Development of massive synthesis method of organic nanotube toward practical use

— Integration of molecular design, molecular synthesis and safety assessment for materials having market competitiveness —

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Organic nanotubes are hollow fibers formed through the self-assembly of amphiphilic molecules in a solvent. Because nanoparticles and proteins can be included within their hollow interiors, such nanotubes can be applied to a wide range of fields. To promote the practical use of organic nanotube, we have developed a strategic scenario that fulfills several conditions, including mass synthesis (by integrating molecular design, synthesis, and self-assembly technologies), practical use, low cost, and safety.

Keywords : Organic nanotube, mass synthesis, self-assembly, inclusion, safety assessment

1 Research objective

Organic nanotubes are hollow-fiber substances formed through spontaneous aggregation (or self-assembly) of amphiphilic molecules that feature both water-soluble (hydrophilic) and oil-soluble (hydrophobic) moieties, much like surfactant molecules.Although the sizes of organic nanotubes differ according to the type of molecule used, in general they possess interior diameters of 10-200 nm, exterior diameters of 40-1000 nm, and lengths of up to several hundreds of micrometers[1].

Amphiphilic molecules have good dispersibility in water because they possess a cylindrically arranged bilayer membrane structure with the hydrophilic parts facing the solvent (Figure 1).

The cyclodextrins, cyclic molecules comprising six to eight glucose molecules arranged in a ring, are used widely in food, drug, and home products. Because various organic low molecular components can be incorporated within the hollow space of a cyclodextrin, unstable substances can be stabilized, drugs and fragrance can be released slowly, and substances that do not dissolve readily in water can be solvated[2]. The hollow space within an organic nanotube is 10 times larger than that within a cyclodextrin; therefore, the former can incorporate large substances, such as proteins, nucleic acids, viruses, and metallic nanoparticles, that do not fit in the cavities of cyclodextrins. In previous studies, we incorporated gold nanoparticles (sizes: 1-20 nm)[3][4] and the spherical protein ferritin (diameter: 12 nm)[5] into organic nanotubes (Figure 2). Through their development and application in agriculture, foodstuffs, healthcare, medicine, and the environment, we suspect that organic nanotube-based materials will become new, commercially competitive products.

The discoveries of the unique structures and properties of fullerenes (1984), which led to the award of the Nobel Prize in Chemistry in 1996[6], and carbon nanotubes (1991)[7] were major nanotechnological breakthroughs; R&D activity continues apace to develop the practical applications of these materials. Notably, although organic nanotubes were developed[8]-[10] in 1984—several years prior to the discovery

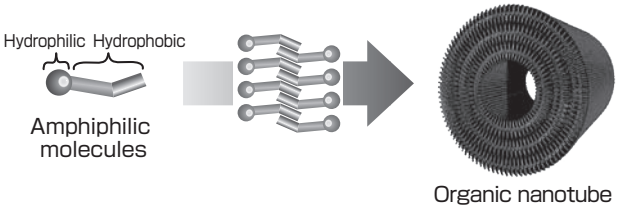

Fig. 1 Schematic representation of the formation of the organic nanotube structure.

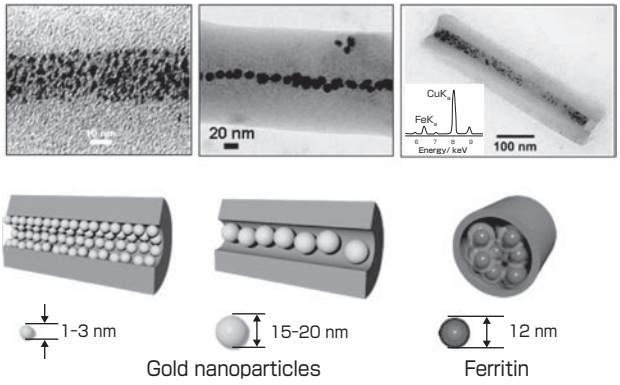

Fig. 2 TEM images displaying the inclusion of (left, middle) gold nanoparticles of various sizes and (right) the spherical protein ferritin within organic nanotubes.

