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Development of a bovine sperm selection procedure for improvement of livestock fertility

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Measurement of mass of aerosol particles

2019

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Highlights of the Papers in *Synthesiology*

Synthesiology is a journal that describes the objectives, specific scenarios, and procedures of research activities that attempt to utilize the results in society, in particular, the process of synthesis and integration of elemental technologies for practical application. To allow the readers to see the value of the papers in a glance, the highlights of the papers that characterize *Synthesiology* have been extracted.

***Synthesiology* Editorial Board**

Research paper: A new process to develop a hydraulic system adapted to biodegradable hydraulic oil for construction machinery
—Case study integrating component analyses and SysML description in failure analyses—
Ohkawa Satoshi *et al.*
From the perspective of environmental pollution, there are many countries that are enforcing the use of biodegradable hydraulic oil or bio-oil in construction machinery. However, the mechanism of failure when bio-oil is used is complex, and it was difficult to conduct analysis that led to countermeasures using conventional failure analyses such as FTA. This paper describes a scenario for using SysML and SafeML to explore the causes of failure when bio-oil is used, and thereby arriving at countermeasures.

Research paper: Development of a bovine sperm selection procedure for improvement of livestock fertility
Nagata MariaPortia B. *et al.*
This is a description of the development of technology to sort highly motile spermatozoa in frozen sperms to improve cattle reproductivity. It is accomplished by gathering sufficient number of spermatozoa that can be used directly for artificial insemination using fluid handling technology. From the planning stage, a synthetic R&D was conducted, and it was assumed that this technology would be applied at sites of animal breeding where conditions such as individual animals and environment cannot be controlled. Moreover, good pregnancy rates were obtained in the demonstration test. It is an interesting paper in which the characteristic of spermatozoa that have advantage in conception was linked to their swimming form.

Research paper: Efforts toward commercialization of antifreeze proteins
Ishii Hirotaka *et al.*
The paper summarizes the efforts toward industrial application of antifreeze protein. It describes the course in which the scenario was changed from the efforts for practical use in the frozen foods field, to the efforts for practical use in new application fields. This paper was initially submitted as a "report" but was published as "research paper" since it showed the authors' originality in the scenario and component elements (selection and integration).

Research paper: Measurement of mass of aerosol particles
Ehara Kensei *et al.*
The paper describes the conception of measurement principles of the aerosol particle mass analyzer to measure aerosol particle mass that fell in the range of 0.1 μg or less that could not be measured before. It also describes cases to which the developed device was used to evaluate the properties of various particles. As the definition of the kilogram was revised for the first time in 130 years, concerning the standard of mass, this is a timely paper in terms of pioneering a new measurement field.

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Contents

A new process to develop a hydraulic system adapted to biodegradable hydraulic oil for construction machinery

—Case study integrating component analyses and SysML description in failure analyses—

Ohkawa Satoshi*, Sunkil Yun, Hibiya Taketoshi and Nishimura Hidekazu

[Translation from *Synthesiology*, Vol.12, No.2, p.57–74 (2019)]

Causes and countermeasures for various kinds of hydraulic system failures in construction machinery generated by biodegradable hydraulic oil (bio-oil) are discussed. Previous component analytical methods can prevent all kinds of failures except hydraulic system malfunction, which requires a holistic approach for analysis. Using the Systems Modeling Language (SysML), the cause of malfunctions can be understood, and the most effective countermeasure can be obtained. Integration of a component analysis method and a holistic analysis method for system development is proposed.

Keywords : Hydraulic system, construction machinery, biodegradable hydraulic oil, malfunction, countermeasure, SysML

1 Introduction

A vehicle type construction machinery can travel by rotating crawlers (or tires) and can excavate using a bucket, as shown in the example of a hydraulic excavator of Fig. 1. The mechanisms for motion and excavation are driven by high-pressure oil (34–45 MPa), and the operator performs complex maneuvers by operating multiple hydraulic pilot valves. Concerning such construction machinery, environmental pollution becomes an issue if the oil is released into the environment when hydraulic oil in the hydraulic system is changed on site or when the hose is damaged by hitting rocks during the operation. Specifically, damages include contamination by oil film of drinking water, death of fish, or withering of plants in forests or agricultural fields.

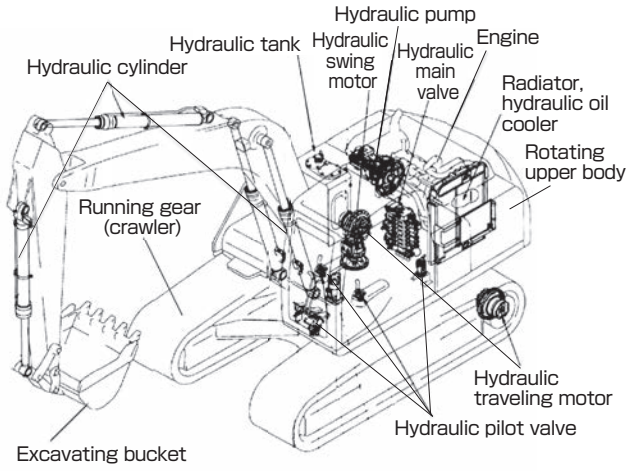

Fig. 1 Exterior of construction machinery and arrangement of hydraulic devices[1]

Therefore, in the 1990s, Germany, Switzerland, and Austria required the use of biodegradable hydraulic oil (hereinafter called bio-oil) in construction machinery, as such oil is broken down in a short period into carbon gas and water by natural microorganisms.[2][3] This was enforced through administrative actions of local governments[5] based on Germany's Federal Water Act.[4] The mandatory use of bio-oil in construction machinery has affected the entire EU regions and spread throughout the environmental protection areas.[3] In 2002, the ISO standard was set for the quality standard of bio-oil.[3][6][7] In 2011, the EU eco-label, which is equivalent to the Japanese eco-mark, was given only to bio-oil products that passed over 40 items of environmental toxicity tests.[8]

Figure 2 is a schematic diagram of the relationships of stakeholders and the environment in which construction machinery is used in Europe. With stringent demands for use of bio-oil from the environmental protection authorities and pressure from neighborhoods, the users pressed the construction machinery manufacturers for a quick response. One of the authors was a lubricants researcher who responded by starting up a company project for bio-oil at Komatsu Ltd. The project team included people from the service, design, testing, factory, and purchasing divisions, and the countermeasures were also proposed with the cooperation of parts manufacturers. As a short-term measure, it was decided that an operation manual for handling bio-oil would be created within half a year, and as a long-term measure, that the hydraulic system would be improved in four to five years.

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The operation manual on bio-oil for service persons and users was prepared with consideration for the EU competition law[9] (equivalent to the antimonopoly or antitrust laws of Japan). Operating conditions of construction machinery are difficult due to rocky ground, forests, and water sources in the environmental protection areas, and oil leakages are likely to occur during maintenance work in poor conditions and the backwoods. The users had to determine the use or non-use of bio-oil according to the instruction of the environmental protection authorities, and selection of bio-oil, operation, and maintenance would be conducted based on the operation manual and guidance of service persons.

For bio-oil, rapeseed oil (glycerol fatty acid ester) and synthetic polyol esters (hereinafter, synthetic esters) are used as main ingredients (base oil). The base oil is decomposed by enzymatic reaction by microorganisms and is taken in as a nutrient (biological constituents) of microorganism (this process is called assimilation).[10] Rapeseed oil and some synthetic esters containing unsaturated fatty acids have low oxidation stability due to the presence of double bonds. Even commercial bio-oil that adopts synthetic esters of saturated fatty acids with high oxidation stability as base oil, the oxidation stability is lower compared to petroleum hydraulic oil. This is due to prohibition of additives by stringent environmental regulations,[1] and antioxidants of hindered phenol and aromatic amines that are generally used in petroleum hydraulic oil, or antioxidants and anti-seize agents of zinc dialkyldithiophosphate (ZDTP)[1] cannot be used from the perspective of aquatic environmental toxicity.[11] That is, the premise is that there is absolutely no oil leakage for petroleum hydraulic oil, while bio-oil is developed with the assumption that it may leak into the natural environmental such as rivers and marshes.

