELSI

- **MEXT: Research study for public acceptance of nanotechnology**
  - **Risk assessment of nanomaterials (AIST)**
  - **Health issues of nanomaterials (NIHS)**
  - **Environmental issues of nanomaterials (NIES)**
  - **Ethical and societal issues of nanotechnology (NIMS)**
  - **Technology assessment for promoting the public acceptance of nanotechnology and economic effects (AIST)**
- **METI–AIST: Standardization of testing methods for evaluation of safety of nanoparticles**
- **METI-NEDO: R&D for Evaluation of properties of nanoparticles (solicitation closed last month)**
- **MHLW: Development of evaluation methods for health impacts of nanomaterials**
- **MEXT-Nanonet: Nanotechnology Support Project**
  - **Dissemination of information and database**
  - **Support of interdisciplinary and international network**

International Collaboration

- International Organization for Standardization (ISO)
- American Society for Testing Materials (ASTM)
- Royal Society
- International Council on Nanotechnology (ICON)
- International Nanotechnology in Society Network (INSN)

Joint Royal Society- Science Council of Japan workshop on the potential health, environmental and societal impacts of nanotechnologies

- **Date and Venue**
  - 1st: July 11-12, 2005, Royal Society (London)
  - 2nd: February 23, 2006, Tokyo Big Sight (Tokyo)
- **Objectives**
  - To share the latest scientific research into the health, environmental and societal impacts of nanotechnology.
  - To identify specific areas where further research is needed and to identify common areas of interest.
  - To determine areas for future collaboration and identify mechanisms for facilitating this collaboration.
  - To discuss appropriate regulatory systems for nanomaterials.
- **Participants from Universities, Industries, Government and National Institutes**
  - UK: Prof. M. Welland, Prof. A. Seaton, Prof. J. Ryan, Prof. K. Donaldson, Prof. V. Stone, Oxonica, DTI, DEFRA, HSE, NPL, …
  - Japan: Prof. T. Kishi, Prof. M. Endo, Prof. T. Tsuda, Prof. Y. Kusaka, NBCI, AIST, NIMS, NIES, NIHS, NIIH, …
  - USA: Dr. J. Moore, Dr. A. Maynard

Joint Royal Society- Science Council of Japan workshop on the potential health, environmental and societal impacts of nanotechnologies - Summary -

- Significant funding for research into the potential negative health and environmental impacts of nanomaterials is urgently needed.
- International and interdisciplinary research collaboration is required. For example, nanomaterial scientists should collaborate with toxicologists.
- A standardized framework for the risk assessment of nanomaterials is required, including standard reference samples and toxicology protocols.
- Industry should work with academia and other stakeholders by sharing information on methodologies and providing samples.
- Research into the potential negative impacts of nanomaterials on the environment is urgently required. There remains virtually no data on them.
- A robust, publicly acceptable regulatory framework for nanotechnologies is more likely to be achieved if appropriate stakeholder engagement are undertaken and the results are incorporated into the policy-making process.

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Research on Biological Impacts of Nanomaterials in Japan

- **Fullerene**
  - **"Biological Effects of Fullerene", Scientific Research for Priority Area "Carbon Cluster", MEXT, 1993-1995**
- **Carbon nanotubes**
  - **"Tissue Responsiveness and Bio-application of Nanotubes and Nano-micro Particles" by Prof. F. Watari (Hokkaido Univ.), et al.**
  - **"Role of Systemic T-cells and Histopathological Aspects after Subcutaneous Implantation of Various Carbon Nanotubes in Mice" by Prof. S. Koyama, Prof. M. Endo (Shinshu Univ.), et al.**
  - **"Toxicity of Carbon Nanotubes Instilled Intratracheally" by Dr. A. Tanaka (Kyushu Univ.), et al.**
- **Metals & Ceramics (Ni, Co, Fe, TiO_2, …)**
  - **Toxicological study from a viewpoint of occupational health by Prof. Y. Kusaka (Fukui Univ.), NIIH, …**

Amount of data is not enough for risk assessment.

Biological Impacts of Fullerene

Research | Article

Manufactured Nanomaterials (Fullerenes, C_{60}) Induce Oxidative Stress in the Brain of Juvenile Largemouth Bass

Eva Oberdörster

Duke University Marine Laboratory, Beaufort, North Carolina, USA; Department of Biology, Southern Methodist University, Dallas, Texas, USA