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of carbon nanotubes–they have not been put to practical use, primarily because organic nanotubes have been difficult to mass-produce successfully, unlike carbon nanotubes; therefore, relatively little research has been performed into the practical applications of organic nanotubes or into comparisons with existing materials. Resolving this issue would make it possible to verify the potential of using organic nanotubes as materials for its practical applications.

The objective of this study was to create a new industry involving the practical use of organic nanotubes. To do so, a mandatory requirement was the development of a method for the mass synthesis of organic nanotubes. Also, it was necessary for us to develop low-cost molecules for the synthesis of the organic nanotubes, to perform utilization development, and to demonstrate their safety.

2 Goals and scenario to achieve the goal

For the practical application of organic nanotubes, it is necessary for companies to actually use these materials in trial runs so that they can be accepted as candidates for product development. To promote trial use by companies, it was necessary to develop a mass synthesis method that enables a steady sample supply and provides functions that can be employed in various fields, while fulfilling such practical considerations as cost competitiveness and safety. Thus, it was necessary to determine an optimal molecular structure for organic nanotube synthesis through molecular design and synthesis and then to produce a low-cost molecule by simplifying the synthetic process to cut costs. Also, it was important to lower the entry barrier for industrial use by conducting a safety assessment and sharing the information (Figure 3).

To achieve the aforementioned goals, we first developed a molecular design and synthesis technology using amphiphilic molecules for the fabrication of organic nanotubes with considerations of economy, safety, and mass production. The policy we employed for molecular design was to use a material resource that was recyclable, naturally occurring, and abundant (i.e., available at low cost from a reagent supplier). We established a working hypothesis that amphiphilic molecules synthesized from naturally occurring raw materials would be safe.

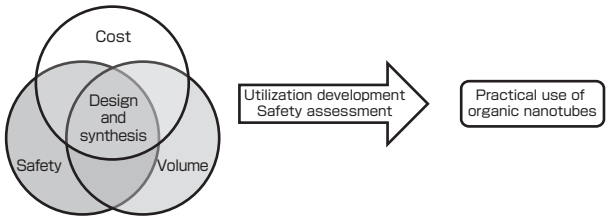

Fig. 3 Schematic representation of the development and application scenario.

Next, we developed a process—an improvement of the established self-assembly method—to enable the mass synthesis of organic nanotubes from amphiphilic molecules. We optimized four phases of the organic nanotube synthesis to increase efficiency and enable mass production: (i) dissolving the amphiphilic molecule in a solvent; (ii) forming organic nanotubes through self-assembly of amphiphilic molecules; (iii) separating the organic nanotubes from the solvent; and (iv) drying the organic nanotubes. Not only would the development of a suitable large-scale synthesis enable both economy and mass production, it would also open up the possibility of developing organic nanotubes in various fields–where investigations have previously been limited by insufficient amounts of materials (e.g., 100 mg or less)[11]–and enabling appropriate safety assessments.

Third, for the practical application of organic nanotubes in various fields, it was important to recognize their superiority through comparisons with the properties of conventional materials. For utilization development, it is important that companies use the materials in trials to determine whether they can be applied in actual situations. Therefore, it was necessary for us to provide companies developing conventional materials with organic nanotubes and to demonstrate the efficacy of organic nanotubes through joint research opportunities. To encourage the companies to experiment with the materials, we undertook an active promotion campaign, presenting organic nanotube technology at conferences and exhibitions.

Finally, because organic nanotubes are new materials, we knew that they would not be accepted in society unless their safety was established–even if their effectiveness was recognized for utilization development[12]. Therefore, we conducted safety assessments of the amphiphilic molecules and organic nanotubes that we synthesized from naturally occurring raw materials. We shared this safety information with relevant companies to lower the entry barrier for this technology.

To satisfy the first and second phases of the scenario, it was necessary for us to conduct several tasks simultaneously. Because organic nanotubes exist in the field of supramolecular chemistry, which studies molecules as component units and investigates their assembly functions based on interactions with other molecules, we developed our molecular design, synthesis, and self-assembly methods by carefully investigating the molecular structure and its potential for self-assembly.