Depending on the type of synthetic ester base oil, some of the synthetic ester bio-oil shows lower viscosity increase against pressure rise (high-pressure viscosity[12]) compared to petroleum base oil.[13][14] Therefore, its oil film is thinner, and there is a disadvantage of causing seizure in the bearing metal of hydraulic motors and hydraulic pumps. Anti-seize agents such as ZDTP that compensate the thin oil film cannot be added due to the aforementioned reason. From such reasons, the commercial bio-oil has various issues such as low oxidation stability, rubber compatibility, a thin oil film and so on.[3][15][16][17] Failures are predicted if bio-oil is used without any measures taken to construction machinery that has complex hydraulic systems.

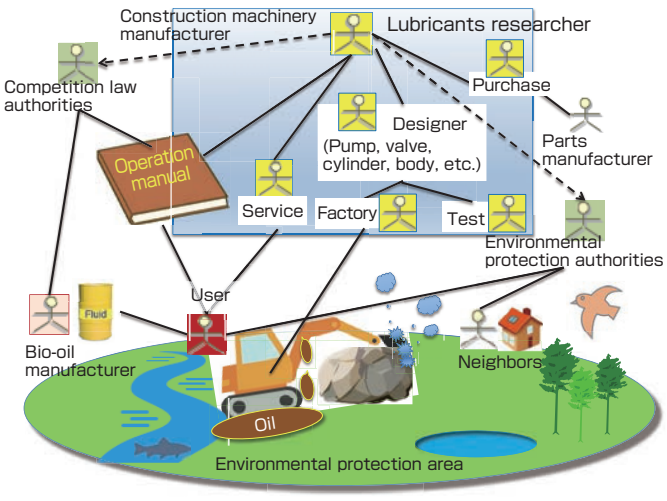

Fig. 2 Schematic diagram of stakeholders and environment in which construction machinery is used in Europe (solid line: direct relationship, dashed line: information gathering)

To develop a hydraulic system to which all bio-oils can be applied, the use of component analysis methods such as fishbone diagrams, fault tree analysis (FTA),[18] failure mode and effect analysis (FMEA)[19] were considered. Based on these analyses, we set out to develop equipment of the hydraulic system (hereinafter, subsystems) that comprises construction machinery that are compatible to bio-oil. FTA is a method for analyzing causes and conditions under which a phenomenon occurs by extracting the causes and factors of the established top events,[18] while FMEA is a method for clarifying the potential failure mode of system performance and its cause and effect.[19] These component analysis methods have been widely used by the automobile and construction machinery manufacturers since around 1980. In general, FTA is used to analyze the cause of failures, while FMEA is used to prevent failures during designing.

2 Scenario for development of construction machinery compatible with bio-oil

The development scenario of this study is shown in Fig. 3. There are several issues in terms of the quality of bio-oil. In the development of the operation manual as a short-term measure, directions for usage of bio-oil in construction machinery for each quality issue were determined, and brands of commercial bio-oil were categorized by quality. The short-term measures were terminated at the point when long-term measures were completed. The subjects of the long-term measures were mostly technologies of the hydraulic system of construction machinery. In relation to the production of construction machinery, there was a problem that bio-oil may mix with hydraulic oil added at the factory. For these issues, the factors of failures caused by bio-oil were analyzed by conventional FTA or FMEA to investigate and derive countermeasures. However, for failures that involve chemical properties (oxidation stability) of the bio-oil, in addition to the complexity of the hydraulic system comprised of multiple subsystems, the cause investigation was difficult using the conventional methods. Also, there was no appropriate method for creating and validating countermeasures for a failure. Therefore, in this paper, we attempted the analysis of this unsolved

Table 1. Problems in quality of bio-oil, and short-term and long-term measures implemented by the construction machinery manufacturers using component analysis method

No.	Required lubricant's qualities	Current bio-oil's quality	Field failure	Failure cause	Countermeasures implemented and content (○: Yes, ×: No)		Remarks
					Short-term measures	Long-term measures	
1	Oil-film strength	Low	Bearing metal seizure	・Low viscosity at high-pressure ・Low content of extreme-pressure additive	○*1	Expand bearing width	*1: Machinery operation manual for bio-oil *2: Recommendation for bio-oil brand
2	Rubber compatibility	Low	・Oil leak at seal ・Hose breakage	Strong affinity of ester base oil with rubber	○*1	Change rubber material	
3	Antirust	Low	Rust forming in hydraulic oil tank	Lack of antirust additive	○*1	Antirust treatment	
4	Anticorrosion for bronze	Low	Abnormal wear of bearing metal	Addition of active sulfur additive	○*1,*2	Improve bronze alloy	
5	Friction coefficient	Extremely low	Poor performance of parking break	Property of ester base oil	○*1	Improve brake material	
6	Compatibility with petroleum hydraulic oil	Precipitation	Filter plugging	Precipitation of calcium carbonate and calcium soap due to the reaction between ester base oil and petroleum hydraulic oil additives	○*1	Bio-oil filling at factory	

problem and the derivation of countermeasures from the perspective of systems engineering[21] of a multidisciplinary approach using the System Modeling Language (hereinafter, SysML),[20] which is a graphical modeling language that supports analysis, specification setting, design, verification and validation of a complex system.

3 Failure analysis and countermeasures using component analyses (FTA and FMEA) for each subsystem of hydraulic system

Table 1 is a summary of the problems No. 1–6 on bio-oil quality, the short-term measures and the long-term measures to develop subsystems compatible to bio-oil. The operation manual was prepared using the component analysis method based on a survey[17][22] of bio-oil quality through chemical analysis and laboratory tests and also investigation results of field failure. Prohibition of use of low-quality bio-oil brands was considered as an short-term measure, but this was not done because it may have led to local bio-oil companies suing the authorities for violation of competition laws. Therefore, as shown in Table 2, the operation manual outlined limitations of oil temperature and pressure during operation, shortening of oil change interval, and recommended brands for each quality level, and it was left up to the users to make the selections.[17] Since the users have come to select brands with

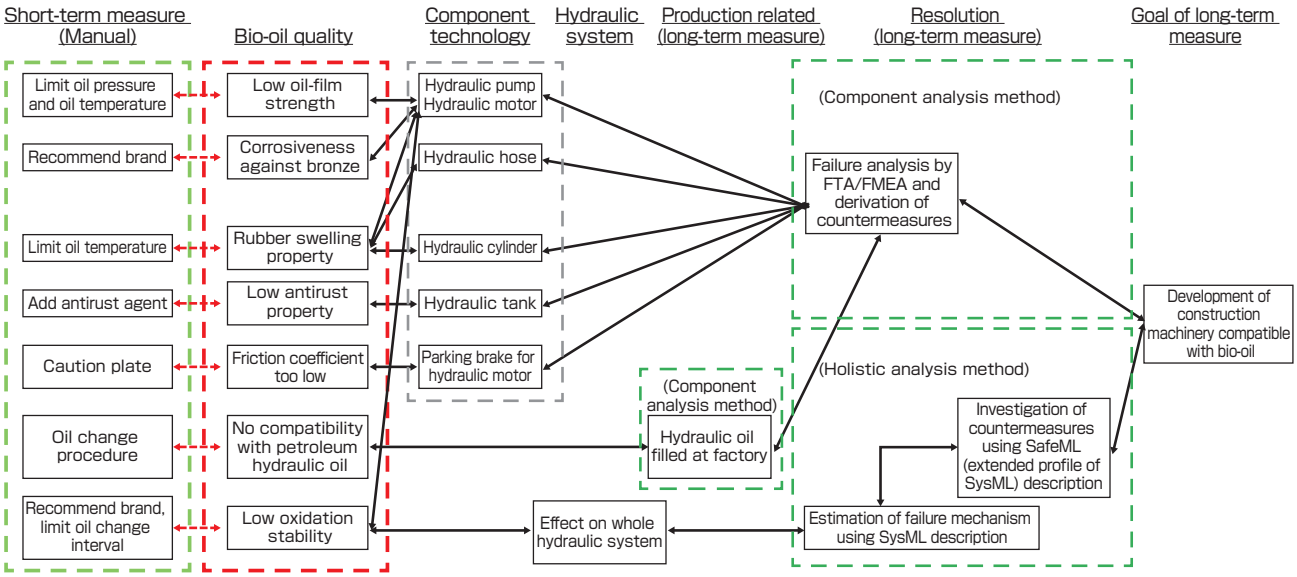

Fig. 3 Scenario for development of construction machinery compatible with bio-oil