Although nanotechnology has vast potential in uses such as fuel cells, microreactors, drug delivery devices, and personal care products, it is prudent to determine possible toxicity of nanotechnology-derived products before widespread use. It is likely that nanomaterials can affect wildlife if they are accidentally released into the environment. The fullerenes are one type of manufactured nanoparticle that is being produced by tons each year, and initially uncoated fullerenes can be modified with biocompatible coatings. Fullerenes are lipophilic and localize into lipid-rich regions such as cell membranes *in vitro*, and they are redox active. Other nano-sized particles and soluble metals have been shown to selectively translocate into the brain via the olfactory bulb in mammals and fish. Fullerenes (C_{60}) can form aqueous suspended colloids (nC_{60}); the question arises of whether a redox-active, lipophilic molecule could cause oxidative damage in an aquatic species. The goal of this study was to investigate oxyradical-induced lipid and protein damage, as well as impacts on total glutathione (GSH) levels, in largemouth bass exposed to nC_{60}. Significant lipid peroxidation was found in brains of largemouth bass after 48 hr of exposure to 0.5 ppm uncoated nC_{60}. GSH was also marginally depleted in gills of fish, and nC_{60} increased water clarity, possibly due to bactericidal activity. This is the first study showing that uncoated fullerenes can cause oxidative damage and depletion of GSH *in vivo* in an aquatic species. Further research needs to be done to evaluate the potential toxicity of manufactured nanomaterials, especially with respect to translocation into the brain. *Key words:* antioxidant defense system, fish, fullerenes, glutathione, lipid peroxidation, manufactured nanomaterials, toxicity. *Environ Health Perspect* 112:1058–1062 (2004). doi:10.1289/ehp.7021 available via *http://dx.doi.org/* [Online 7 April 2004]

Nanomaterials are defined by the U.S. National Nanotechnology Initiative as materials that have at least one dimension in the 1- to 100-nm range. Nano-sized materials are naturally present from forest fires and volcanoes, viral particles, biogenic magnetite, and even protein molecules such as ferritin. Recently, anthropogenic sources have also produced nano-sized materials—unintentionally from combustion by-products and intentionally as manufactured nanomaterials. Engineered nanomaterials are useful because of their large surface area:mass ratio, which makes them important as catalysts in chemical reactions, and they have desirable properties as drug delivery devices, as imaging agents in medicine, and in consumer products such as sunscreens and cosmetics (Colvin 2003).

The aquatic environment may be contaminated from consumer products (e.g., sunscreens and cosmetics), as well as spillage from manufacturing and shipping. It is unknown at what quantities these nanomaterials may be found in the environment, and it is especially difficult to predict as the number of products that use nanomaterials increases. As a comparison, lipophilic chemicals such as polycyclic aromatic hydrocarbons can be found at up to 4 ppm in produced water [International Association of Oil & Gas Producers (OGP) 2002], although steady-state concentrations are usually in the low ppb range and below [OGP 2002; San Francisco Estuary Institute (SFEI) 2003]. Because fullerenes are being produced by the ton (Colvin 2003), it is likely that they will eventually be found in the environment at measurable concentrations. Fullerenes can also be coated at the time of production with a variety of biocompatible materials, but it is unknown how long those coatings will stay on the fullerenes during weathering in an environmental setting, and what will happen to the fullerenes once the coating is removed. The likelihood of coating breakdown has been shown in cell culture systems, where quantum dots with cadmium-selenium cores were initially rendered nontoxic with coatings, but if the quantum dots were either exposed to air or ultraviolet radiation for as little as 30 min, they became extremely cytotoxic (Derfus et al. 2004). Therefore, this study was designed to evaluate the toxicity of uncoated fullerenes to an environmentally relevant species, the largemouth bass.

There are three areas of primary concern in terms of toxicity of fullerenes and engineered nanomaterials: *a*) some manufactured nanomaterials (especially the fullerenes) are engineered to be redox active (Colvin 2003); *b*) nano-sized particles partition into cell membranes and especially mitochondria both *in vivo* and *in vitro* (DeLorenzo 1970; Foley et al. 2002; Li et al. 2003); and *c*) research on nano-sized particles in mammalian systems shows that there is a selective transport mechanism from the olfactory nerve into the olfactory bulb (Bodian and Howe 1941; DeLorenzo 1970; Howe and Bodian 1941; Oberdörster et al. 2004). This pathway also exists in rodents and fish for soluble metals (Tjälve and Henriksson 1999; Tjälve et al. 1995).

I hypothesized that this neuronal translocation pathway could also exist in fish for redox-active, lipophilic fullerenes, causing oxidative damage in the brain. I show here that juvenile largemouth bass exposed to 0.5-ppm aqueous uncoated fullerenes (nC_{60}) for 48 hr had a significant increase in lipid peroxidation of the brain, and glutathione (GSH) depletion in the gill.

Materials and Methods

Fullerenes. Uncoated 99.5% pure fullerenes (SES, Houston, TX) were water solubilized using standard methods (Deguchi et al. 2001) by the Center for Biological and Environmental Nanotechnology, Rice University (Houston, TX) and were a generous gift for this study. Briefly, fullerenes (100 mg/L) were dissolved in tetrahydrofuran (THF), sparged with nitrogen, stirred overnight in the dark, and filtered through a 0.22-μm nylon Osmonics filter (GE Water Technologies, Fairfield, CT); MilliQ water (Millipore Corp., Bedford, MA) was added to an equal volume of C_{60} in THF. THF was eliminated using a Buchi rotovapor (Büchi Labortechnik AG, Flawil, Switzerland) by reducing the volume to 450 mL and adding 550 mL MilliQ water. This was repeated twice, and the final solution was evaporated to 500 mL and stored overnight. The solution was filtered through a 0.22-μm nylon filter, yielding a working nC_{60} suspension of 3.8 ppm. This suspension consisted of stable 30- to 100-nm aggregates in which the fullerenes facing the water were most likely partially modified, but the central core of the aggregate contained unmodified fullerenes (Colvin et al. 2004).