The third phase of scenario was to be investigated after completing the first and second phases. Because heteronomous factors had to be solved by comparing the third phase with first and second phases, organized and strategic efforts were necessary for rapid assessment.

The fourth phase was an extremely important aspect of the potential practical application of the organic nanotubes. Although the safety assessment items differed from field to field, we selected common features in the initial phase to motivate the manufacturers to employ our organic nanotubes.

3 Functions to be realized and synthesiological method

3.1 Main elemental technologies

We selected the following five elemental technologies for the practical application of organic nanotubes: (1) technology for the molecular design and synthesis of amphiphilic molecules for the fabrication of organic nanotubes using naturally occurring, recyclable raw materials; (2) technology for the efficient synthesis of organic nanotubes through the self-assembly of amphiphilic molecules; (3) selection of elemental technologies necessary for utilization development; (4) selection and implementation of safety assessment items to widely promote organic nanotubes; and (5) selection of a timely technological transfer policy and appropriate research management for product realization research.

3.2 Integrated system and realized function

Each elemental technology should ideally proceed sequentially, from (1) molecular design and synthesis to (2) self-assembly to (3) utilization development to (4) safety assessment and then to (5) product realization. In actual research, however, things almost never progress in an orderly manner. For the progress of an elemental technology in actual research, planning of the working hypothesis and verification through experiment are repeated between stages (1) and (2). New knowledge is obtained through such repetition (*Type 1 Basic Research*); the research process moves on to stages (3) and (4) when satisfactory answers are obtained. In our R&D study for the practical applications of organic nanotubes, after developing the elemental technology in stage (3), heteronomous factors increased because the process progressed to a phase in which goal achievement became a prime motive (*Type 2 Basic Research*). Specifically, technology was refined further by feedbacks through fusion with conventional technologies to meet demand, comparison with conventional competitive materials, and managerial decisions (Figure 4). For stage (4), existing information using conventional assessment technologies and comparable assessment methods should be selected at the initial phase because there is very little room for the development of new technology. When utilization development progresses in stage (3) and demand arises in new fields, new safety assessment methods must be developed in stage (4) in collaboration with practitioners in relevant fields.

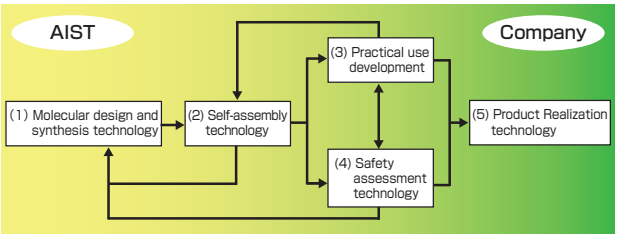

Fig. 4 Conceptual representation of the integration of the main elemental technology for the development and application of organic nanotubes.

4 Research results

4.1 Molecular design and synthesis of amphiphilic molecules for the fabrication of organic nanotubes

A few years ago, we discovered a way to selectively form organic nanotubes through self-assembly in water using cardanol/glucose-based amphiphilic molecule 1 synthesized from glucose and cardanol extracted from cashew nut shells.[13] The characteristic function of this amphiphilic molecule was the selective formation of tubular structures in water; nevertheless, the thermal stability was low, with a gel–liquid crystal phase transition temperature in water of 40 °C. In other words, when the sample was heated to 40 °C, the structure changed from tubes to liposome-like spheres; we determined that we could not put these organic nanotubes to practical use. Therefore, we designed and synthesized the amphiphilic molecule 2, in which hydrogen bonding can occurs at the amide group, by replacing the benzene ring linking the glucose unit and the alkylene chain in amphiphilic molecule 1 with an amide unit. As expected, the organic nanotubes created from the amphiphilic molecule 2 was much more heat-stable than molecule 1, with its gel–liquid crystal phase transition temperature being ca. 70 °C[14]. Although we had solved the issue of thermal stability by using the