Table 2. Bio-oil classification and recommended operating condition[17]

Quality grade	Base oil	Oil temperature °C	Oil pressure MPa	Oil change interval h	Parking brake
1	Rapeseed oil	-10+80	32 or less	1,500 or less	Attach caution plate, or replace to improved brake material
2	Rapeseed oil or synthetic ester		35 or less	3,000 or less	
3	Synthetic ester	-30+100			
4			42 or less	5,000 or less	Continue use as is

Table 3. Cause of bio-oil oxidation and short-term measures

Possible field failures	Causes of failure occurrence	Short-term measure
Discoloration of oil	・Unsaturated ester base oil ・Lack of anti-oxidant	Shortening of oil change interval in Table 2
Lead dissolving from bronze bearing	Corrosion and dissolution of lead by oxidation product	

quality grade 3 based on the recommendations, the operation manual was effective.

For the development of subsystems that allow the use of bio-oil, the long-term measures were planned and conducted using the component analysis method as shown in Table 1. For problem No. 1, low oil-film strength, we conducted design change of expanding the width (surface area) of bearing metal to eliminate complex verifications. For No. 2, rubber compatibility with bio-oil, it was found that the strengths of nitrile rubber (NBR) with low nitrile content and chloroprene rubber (CR) decrease significantly.[16] Therefore, these rubber parts were replaced to materials of hydrogenated NBR (HNBR) or NBR with high nitrile content for high oil resistance. For No. 3, poor antirust property of bio-oil, an antirust coating was applied inside the hydraulic oil tank. For No. 4, corrosion of bronze, an improved bearing material, which has anticorrosive property against hydrogen sulfide, was developed maintaining the bearing property by adding zinc to the metal composition.[23] For No. 5, excessive low torque of multi-disk wet parking brakes, a new brake material was developed and was adopted for producing high torque in bio-oil in collaboration with a brake material manufacturer. For No. 6, filter plugging caused by a chemical reaction between bio-oil and petroleum hydraulic oil additives, we took measures to prevent mixing with petroleum hydraulic oil by shipping the machinery with factory filled bio-oil to users who requested bio-oil.

For the low oxidation stability of bio-oil, only the discoloration (darkening) of the rapeseed oil was the failure encountered in the field, as seen in Table 3. Although the possibility of lead dissolving from the lead bronze bearing was considered, there were no failures in the market. Therefore, the authors determined that there was no problem other than instructing the proper oil change interval to the users. As a long-term measure, a new bio-oil with high oxidation stability with the goal of achieving quality grade 4 could be developed,[15][17] but it was unable to clear the European environmental toxicity regulation[8] and was not introduced to the European market.

The following problem occurred after the aforementioned series of countermeasures. In construction machinery operated in the field, malfunctions of hydraulic valves caused by bio-oil occurred, and lack of pressure in the hydraulic pumps and malfunctions of hydraulic cylinders and hydraulic motors became problems. From the surveys of these matters, the cause was suspected to be abnormal wear and sticking of the hydraulic valves due to wear debris produced inside and sand dust that entered from outside (hereinafter, oil dust), but the direct relationship to bio-oil was unclear. It was thought that because of low oxidation stability of bio-oil, oil-insoluble oxidative polycondensation products[1] (hereinafter, lacquer) were formed, which then adhered to the hydraulic valve to cause malfunctions. However, there was no lacquer adhesion upon inspection of the malfunctioned hydraulic valve. It seemed that the malfunction phenomenon subsided, but the cause of hydraulic valve malfunction was never clarified. Due to this fact, we were unable to complete the development of the construction machinery that was compatible with bio-oil.

4 Application of holistic analysis method using system model for cause investigation and countermeasure for the whole system

As described earlier, it was necessary to newly conduct a holistic analysis using a system model described by SysML for the failures involving oxidation stability of bio-oil, in addition to the complex hydraulic system. Since SysML was a modeling language that enabled graphic description of system requirements, structure, and behaviors, and supported verification and validation, we thought it would be effective to combine system models described by SysML and SafeML (extended profile of SysML, details will be explained later) for cause investigation and countermeasures, as shown in Table 4. It is difficult to apply the conventional component analysis method to the analysis of the whole system in cases in which the cause is particularly complex, and the mechanism cannot be graphically rendered. The component analysis method has the disadvantage that the descriptive technical terms cannot be understood or reviewed unless one is an expert. In contrast, SysML allows description even if the cause is complex, and simple descriptions can be used for technical terms (for example, a “hydraulic valve” is described as “a subsystem to control the flow of oil”), and it can be easily understood in graphical form even by multidisciplinary team members. Although SysML allows validation of a countermeasure, it cannot create or verify

Table 4. Comparison of previous methods of analyses and countermeasure and a new holistic method using system model (○: Possible, △: Partially possible, ×: Impossible)

No.	Previous methods or modeling language		Cause investigation			Countermeasures			Multidiscipline discussion
			Case study of cause investigation for failures	Application to complex systems and causes	Visualization of failure mechanism (behavior)	Resolution planning	Verification	Validation	
1	Fishbone diagram		Yes	×	×	×	×	×	×
2	FTA		Yes	×	×	×	×	○	×
3	FMEA		Yes	×	×	×	×	○	×
4	SysML	SysML	No	○	○	○	×	○	○
5		SafeML	Yes	△	×	△	○	○	○

the countermeasure. SafeML is a language that defines the combination of hazards, hazardous context, and harm as risks and can clearly describe defense (countermeasure) methods. However, SafeML cannot illustrate in detail the failure mechanism. SysML and SafeML allows multidisciplinary investigation by a team even with some people who are not engineers.

Figure 4 shows the integrated methods of both analysis and development on the hydraulic subsystem and system against bio-oil using the conventional component analysis method and the holistic analysis method. While the component analysis is effective for cause investigation and countermeasure planning focusing on individual subsystems, it cannot handle complex problems like a malfunction of a hydraulic valve that needs to be solved observing the state of bio-oil, while looking at the whole hydraulic system.

In this paper, the action of the hydraulic system is described and understood as a systems model using SysML. Next, the mechanism of a hydraulic system that leads to failure (hereinafter, behavior) is described, the relationship with the bio-oil state transitions is grasped, and the cause is assumed.

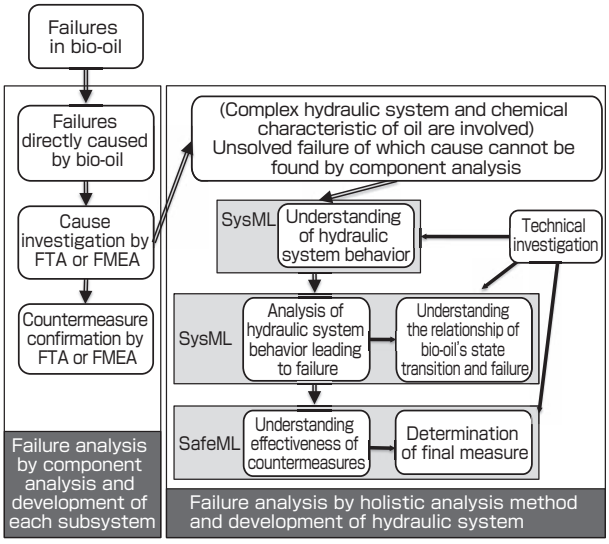

Fig. 4 Development of each subsystem against bio-oil, and development method of hydraulic system using system model described by SysML

Also, the relationship with failure is understood from the bio-oil state transitions.