Fish exposures. Juvenile largemouth bass (*Micropterus salmoides*) were cultured at

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The author declares she has no competing financial interests.

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Oberdörster, E. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of juvenile largemouth bass. *Environ. Health Perspect.* 112, 1058-1062 (2004).

For Better Toxicological Evaluation

- **To Provide Series of Standard Materials for Organized Toxicological Evaluation**

- **To Develop Methods of Dispersion of Nanomaterials in Air and Water for Appropriate Inhalation and Injection**

- **To Understand Existence of Nanomaterials in Actual Environments**

Aqueous Dispersion of C60 with Using THF

THF: tetrahydrofuran

- **Procedure**
  1. **Mix a saturated solution of C60 in THF and water (Sample 3).**
  2. **THF removal by purging gaseous N_2 (Sample 4).**
- **Merits**
  - **Long term stability - remain dispersed for years**
  - **No dispersing agent necessary**
  - **Flocculate rapidly in the presence of salts.**
- **Problem**
  - **Residual solvent (THF) may be responsible for the toxicity**

Sample 1: saturated solution of C60 in THF
Sample 2: water
Sample 3: mixture of sample 1 and 2
Sample 4: sample 3 after THF removal
(contains C60 and water)

S. Deguchi, R. G. Alargova, K. Tsujii, *Langmuir* **17**, 6013 (2001)

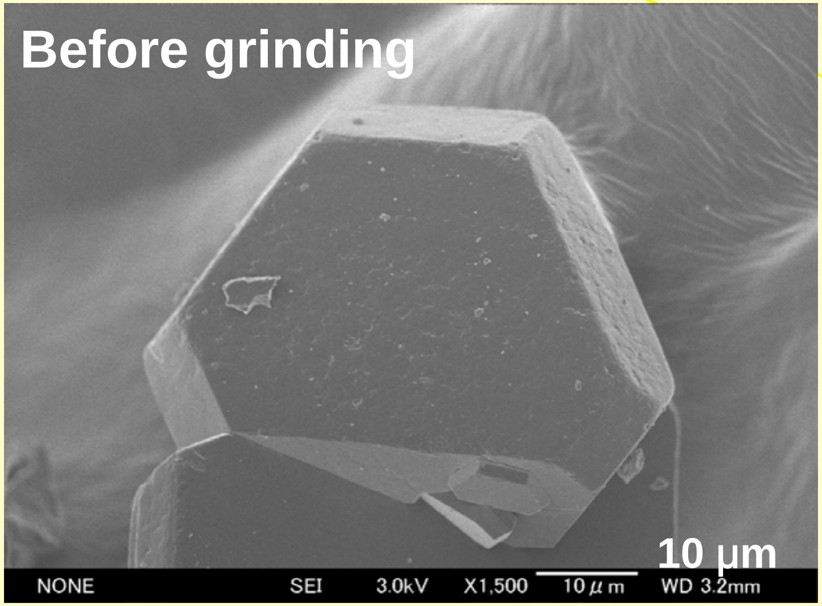
Solvent-free　Aqueous Dispersion of C60

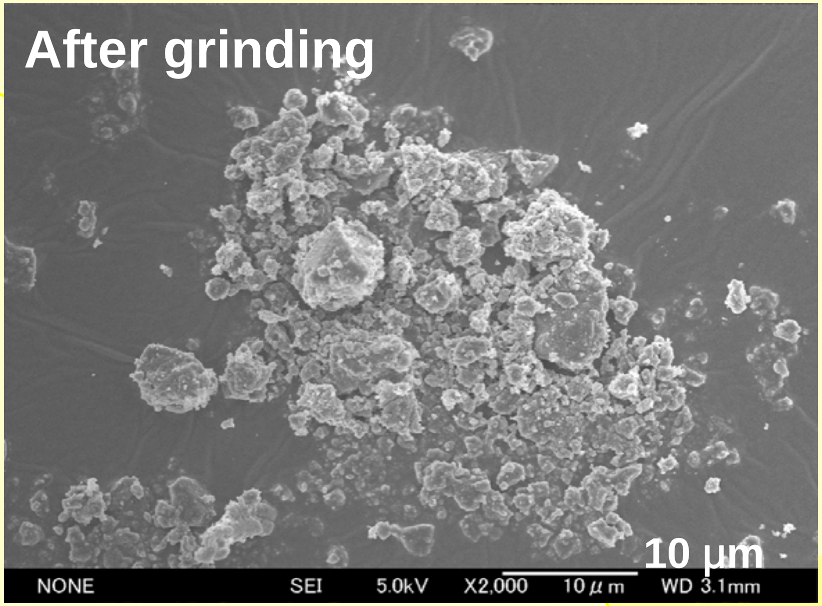
- **Using Agate Mortar**
  1. **Mix ground C60 and water**
  2. **Sonication for 30 min or stirring overnight**
  3. **Filtration (pore size: 5 μm)**