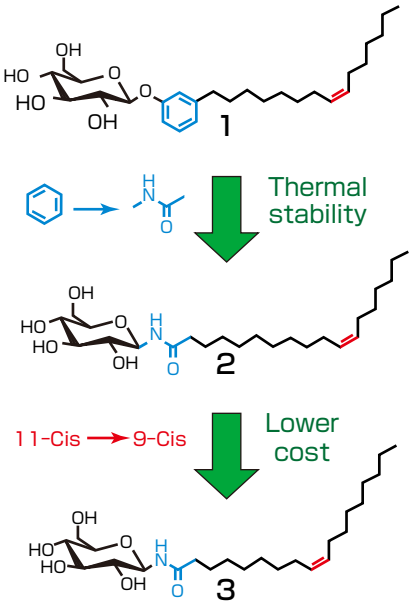

Fig. 5 Molecular design for practical application of amphiphilic molecules.

amphiphilic molecule 2, we departing from our initial plan of using naturally occurring, low-cost materials: the fatty acid raw material for molecule 2, cis-11-octadecenoic acid (*cis*-vaccenic acid), was expensive at 30,000 yen per gram. In an attempt to further optimize the molecular structure, we found that *cis*-9-octadecenoic acid (commonly known as oleic acid), which differs from cis-vaccenic acid only in the position of its carbon-carbon double bond, was available from olive oil at low cost; gratifyingly, the organic nanotubes synthesized using the amphiphilic molecule 3 incorporating this fatty acid exhibited its gel-liquid crystal phase transition at a temperature of ca. 70 °C, i.e., it exhibited satisfactory thermal stability (Figure 5).

4.2 Self-assembly of organic nanotubes

With the discovery of the amphiphilic molecule 3 that was thermally stable and could be synthesized from naturally occurring, low-cost materials, we set out to investigate an efficient method for the fabrication of organic nanotubes.

In the conventional method, organic nanotubes are obtained after heating amphiphilic molecules in water until they dissolve, waiting until the organic nanotubes had formed through self-assembly in solution and precipitation, and then collecting and drying them. There were three problems with this synthetic method: the solubility of the amphiphilic molecule in water was poor; a long time was required for the organic nanotubes to form through self-assembly in solution; and it was difficult to dry the organic nanotubes collected from the solution.

To overcome these drawbacks, we investigated the self-assembly of this amphiphilic molecule in other solvents. Indeed, we found that using alcohol as the solvent solved each of these problems; i.e., alcohol dissolved the amphiphilic molecules well, the self-assembly progressed rapidly, and the collected organic nanotubes were readily dried[15].

Using this new synthetic method, we could easily synthesize over 100 g of the organic nanotubes in the laboratory, whereas previously it required much effort to produce 1 g (Figure 6).

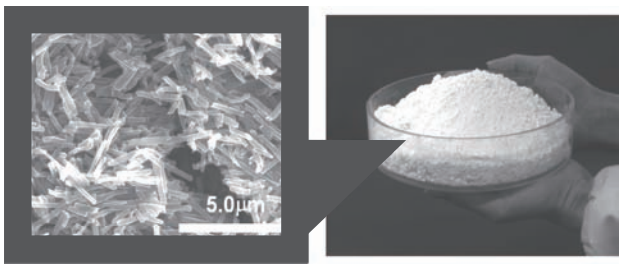

Fig. 6 SEM image (average exterior diameter: 300 nm; average interior diameter: 90 nm) and a photograph of the organic nanotubes prepared in the form of a white solid power (weight: ca. 140 g).

4.3 Utilization development of organic nanotubes

Once we were able to mass-synthesize the organic nanotubes, we conducted a publicity campaign—through press releases and announcements at exhibitions—to attract companies that might have been open to using the material. We made preparations and began to supply samples to various companies in 2007. When providing samples, a material transfer agreement (MTA) was signed between the research institute and each company. The agreement required a specific description of the utilization development and took appropriate measures to understand the fields in which conflicts of interest in intellectual property rights may occur in the future.

As result of sample provision, issues in utilization development for different fields became apparent, and we also found that the time required for practical application differed from field to field. Where practical application could be determined quickly, it was important to optimize supply with demand to enable rapid technological transfer.