Although there are several attempts to integrate SysML with safety analysis,[24]–[27] SafeML proposed by Biggs *et al.*[27] is the most practical. SafeML is an extended profile of SysML specific to safety information, and can clearly describe system risks, countermeasures taken for the risks, and risk management results.[28] Using this SafeML, the countermeasures are created for the situation in which a failure occurs, and the effect of such countermeasures, validation, and also countermeasure costs are considered. The safety score (will be explained later) is calculated according to the items pertaining to the safety of system behavior that is clarified here. In this system model, descriptions are written at the parts level of the hydraulic system, to allow easy understanding by the parts manufacturers of the subsystem.

5 Understanding of hydraulic system by holistic analysis method using system model and result of technical investigation of failure factor

5.1 System model description using SysML

Behavior of the whole construction machinery is shown in the activity diagram of Fig. 5. The activity diagram shows behavior using input flow, output flow, control flow (dashed line), object flow (solid line), and action blocks.[20] The "Engine" drives the "Hydraulic System" by output of "Generate Power" action. The operator controls the hydraulic system by "operator command force" flow, and transfers hydraulic pressure into force or rotational torque to the "Work Component" partition by the "Provide Hydraulic Force" action. The work components are set in motion to perform actions such as "Excavate Earth," "Rotate Excavating Direction," or "Run Machinery." To adjust air pressure of the hydraulic system, a small amount of "Air" is taken in a "discrete" manner.

Similarly, the context of the hydraulic system is shown in the internal block diagram of Fig. 6. The hydraulic pump "block1 pump: Oil Press Generation Subsystem" is driven by the "Engine Power," and high-pressure (35 MPa) oil "HP_oil" and middle-pressure (3 MPa) pilot oil "Pilot_

oil" are produced. The operator adjusts the pressure of the hydraulic pump and controls the main valve "block2.2 main-valve: Actuator Moving Device," through the pilot valve "block2.1 pilot valve: Pilot Valve Device" in the hydraulic valve "block2 valve: Oil Flow Control Subsystem." The main valve is composed of multiple units that control the flow direction, flow rate, and oil pressure of the high-pressure oil. The controlled high-pressure oil "CHP_oil" actuates the hydraulic motor device and hydraulic cylinder device in the subsystem that moves the work component "block3 actuator: Work Component Actuation Subsystem." The high-pressure oil after the actuation returns to the main valve and becomes low-pressure (0.1 MPa) oil "LP_oil," and enters the oil feed subsystem "block4: Oil Feed Subsystem." Since the oil temperature increases due to heat release as high-pressure oil becomes low-pressure oil, as well as due to friction of various parts and heat generation by viscous resistance, oil cooling is provided by "Oil Cooler Device." Oil dust in the low-pressure oil is eliminated by the filter "block4.1.1 filter: Dust Eliminating Unit" and returns to the "Oil Tank Unit."