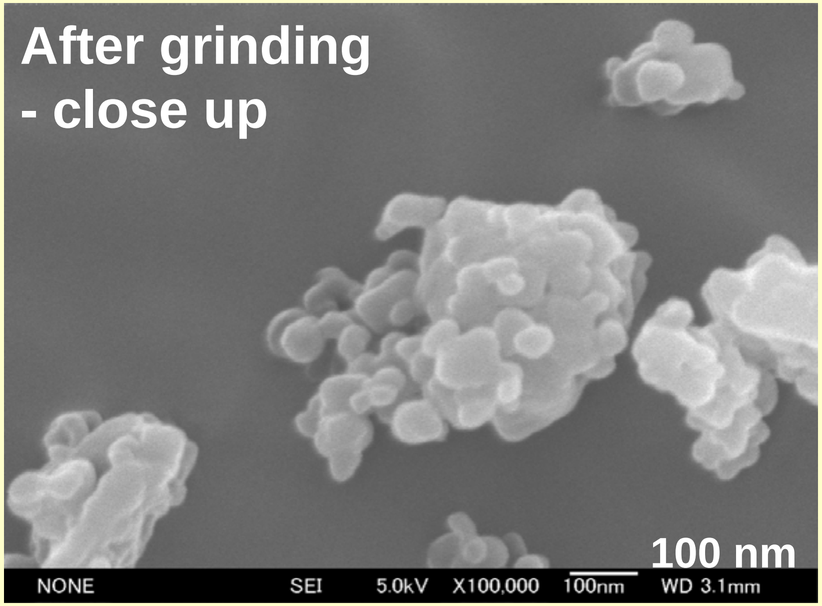
Sample 1: dispersion in water containing 40 mM of sodium dodecylsulfate, $[C_{60}] = 6.56 \times 10^{-4}$M
Sample 2: tenfold dilution of Sample 1
Sample 3: 100-fold dilution of Sample 1
Sample 4: dispersion of as-received C_{60}, $[C_{60}] = 1.25 \times 10^{-5}$M
Sample 5: dispersion in pure water, $[C_{60}] = 3.27 \times 10^{-4}$M

Courtesy to S. Deguchi, JAMSTEC

SEM Images







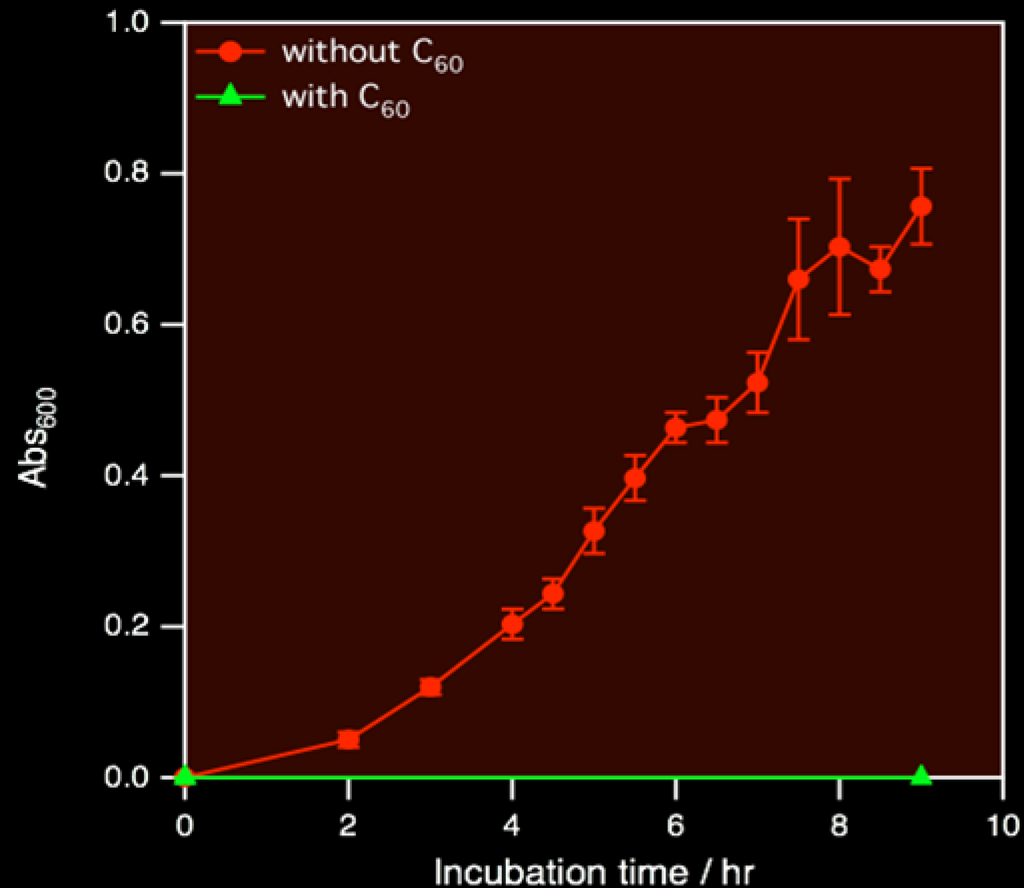
S. Deguchi et al, *Adv. Mater.*, **2006**, *18*, 729.