For our own utilization development of the organic nanotubes, our research team investigated (a) the preparation of fluorescent organic nanotubes and (b) methods for the decomposition of organic nanotubes under mild conditions, both useful R&D tools in the field of nanobiology, in addition to studying their dispersibility in water and guest inclusion properties.

(a) Development of fluorescent organic nanotubes. This technology involves creating fluorescent organic nanotubes by adding fluorescent molecules during the self-assembly of amphiphilic molecules in organic solution, i.e., during the mass-synthesis of the organic nanotubes (Figure 7). We suspect that these materials will be useful as a research tool for utilization development in the field of nanobiology, where important information can be obtained regarding the stability and behavior of organic nanotubes in vivo through observation of cells tagged with fluorescent organic nanotubes.

(b) Method for the decomposition of organic nanotubes. The organic nanotubes form spherical structures when they are heated above their gel-liquid crystal phase transition temperature (ca. 70 °C) in water. While investigating a mild and safe decomposition method, we found that the organic nanotubes transformed into a plate structure when a cyclodextrin solution was added. The tube structure

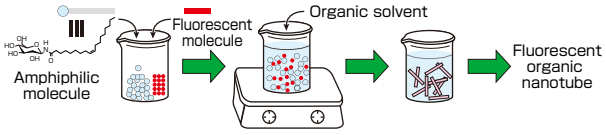

Fig. 7 Cartoon representation of the process used to manufacture fluorescent organic nanotubes.

disassembled when the amphiphilic molecules that comprised the organic nanotubes were included into the cavities of the cyclodextrin (Figure 8). We suspect that the range of applications of these organic nanotubes as functional materials will increase with the discovery of this simple decomposition method performed under mild conditions.

4.4 Safety assessment of organic nanotubes

The safety assessment items for our organic nanotubes were discussed and decided in conference at AIST with departments related to technology transfer, such as the Collaboration Promotion Department, Intellectual Property Department, Technology Licensing Organization, and Technology Information Department.

As result, we conducted four safety tests: (i) biodegradation tests using environmental microorganisms according to "Methods of Testing New Chemical Substances," Chemical Substance Control Law (CSCL), which is required if materials are to be synthesized at scales greater than 1 ton; (ii) oral acute toxicity tests using rats to meet requirements for use in food and drugs; (iii) ecotoxicity tests to assess the impact on aquatic organisms that are most likely to be influenced by exposure to the substance in the environment; and (iv) reverse mutation tests to assess mutagenicity[Note 1)].

In the biodegradation tests using environmental microorganisms, we found that our organic nanotubes had almost no effects on humans, animals, or plants because they were almost completely degraded by environmental microbes within 28 days of release into the environment. In the oral acute toxicity tests, we observed no fatalities in the 2 weeks following oral administration of 5000 mg/kg organic nanotubes to rats. The acute toxicity was extremely low, with a least lethal dose (LDLo) of 5000 mg/kg or higher for both male and female rats. In the ecotoxicity tests using algae, water flea (*Daphnia magna*), and orange rice fish (*Oryzias latipes*), we tested a 100 mg/L organic nanotube solution for its 72-hour growth inhibition, 48-hour acute mobility inhibition, and 96-hour acute toxicity; we observed no growth inhibition, no mobility inhibition, and no acute toxicity. The reverse mutation test for mutagenicity was negative.

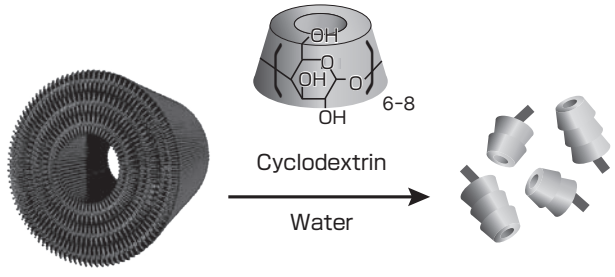

Fig. 8 Decomposition of organic nanotubes through the addition of cyclodextrin.