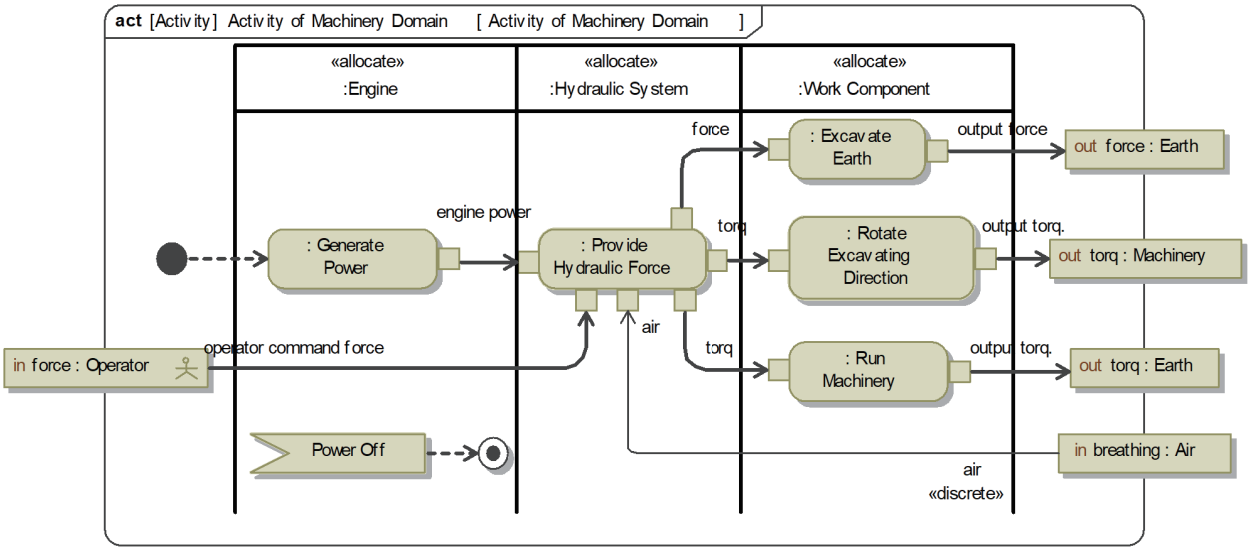

Fig. 5 Activity diagram of construction machinery domain

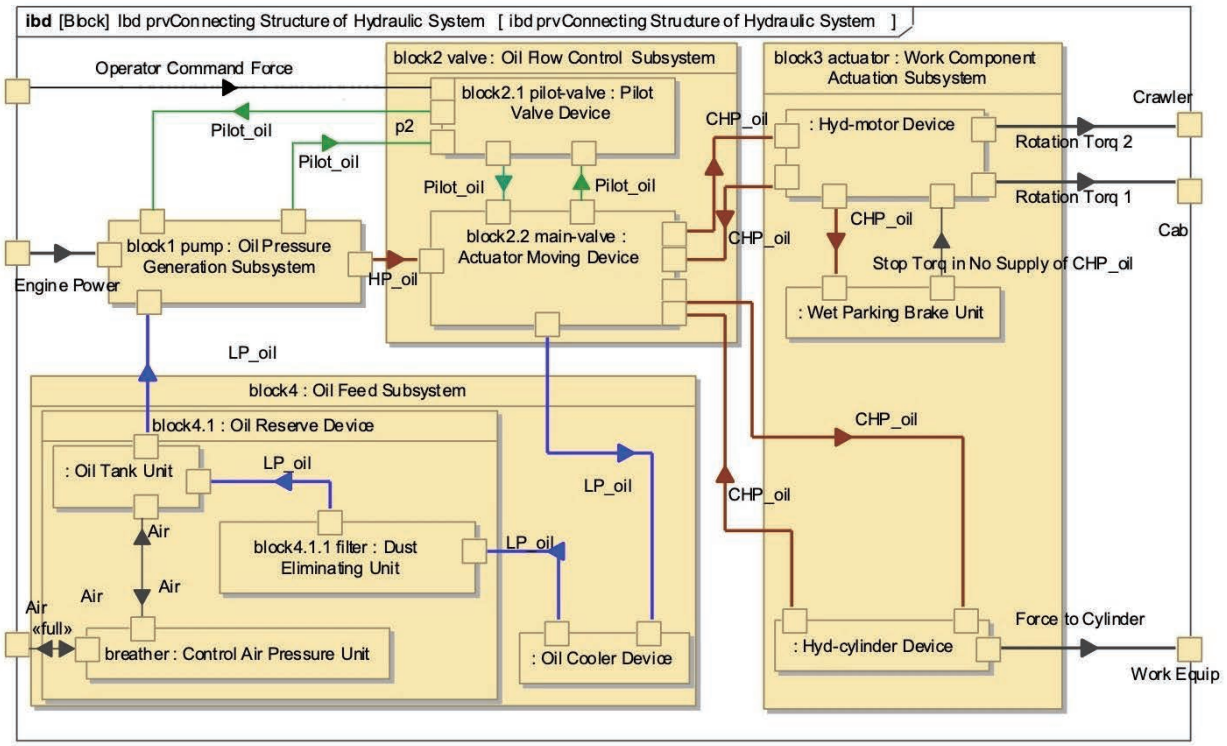

Fig. 6 Internal block diagram of hydraulic system of construction machinery

5.2 Technological investigation on failure factor

5.2.1 Consideration of subsystem (pump and valve) that causes oxidation of bio-oil

There was no long-term countermeasure for the poor oxidation stability of the bio-oil, and it was thought that the oxidation stability had something to do with hydraulic valve malfunctions. Therefore, to understand the cause of hydraulic system malfunctions, it was necessary to clarify in which subsystem the bio-oil underwent oxidation. In Fig. 6, the subsystem in which oxidation is most likely to occur is the hydraulic pump with high load and temperature, but no investigation has been done on the possibility of oxidation in the hydraulic motor, the hydraulic cylinder in "block3 actuator" and in the main valve "block2.2 main-valve." Kazama *et al.*[29] conducted temperature measurement of the swash-plate-type axial piston hydraulic pump (21 MPa) used in construction machinery, and observed a temperature increase of 30 ºC or higher at the cylinder block (increase to 110 ºC when oil temperature was 80 ºC). In cases in which there were bubbles in the oil, hot spots of 1,400 ºC or higher (in cases of 35 MPa) were produced due to adiabatic compression, and the surrounding bio-oil became heated.[30] It could be assumed that the bio-oil underwent oxidation to become lacquer.

The energy loss of all subsystems during the operation of construction machinery may reach 60–75 %,[31][32] and according to the authors' study, it was about 15 % at the hydraulic pump, and about 25 % at the main valve. Since energy loss leads to increased oil temperature, it can be assumed that the oil temperature inside the main valve becomes higher than the hydraulic pump. There is a relief valve that releases high-pressure oil into a low-pressure circuit in the main valve. When the motion of the hydraulic cylinder or the hydraulic motor is stopped when there is excessive load on the hydraulic system, as when construction machinery is removing a large rock with an excavating bucket, excess high-pressure oil is released into the LP_oil circuit of the Oil Feed Subsystem from the relief valve in "block2.2 main-valve," and the kinetic energy is converted to heat. When the heat value at the relief valve is calculated according to Equation (1),[33] the oil temperature reaches about 100 ºC (from oil temperature 80 ºC), and this is equivalent to the oil temperature of the hydraulic pump.

$$H = p \bullet Q \qquad (1)$$

Here, H is the heat value (KJ/min) from the relief valve, p is relief pressure (MPa), and Q is relief flow rate (L/min).

Imanishi *et al.*[34] conducted simulations of the action of the main valve "block2.2 main-valve," and showed that the energy loss is large, equivalent to the relief valve unit, even at the unit that controls the direction of the flow of high-pressure oil from the main valve to the actuator "block3 actuator." The oil flows at flow rate 100 m/s or more at the unit that controls the flow direction of high-pressure oil,[1] and severe cavitation occurs. This may cause damage to the quenched or carburized steel parts.[1] In such severe cavitation, high-temperature and high-pressure (about 4,700 ºC and 100 MPa) hot spots[35] may occur due to the collapse of bubbles. In this situation, molecules may be broken down almost to the atom level or carbon bonds may be randomly split.[35]–[37] It is assumed that this causes surrounding bio-oil to become oxidized and lacquer is formed. Also, damage by cavitation may occur inside the hydraulic pump although at a smaller scale.[38] From these investigations, the authors found that lacquer was formed in the hydraulic pump and at the hydraulic valve.

Although lacquer formation may occur in the hydraulic cylinder and the hydraulic motor, the load factor is lower compared to the hydraulic pump or the main valve. In the past, problems were caused by cavitation during rotation switching from left to right in the hydraulic swing motor, but currently countermeasures are taken by improving the hydraulic valve.[31] Therefore, it is assumed that lacquer formation is minimal in the hydraulic motor and the hydraulic cylinder. The formed lacquer does not dissolve in bio-oil, is captured in the filter, and may cause plugging.

5.2.2 Consideration of oil dust and filter

Figure 7 shows the structure of the hydraulic tank, filter, and breather "block-4.1" in Fig. 6. The filter "block-4.1.1 filter" captures oil dust during by passing of low-pressure oil about 20–100 times. According to our experiments conducted by the authors using actual machinery, conventional cellulose filters (explained later) can capture 50 % or more oil dust of 5 μm or more in a few hours. The number of particles of oil dust (cleanliness level) can be kept below the upper limit required for the hydraulic system. It is designed so that when the filter is plugged and pressure increases, the filter bypass

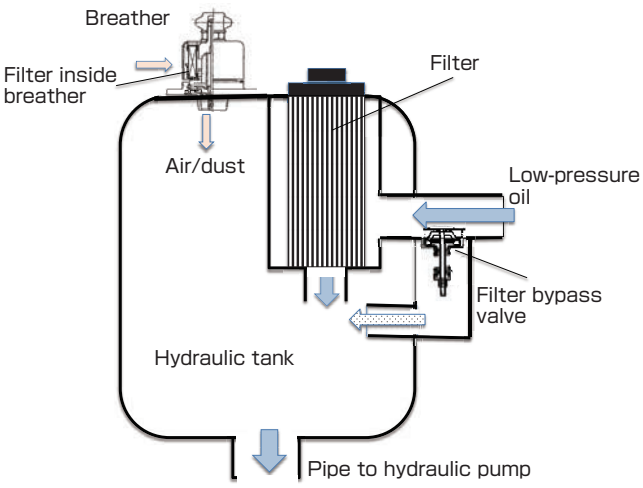

Fig. 7 Schematic diagram of hydraulic tank and filter/ breather

Table 5. Limit value of particle count (cleanliness level) for each particle diameter of oil dust, and typical particle count values in hydraulic oil where failure occurred

Particle size μm	5-15	15-25	25-50	50-100	>100
Upper limit of particle numbers mL^{-1}	500,000	32,000	4,000	1,000	100
Examples of contamination where failure occurred mL^{-1}	3,490,150	96,990	3,120	140	0

valve opens, and the low-pressure oil does not go through the filter and enters the hydraulic tank directly. The timing of filter replacement is set at 250–500 h, and this is calculated from the volume of oil dust captured.

As mentioned before, oil dust is composed of outside dust and metal wear debris from the interior. The outside dust enters as dust particles and muddy water along with air intake, from the filtered breather[39] in "block4.1" that is installed in the hydraulic tank. Since the air goes through the breather filter only once, fine dust cannot be prevented and this becomes the source of oil dust. This filter is made of a cellulose material the same as the oil filter. The outside dust may enter during oil feeding, filter exchange, hydraulic hose exchange, and subsystem repair at sites with poor conditions.

Therefore, oil dust contains hard components (Vickers hardness HV 600 or more) such as oxidized iron and steel that are metal wear debris, as well as silica sand (quartz) or feldspar from dust. Table 5 shows the cleanliness limit values[1] for oil dust that were prepared by the authors,[40] and also provides examples of typical cleanliness levels at which failures occurred. When limits are exceeded, malfunction due to abnormal wear or sticking of the hydraulic valve may occur.[33] Since the gap of the movable parts such as the main valve and the pilot valve is about several μm to 30 μm,[1] oil dust may enter the gaps even if it is within the cleanliness limit. In the case of a field pump failure, the number of oil dust reached at maximum seven times the cleanliness limit.