Growth Inhibition of E. coli in Water with C60 Dispersed with Using THF

	E. coli	
	aerobic	anaerobic
control	+	+
0.04 μg/mL C_{60}	+	+
0.4 μg/mL C_{60}	–	–
4 μg/mL C_{60}	–	–
5 μg/mL $C_{60}(OH)_{24}$	+	+

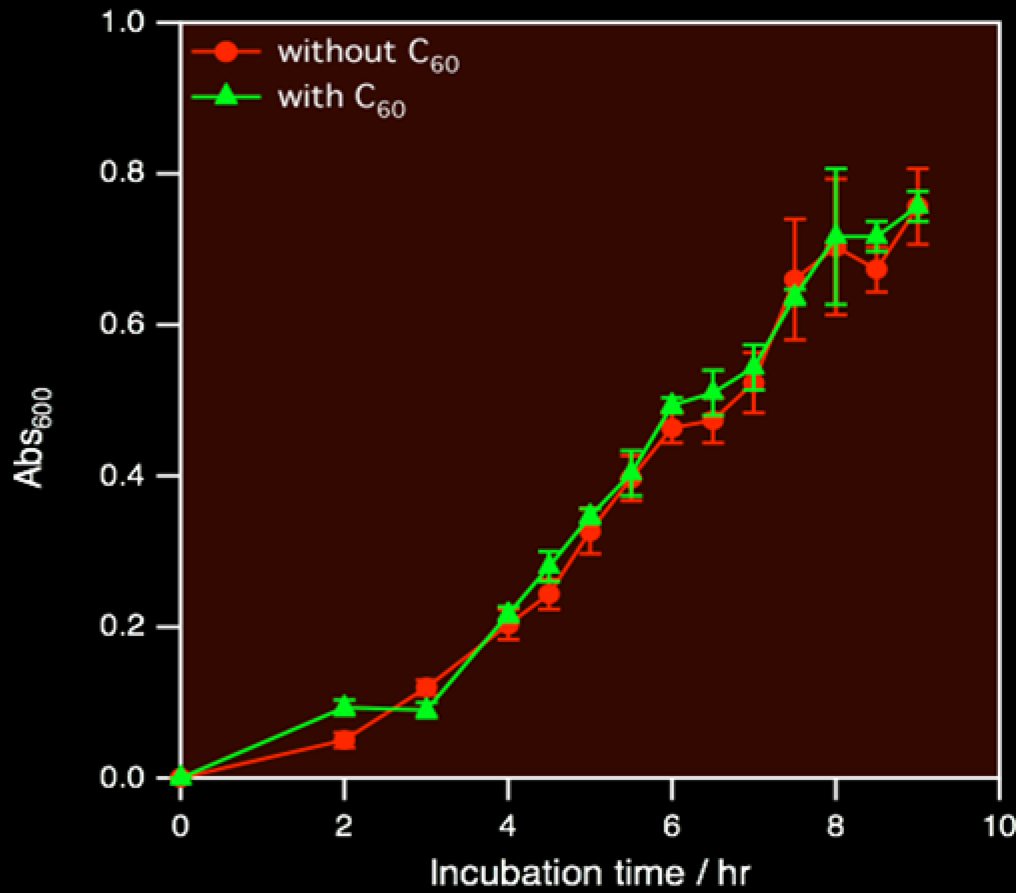
J. D. Fortner et al, *Environ. Sci. Technol.* **2005**, *39*, 4307-4316.