5 Discussion: Comparison of research results and scenario

By conducting *Type 1 Basic Research* into the development of a molecular design and synthesis technology for amphiphilic molecules that self-assemble into organic nanotubes, we succeeded in designing and synthesizing low-cost amphiphilic molecules using naturally occurring, recyclable raw materials. Moreover, by integrating *Type 1 Basic Research* into the development of a self-assembly technology with this molecular design and synthesis technology, we achieved the mass synthesis of organic nanotubes (*Type 2 Basic Research*). With these results, we enabled R&D into the practical use of these organic nanotubes.

To solve the issues relating to utilization development, we are in the process of providing samples to companies, organizing issues that became apparent through communications with companies, and working on R&D to solve problems. To overcome the unique issues that arise in each field of utilization development, we will continue to cooperate with related fields and companies and develop appropriate research systems.

For safety assessment, we conducted four tests: biodegradation tests using environmental microorganisms, oral acute toxicity tests using rats, ecotoxicity tests to assess the impact on aquatic organisms, and reverse mutation tests to assess mutagenicity. Safety was confirmed in all tests. The results have been regarded highly by many people when we have provided samples to companies; in addition, consideration of safety concerns from an early stage has proven to be useful in lowering the entry barrier to industry. Also, safe use of amphiphilic molecules synthesized from naturally occurring raw materials, as we had hypothesized in the molecular design phase, was proven in this case.

For technological transfer policy, it was difficult for us to set a timeline when creating the schedule and, at present, it remains difficult to provide an optimal solution because so-called people, thing, and money factors are involved, and there are complex time factors that depend on utilization development. In this research subject, as a result of seeking potential applications of our organic nanotubes by transferring the material widely, it became clear that some fields were capable of implementing utilization quickly while others needed considerably more time. Our priority in advancing technological transfer was to optimize supply with demand by extracting R&D elements for fields that could implement utilization most quickly. Moreover, for rapid technological transfer, we decided to establish a joint research system based on a material transfer agreement, developed a system for open-type joint research, and began our own R&D examinations.

6 Future issues

For research into the practical use of organic nanotubes, a new material that has never been used in the world, we selected an open R&D method where utilization development was sought openly through material transfers, rather than a closed R&D method within a research institute, because we expected that applications would be possible in a wide range of fields. This approach became feasible after we had achieved the mass synthesis of organic nanotubes. At the same time, this mass synthesis enabled safety assessments to be performed at early stages of the R&D, enhancing the potential for practical use of our organic nanotubes and helping to raise the awareness and acceptance by companies.

In the future, based on feedback obtained from the companies that accepted our samples, we shall (i) extract elemental technologies for utilization development and investigate solutions and (ii) develop Product Realization Research through collaborations with these companies. Also, we shall seek out fields where markets might form quickly and slowly. In the quick fields, we must prepare a system for supply to meet demand; in the slow fields, we must conduct R&D to accelerate the process through collaborations with universities. In particular, for the creation of a new industry, we must consider the development of new safety assessment methods and industrial standards for organic nanotubes, and we shall continue R&D through collaborations with practitioners in related fields and to collect/accumulate necessary information. Also, based on the concept of minimal manufacturing[Note 2)], we shall refine the synthetic method for the preparation of the organic nanotubes and create a new industry through the practical application of organic nanotubes by providing high added value through efficient synthesis, process development, and size control.

Acknowledgements

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Notes

Note 1) Tests were subcontracted to specialized testing organizations; they were conducted according to the following methods:
Biodegradation tests: "Biodegradation Test of Chemical Substance by Microorganism" in *Methods of Testing New Chemical Substances*, CSCL.
Oral acute toxicity tests using rats: "On Guidelines for Single and Multiple Dose Toxicity Test," appendix to *Guideline for Toxicity Test for Drugs* and "On Revisions to Guidelines for Single and Multiple Dose Toxicity Test."
Ecotoxiciy tests: For acute toxicity tests of fish, "Fish, Acute Toxicity Test," *OECD Guideline for Testing of Chemicals 203* (1992); for the sea flea, "*Daphnia* sp., Acute Immobilization Test," *OECD Guideline for Testing of Chemicals 202* (2004); for algae growth inhibition tests, "Freshwater Algae and Cyanobacteria Growth Inhibition Test," *OECD Guideline for Testing of Chemicals 201* (2006).