6 Investigation result of hydraulic system behavior leading to failure

6.1 Normal behavior of bio-oil in hydraulic system

Figure 8 shows the activity diagram of the normal behavior of the hydraulic system. The activity partitions in this figure match the subsystem blocks in Fig. 6. The hydraulic pump "block1 pump" sends high-pressure oil "HP_oil" to the hydraulic valve "block2 valve." The operator controls the hydraulic valve to adjust the pump pressure by pilot oil and controls the direction and flow rate of the high-pressure oil. This controlled high-pressure oil "CHP_oil" moves the actuator "block3 actuator" such as the hydraulic motor and the hydraulic cylinder. This moves the "Work Component." The high-pressure oil used in the work returns to the oil feed subsystem "block4" as low-pressure oil "LP_oil" through the hydraulic main valve, becomes cooled as "Cool LP_Oil," filtered as "Filtrate LP_oil," goes to the oil tank "Reserve LP_Oil" as clean low-pressure oil "clean LP_oil," and enters the hydraulic pump to become pressurized gain. However, when the viscosity of the bio-oil is high such as during low-temperature start-up, the filter differential pressure becomes high "filtration pressure => 0.15 MPa," and the bypass valve in the filter temporarily opens. Then, the bio-oil will not pass

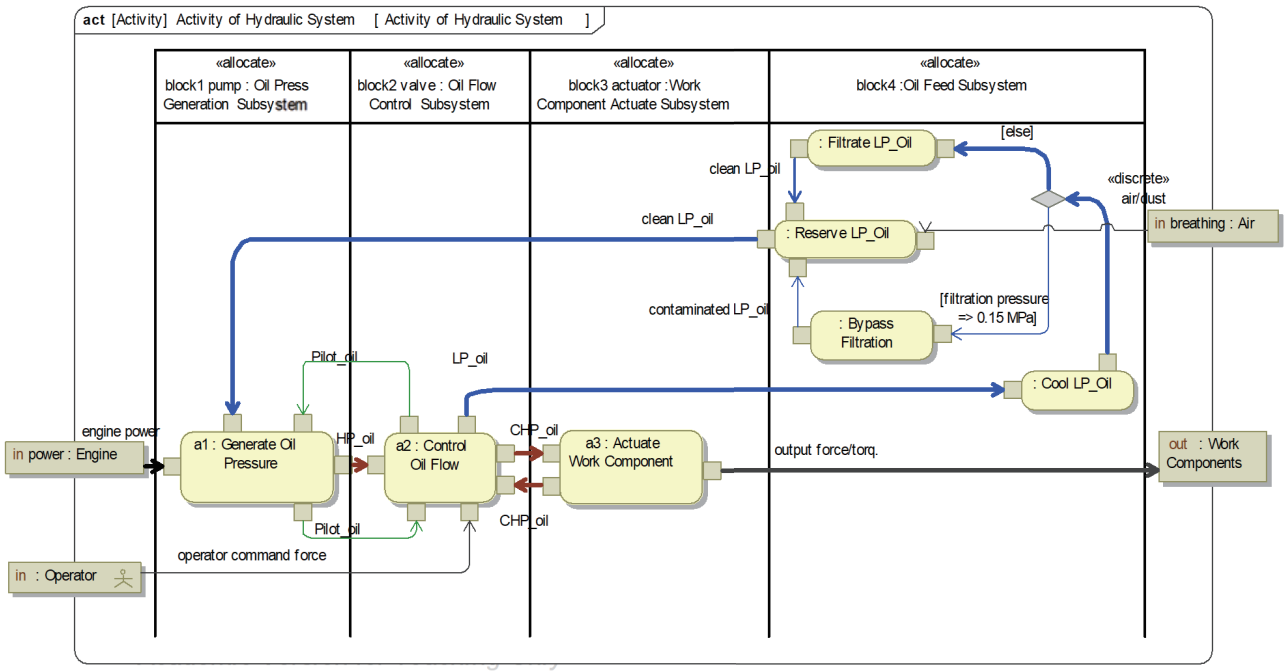

Fig. 8 Activity diagram of normal state of hydraulic system

through the filter "Bypass Filtration," and enters the oil tank while containing oil dust "contaminated LP_oil." The oil temperature increases to 60 ºC or more in about 30 min after start-up, the low-pressure oil will start passing through the filter, and the amount of oil dust decreases immediately.

6.2 Investigation result of behavior of hydraulic system malfunction by bio-oil oxidation

The activity diagram of Fig. 9 shows the formation of lacquer by oxidation of bio-oil and its behavior in the hydraulic system. (1) In the hydraulic pump with increased oil temperature, lacquer forms in the high-pressure oil due to adiabatic compression of bubbles "Generate Oil Pressure and Generate Lacquer in HP_oil." The high-pressure oil containing lacquer "HP_oil + lacquer" flows into the hydraulic valve. (2) Severe cavitation occurs at the hydraulic valve, lacquer is formed in low-pressure oil "Control Oil Flow and Generate Lacquer in LP_Oil," and this flows into the oil feed subsystem. (3) The high-pressure oil containing lacquer flows into the actuator, but the effect on the actuator may be small. (4) The lacquer is captured in the filter "Filtrate LP_oil" of the oil feed subsystem, and clean low-pressure oil returns to the hydraulic pump. However, as the lacquer gradually accumulates in the filter, the filter differential pressure becomes 0.15 MPa even at oil temperature of 60ºC or more, and the filter bypass valve remains open and the filter is plugged "plug filter with lacquer." As a result, oil dust and lacquer are not filtered and mix into the low-pressure oil as "contaminate LP_oil," and the low-pressure oil contaminated with oil dust flows.

The behavior of the contaminated bio-oil in the hydraulic system is shown in Fig. 10. The contaminated low-pressure oil is sucked into the hydraulic pump and becomes high-pressure oil and pilot oil containing oil dust and lacquer "Generate Contaminated HP_Oil and Pilot_Oil," and these are sent to the hydraulic valve. The wear debris concentrates in the contaminated high-pressure oil "contaminated HP_oil" at the actuator, the oil returns to the oil feed subsystem as low-pressure oil. In the "Reserve LP_Oil," outside dust " air/dust" is entered and is concentrated. This is repeated and when the concentration of oil dust surpasses the upper limit of cleanliness "exceed cleanliness limit" (◇ below ●), malfunction occurs "Malfunction by Valve Sticking or Wear" by sticking or abnormal wear as the oil dust enters the gap of the hydraulic valve, including both the main valve and the pilot valve. The flow ends (⊗) and repair becomes necessary. It was found that the hydraulic valve malfunction causes faulty or no movement of the hydraulic pump, the hydraulic motor, or the hydraulic cylinder.