E. coli in Water with C60 Dispersed with Using THF



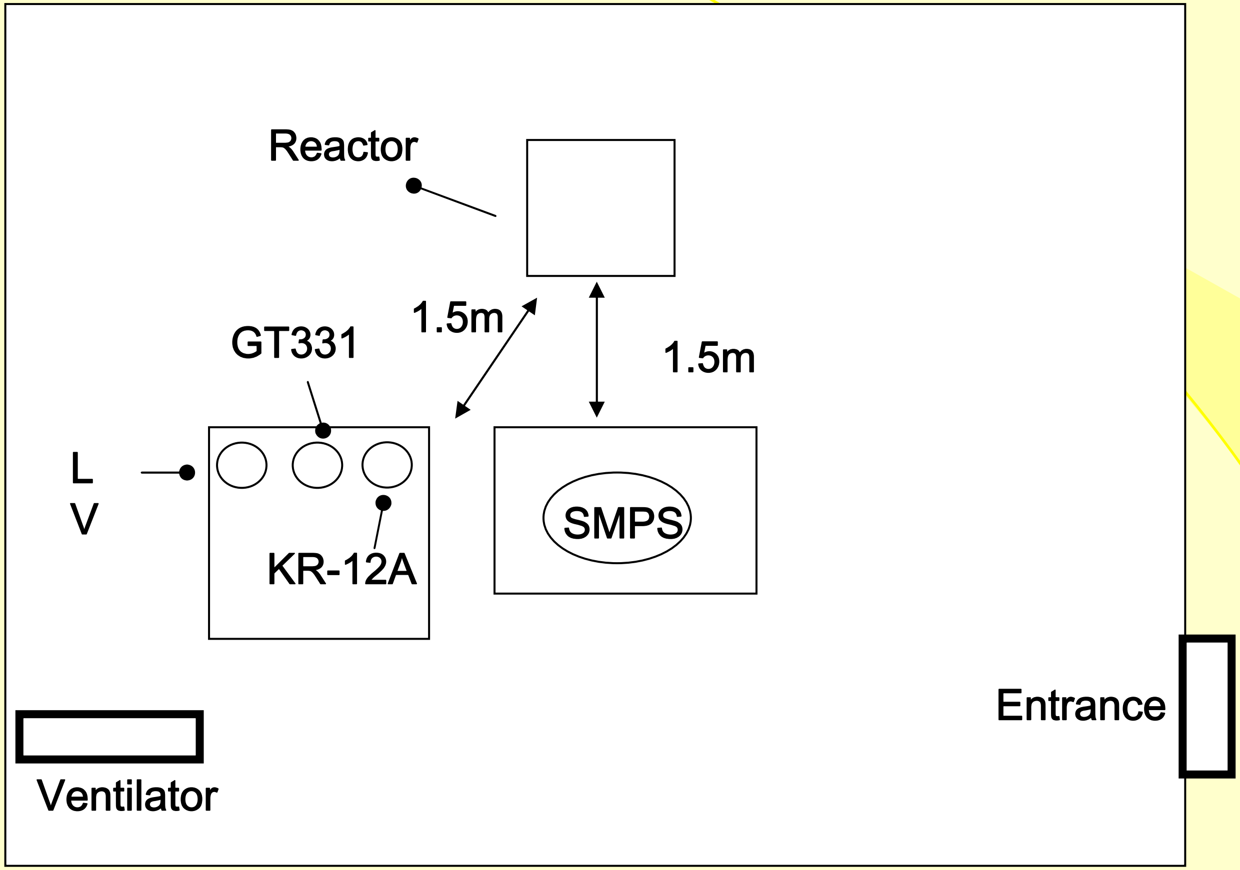
Schematically drawn based on research of J. D. Fortner et al. by S. Deguchi, JAMSTEC

E. coli in Water with C60 Dispersed without Using THF



S. Deguchi et al, *Adv. Mater.*, **2006**, *18*, 729.

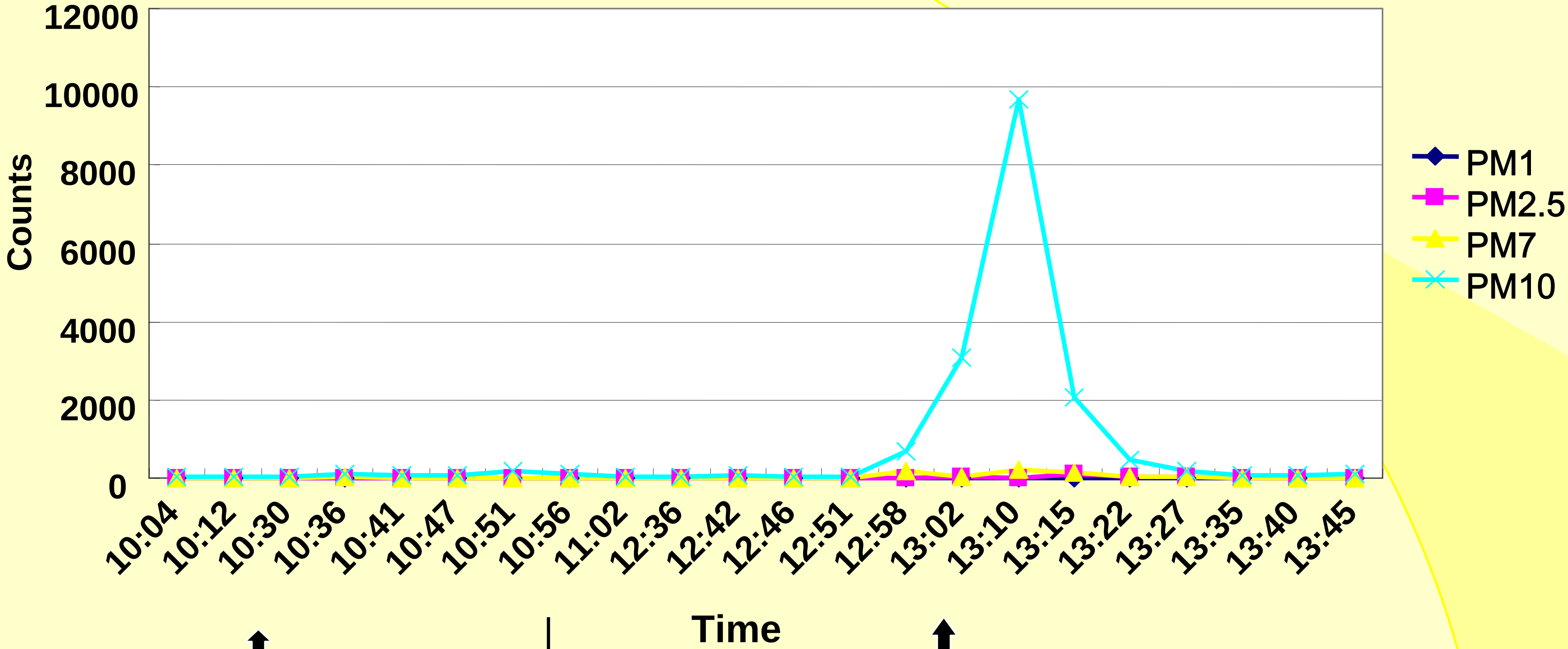
Particle Measurement in Fullerene Factory