Note 2) National Institute of Advanced Industrial Science and Technology: Part III 3: Nanotechnology, Material, and Manufacturing Fields, Kenkyu Senryaku (Phase 2 Research Strategy 2008). http://www.aist.go.jp/aist_j/information/strategy_revise.html

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Masumi Asakawa
Joined the National Institute of Materials and Chemical Research, AIST, in 1996. Engaged in research on molecular elements and molecular assembly using supramolecular chemistry methods to study assembly function based on molecular interactions. After experiencing major responsibilities as head of the planning section in 2004, started the development and practical applications of the mass synthesis of organic nanotubes. In this paper, worked on the mass synthesis, safety assessment, utilization development, and promotion for practical use, and was in charge of the overall conceptualization.

Masaru Aoyagi
Joined AIST in 2001. Worked on research into molecular recognition (self-assembly, inclusion chemistry) by monomolecular membranes at air-water interface and the development of related sensor systems. Recently worked on refining the organic nanotube synthesis and investigating and assessing phenomena (e.g., adhesion, release) caused by organic nanotubes and other substances. In this paper, worked on developing the synthesis process and practical applications.

Naohiro Kameta
After joining the JST-SORST project, has worked on the development of chemical processes for tailor-made organic nanotubes that can include and release biomacromolecules (e.g., proteins, DNA) upon exterior stimulus. Also working to clarify the properties of nanospace, such as assessing the dynamic behavior and stability of protein inclusion in the cylindrical hollow spaces inside organic nanotubes. In this paper, worked on manufacturing the fluorescent organic nanotubes that may become major tools in the field of nanobiology.

Masaki Kogiso
After joining the National Institute of Materials and Chemical Research, AIST, in 1995, has been working on research into one-dimensional nanostructure formation through self-organization of peptide lipids; i.e., forming nanostructures never seen in the world before, through simple methods, from simple compounds. As result, was the first to mass-produce organic nanotubes, leading to this *full research project* from basic research performed in the laboratory. Currently planning the construction of an organic nanotube library with various surface functional groups using a simple peptide lipid incorporating glycylglycine. In this paper, worked on the development of the mass synthesis and its practical uses.

Mitsutoshi Masuda
After joining the Research Institute for Polymers and Textiles, AIST, in 1992, worked on the functionalization through polymerization and formation of nanofibers and the preparation of nanofibers through self-organization of, for example, dual-headed glycolipids and aromatic amides. Developed asymmetrical interior/exterior surfaces of organic nanotubes and selective modification of their interior surfaces. Currently planning elemental technology to determine the physical properties and practical applications of nanospaces within organic nanotubes. In this paper, worked on the molecular design and synthesis technology of amphiphilic molecules.

Hiroyuki Minamikawa
After joining the Research Institute for Polymers and Textiles, AIST, in 1988, worked on molecular design and synthesis, lipid molecule assembly, liquid crystal structures, functional analysis, and colloid chemistry using functional lipids (e.g., glycolipids). Currently working on interactions between lipid assemblies and biomacromolecules. In this paper, worked on the structural correlation assessment and property assessment based on the molecular design of the organic nanotubes.

Toshimi Shimizu
Joined the Research Institute for Polymers and Textiles, AIST, in 1977. Head of the Nanoarchitechtonics Research Center, AIST, from 2001. Coordinator of the Nanoarchitechtonics Research Center from 2008. Worked as a researcher representative of JST-CREST and JST-SORST of the Industrial Science and Technology Frontier Program from 1996, and has made a steady effort to pioneer and develop bottom-up nanotechnology. In this paper, worked as research coordinator for optimizing the molecules for fabricating the organic nanotubes and their nanobiological applications.

Discussion with Reviewers

1 Thinking on the range of R&D that should be done by AIST leadership

Question (Kazuo Igarashi)

Item (5) of Figure 4 is Product Realization technology, but after reading the paper to the end, I see only mention of technological transfer policy for Product Realization. Please explain the positioning of Product Realization as described here and the range of R&D that should be done by AIST as perceived by the Authors.