7 Investigation result of relationship between bio-oil state transition and failure

7.1 System model description of bio-oil state transition

Figure 11 is a state machine diagram that shows the behavior of state transition pertaining to bio-oil oxidation and dust inclusion. When oxygen is dissolved in bio-oil, part of the bio-oil becomes radicals due to oxidation even at oil temperature of around 100 ºC.[41] Radicals are short-lived molecules that are activated by the break in parts of the bond in oil molecules,[42] and tend to accelerate oxidation in a chain reaction. The radicals of bio-oil cause oxidation by a reaction mechanism similar to petroleum hydraulic oil.[43]

The authors have confirmed in oxidation stability tests[44] that lacquer begins to form when bio-oil was oxidized

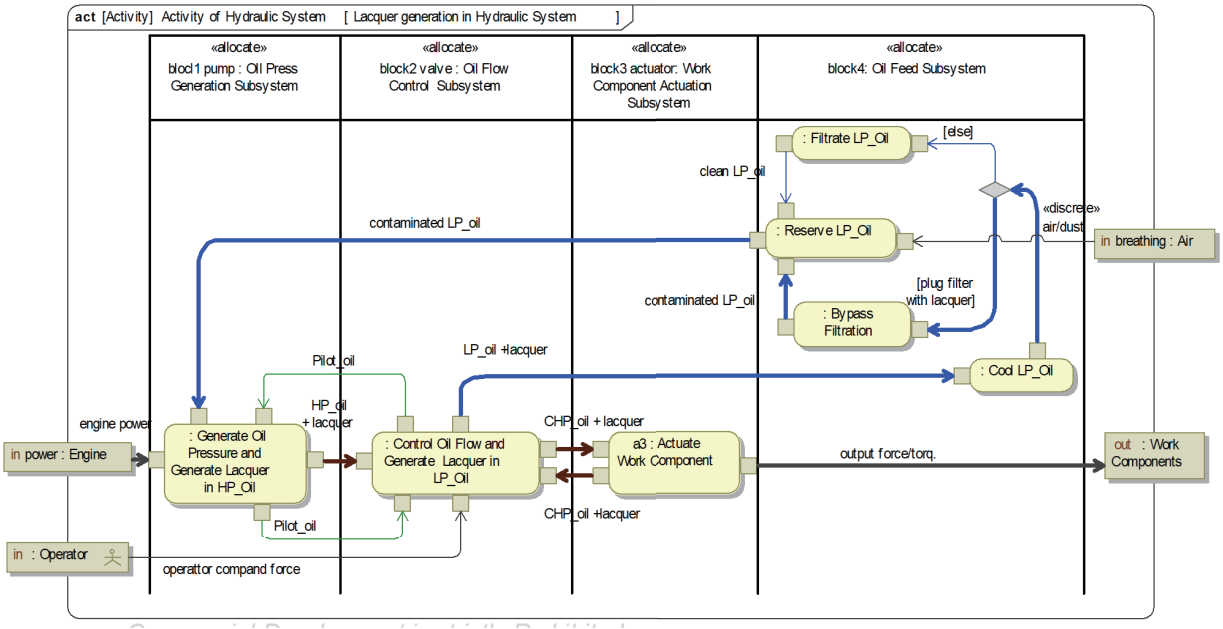

Fig. 9 Activity diagram for lacquer formation and its behavior of bio-oil in hydraulic system

in conditions of 135 ºC × 500 h or more.[15][22] No lacquer formation was observed in this condition for petroleum hydraulic oil. The maximum oil temperature of the hydraulic system for construction machinery is low at 110 ºC,[1] and will not reach 135 ºC. Therefore, the authors assumed that rapid radical formation, oxidation, and lacquer formation occurred in bio-oil only after emergence of local high-temperature regions, or hot spots, due to adiabatic compression of bubbles[30] and cavitation[35] as mentioned earlier. Bio-oil containing lacquer is in a deteriorating state. If lacquer is removed by a filter, it is considered as a "Normal" condition. Lacquer is removed up to the upper limit of the filter capacity

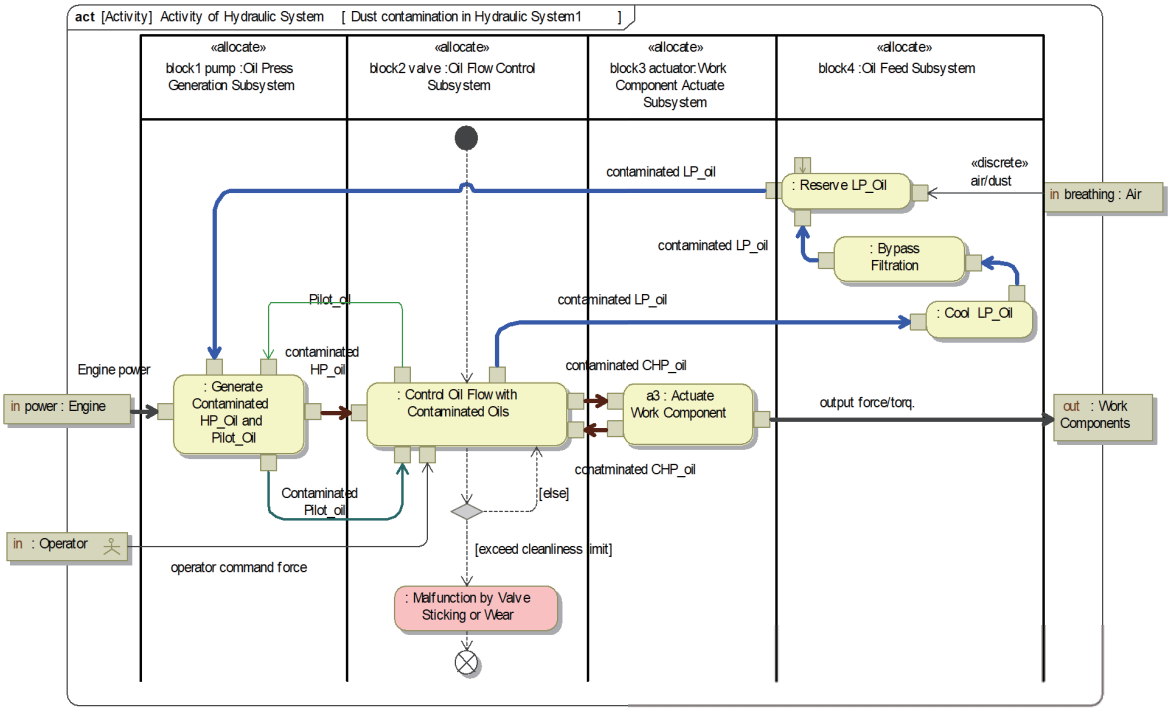

Fig. 10 Activity diagram of contaminated bio-oil and malfunction occurrence of hydraulic system

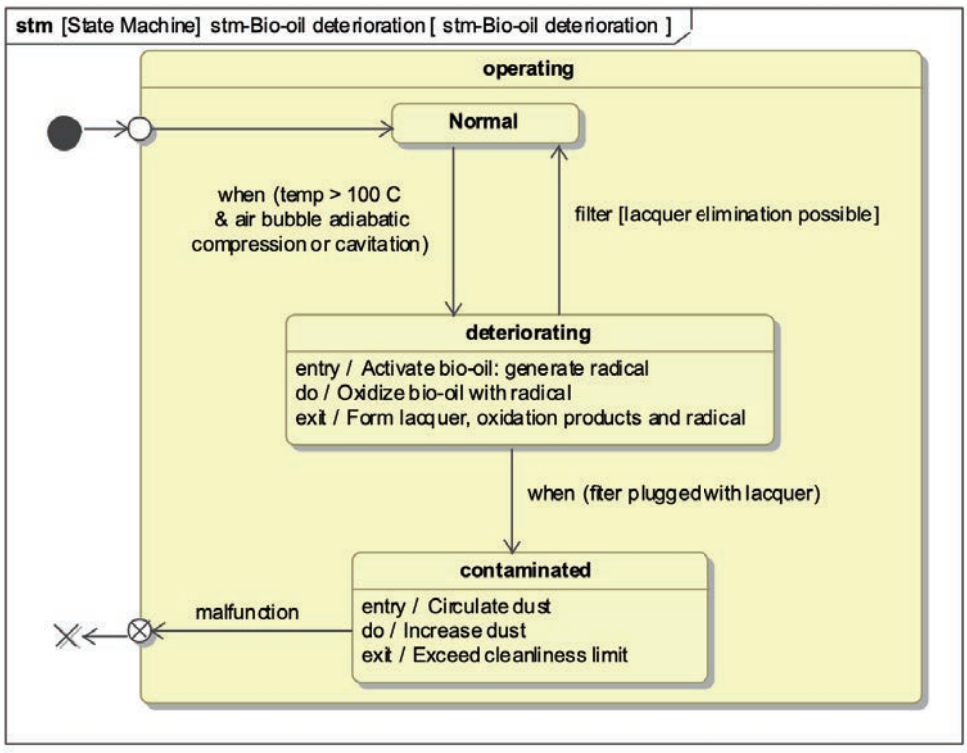

Fig. 11 State machine diagram showing state transition of bio-oil

"lacquer elimination possible." When lacquer accumulates in the filter and the filter differential pressure increases, the filter becomes plugged as "filter plugged with lacquer" in Fig. 9, and the bio-oil is contaminated with oil dust and lacquer. Ultimately, it can be derived that contamination by oil dust means to exceed the limit of cleanliness of the hydraulic system, even in the study described by the state machine diagram.