SMPS :Scanning mobility particle sizer (TSI Model 3034)
KR-12A: Optical particle counter (RION)
GT331: Particle mass monitor (Sibata)
LV: Low volume air sampler (Sibata)

Courtesy to T. Kobayashi, NIES

Particle Counts during Handling in Fullerene Factory



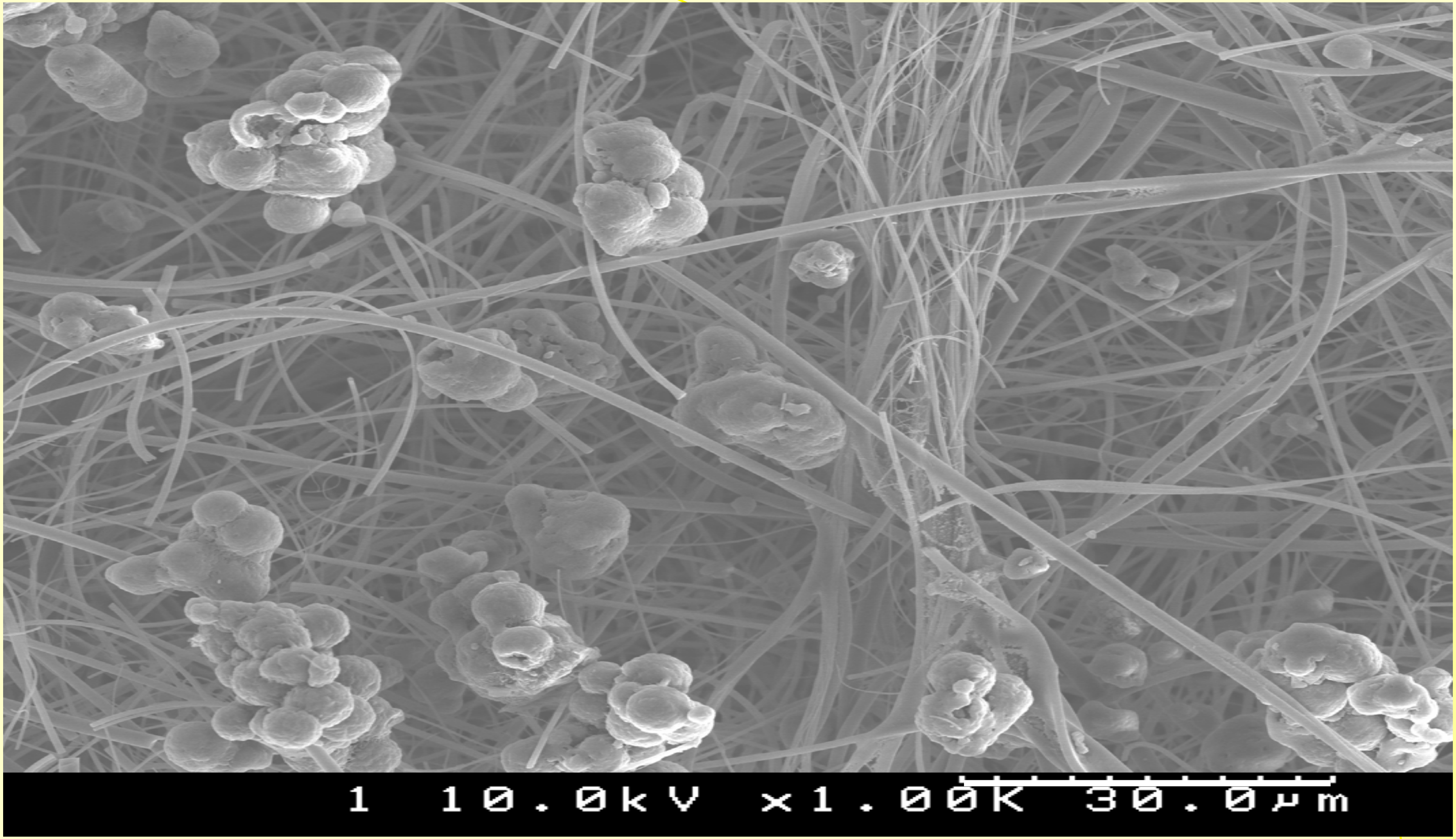
collecting & packing