Answer (Masumi Asakawa)

In Figure 4, the transfer of the contributions from the AIST to the companies is expressed as a color change from yellow to green. We believe that Product Realization in stage (5) should be performed primarily by the companies. Therefore, in this paper, we discuss the need to establish a technological transfer policy and research management system that enables rapid Product Realization by the companies after phases (3) and (4).

Therefore, the issue remains regarding the extent of AIST's involvment in certain phases, but, because we believe that the companies should take initiative in Product Realization, in this paper we describe the phases immediately prior to Product Realization.

2 Measures against nano-risk for organic nanotube

Question (Kazuo Igarashi)

On safety assessment technology in Figure 4, it is written: "Existing information using conventional assessment technologies and comparable assessment methods should be selected at the initial phase because there is very little room for the development of new technology." However, I do not think assessment methods differ by each development phase. Does this mean the level of

evaluations to be done?

Also, in Section 4, you conducted safety tests on rats, and according to the paper, it seemed that the Authors conducted the tests in their own laboratory. There is no problem if this is correct, but if the tests were subcontracted to outside institution, you should state clearly that they were subcontracted. In addition, what is the reliability of the tests?

Answer (Masumi Asakawa)

Safety assessment technologies for nanosized materials, including organic nanotubes, have not been established firmly in every field. While the reactivity and permeability are expected to increase as a result of the nanoscale dimensions, unexpected effects must also be considered. For example, when using a nanomaterial in food or medical fields, while we can expect certain effects that are due to the size, it is necessary to develop assessment technologies to protect against unexpected effects.

Currently, we are working on an investigation system with researchers from the National Agriculture and Food Research Organization. We have also established a new subcommittee, "Food Nanotechnology," in the Materials Forum at AIST to investigate the relationship between food and nanotechnology.

We subcontracted the safety tests to outside institutions, as stated in Note 1. Concerning their reliability, we have added additional information regarding the test methods.

3 Understanding of *Type 1* and *Type 2 Basic Research* and scenario composition

Question (Hisao Ichijo)

I understood that design, synthesis, and structure-function analysis conducted in the early stage of organic nanotube research are *Type 1 Basic Researches*, and "mass synthesis" conducted based on these findings is *Type 2 Basic Research*, but were they positioned as *Type 1 Basic Research*? In Section 2, it is mentioned that working hypothesis and experiments were repeated between (1) and (2), but does this mean that optimal method was found by trial-and-error? Moreover, it is written that utilization development is *Type 2 Basic Research*, and Figure 4 shows it is done mostly by companies. It is described that only the development of Fluorescent organic nanotube is utilization development and no other specific examples are given. Are there other researches?

Answer (Masumi Asakawa)

(1) *Type 1 Basic Research* and positioning:

If it is judged that "mass synthesis" was achieved through the integration of two forms of *Type 1 Basic Research*, namely (i) molecular design and synthesis technology and (ii) self-assembly technology, then I believe that it can be considered as *Type 2 Basic Research*.

(2) Finding optimal method by repeating working hypothesis and experiment, and by trial-and-error:

Yes, the optimal procedures and materials were developed this way. Once the concept and landing point of a research program have been determined, no matter where the starting point is, the landing point is approached using a so-called PDCA (plan-do-check-act) cycle that involves planning out a working hypothesis, doing the experiments to investigate the hypothesis, checking the results, and acting on the issues. The optimal solution for the starting point obtained between stages (1) and (2) will become a greater cycle as it progresses on to stages (3) and (4), and we can bring stage (5) closer to an optimal solution that will satisfy our customers. I believe that how we determine the starting point depends to a great extent on the background of the research conducted so far, as well as on the experience and insight of the researcher.

(3) Other research:

In Figure 4, we present, in a mixture of yellow (AIST) and green (company) hues, how much can be achieved through collaboration of the AIST with external companies for utilization development. Other research includes the development of suitable methods for organic nanotube decomposition through the addition of a cyclodextrin. We have described a specific example.