agitation

(Arashidani et al)

Courtesy to T. Kobayashi, NIES

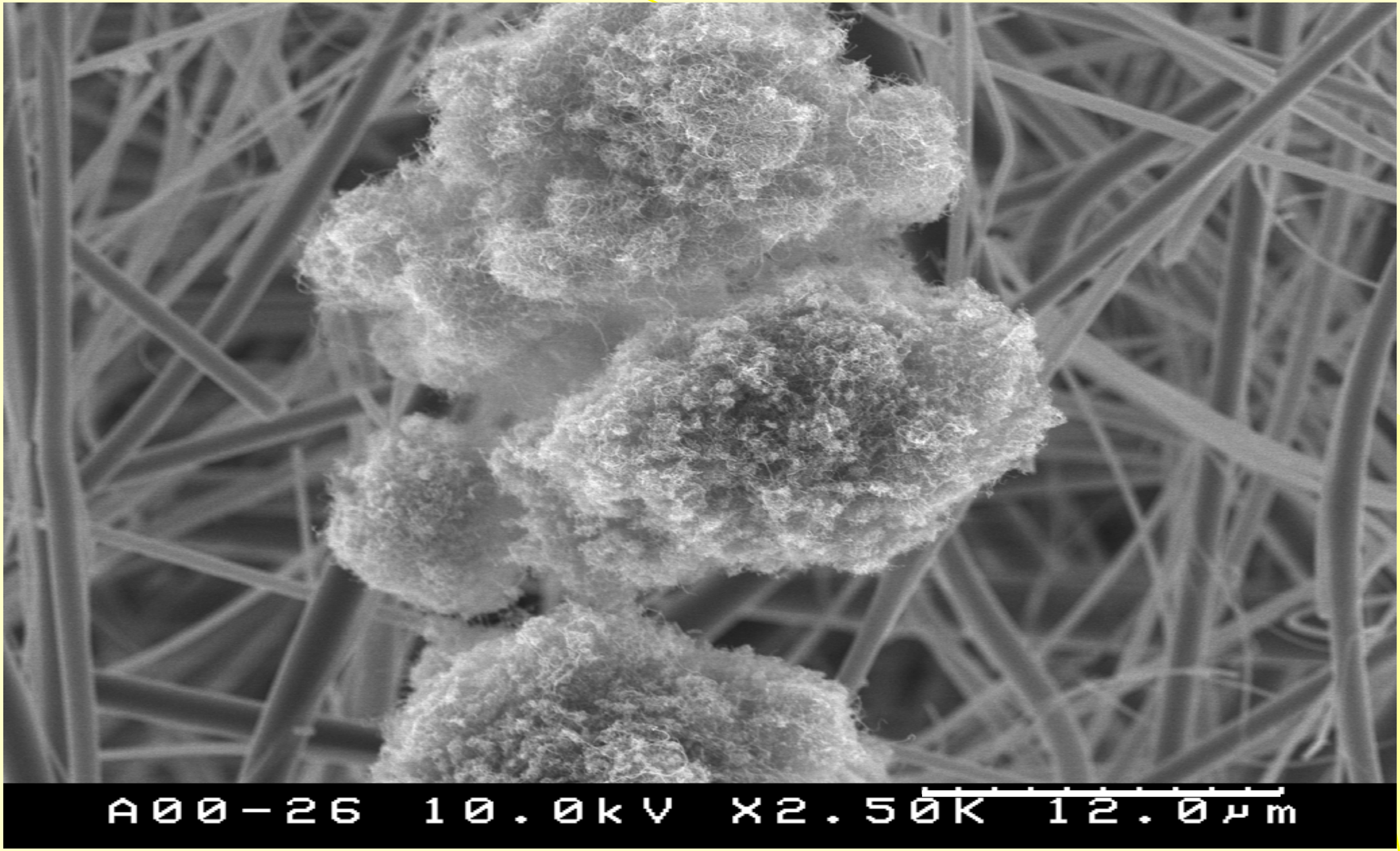
Agglomerated Fullerene Particles



(Arashidani et al)

Courtesy to T. Kobayashi, NIES

Agglomerated MWCNT Particles



(Arashidani et al)

Courtesy to T. Kobayashi, NIES

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