Oxidation products,[45] that are produced by oxidation reaction, and radicals dissolve in oil, move past the filter, and circulate the hydraulic system. High-reactive radicals have short lifespans (1 ns to several hours), while low-reactive radicals have lifespans of a year or more.[46][47] It is thought that not too many highly reactive radicals that may produce lacquer accumulate in oil. However, oxidation products become concentrated.

7.2 Technological investigation result of bio-oil state transition

7.2.1 Acid value increase in bio-oil field test

To study the effect of concentration of oxidation products of bio-oil on lacquer formation, we conducted a field test of construction machinery for commercial bio-oil with base oil of saturated fatty acid synthetic ester that has higher oxidation stability.[48] The result is shown as the solid line of Fig. 12. The acid value of bio-oil, which is the index for oxidation product concentration, approaches the use limit of petroleum hydraulic oil when it exceeds 3,000 h. The acid value is the index of the acidic component or the amount of free fatty acids in the lubricating oil, and it is expressed by the amount of potassium hydroxide needed for neutralization. When this limit is exceeded, the oxidation product corrodes and dissolves the lead in the bronze bearing metal, and seizure occurs.[49] Therefore, the authors set a standard that the recommended oil change interval of saturated fatty acid synthetic ester bio-oil should be every 3,000 h (Table 2) for standard European products, to maintain the acid value below the limit value. Regarding petroleum hydraulic oil, since rapid acid value increase does not occur as shown in the dashed line in the figure, the oil change interval is set at every 5,000 h. In the next section, we consider whether the increased oxidation in bio-oil is related to lacquer formation.

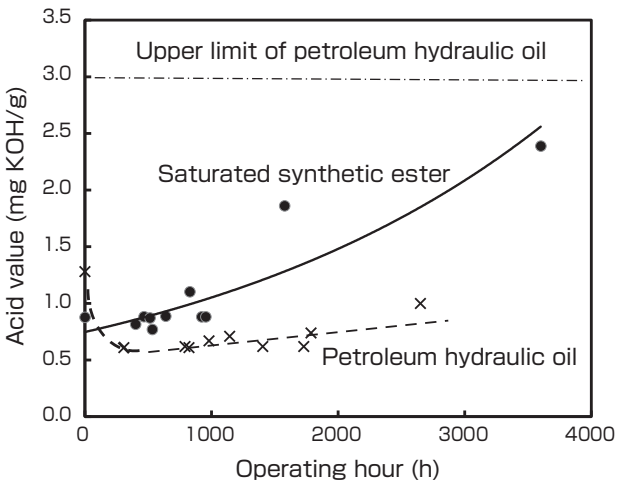

Fig. 12 Acid value change of bio-oil of saturated fatty acid synthetic ester base oil[48] and that of petroleum hydraulic oil during field test of construction machinery,

It is said that in synthetic ester bio-oil, hydrolysis occurs with a mixture of a few percent of water and the acid value increases.[50] However Totten *et al*.[38] determined that hydrolysis does not occur since water entering the hydraulic excavator is 0.1 % or less. The authors similarly obtained results that hydrolysis did not occur in the field tests,[48] and therefore, only the state transition of oxidation is shown in Fig. 10.

7.2.2 Investigation of filter plugging in cases other than bio-oil

To investigate the mechanism of lacquer formation, the authors studied lacquer formation in petroleum hydraulic oil and engine oil.

In the field tests of extending the filter change interval from 500 h to 5,000 h in large construction machinery, which was a wheel loader using petroleum hydraulic oil, significant lacquer adhesion on the filter was observed (Fig. 13). Brown lacquer adhered to the white endplate and the yellow cellulose filter material and the filter material was plugged with lacquer. Since petroleum hydraulic oil has low acid value even at 4,000 h (Fig. 12), it is thought that the increased acid value was not related to lacquer formation, but the filter was plugged by lacquer formation near the hot spots.

The authors also clarified the cause of similar failure in engines.[51] Radicals are formed in the engine oil due to nitrogen oxides (NOx) in the combustion gas, and a large amount of lacquer is formed, and excessive wear of engine parts occurs due to early filter plugging. The engine oil used in this study did not contain effective antioxidants against

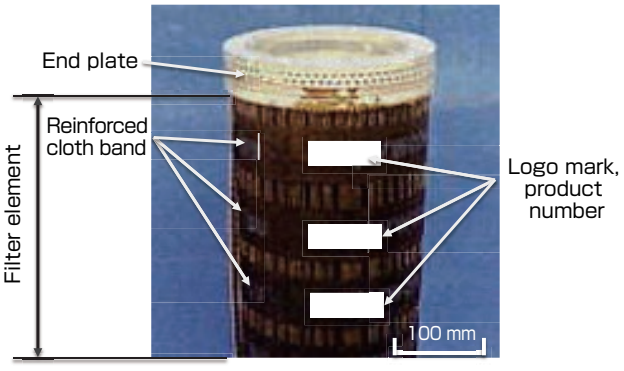

Fig. 13 Lacquer adhered on filter element in extension test (4,200 h) for filter change interval

NOx oxidation. It is the same situation as bio-oil that does not contain effective antioxidants against radicals. Such filter plugging by lacquer formation backs the assumption of the behavior in the state transition of bio-oil.

8 Proposal of countermeasures for malfunction using extended SafeML

8.1 Extension of SafeML

Hazardous situations were described using SafeML based on the behavior of malfunctions described by SysML, and countermeasures were investigated. However, conventional SafeML did not target hazard sources of failure of a product itself.[28] Therefore, the authors extended the language by adding "Short-term defence," "Long-term defence" (hereinafter, countermeasure), and market survey "Field Survey" to understand the effect of short-term measures that are shown in three purple elements as "Defence elements" of SafeML (Fig. 14). This paper will not address the short-term measures and the field survey. Biggs *et al.*[27] attempted calculation of the relative and quantitative "Safety Score" (see Appendix A) for multiple measures. Here, we attempt the application of the safety score for selecting the countermeasures.

8.2 Proposal for malfunction and countermeasures describing by extended SafeML

Three countermeasures can be proposed as described by the extended SafeML, as shown in Fig. 15. The countermeasures against the hazardous/harmful situations are described in Figs. 8–10. The use of unacceptable bio-oil, which is easy to deteriorate, that is inappropriate for construction machinery (pink element) is a "Hazard," and the malfunction of the hydraulic valve due to oil dust (red element) is "Harm." The source of the hazard is bio-oil, and it is shown in association as <<deriveHzd>>. "Harm Context" (yellow element) is the occurrence of sticking or abnormal wear of the hydraulic valve caused by oil dust as the filter is plugged prematurely due to the deterioration of the bio-oil. Since the element where the harm context occurs is in the hydraulic valve, it is shown in association as <<deriveHC>>. The long-term measures (green element <<Long-term defence>>) are proposals for preventing the harm context, and specific effects are shown in the result of long-term measures (blue element <<Defence Result>>). The five tag values are entered into the red, yellow, green, and blue elements. These include investigations of the probability of success, probability of occurrence, probability of harm, and range of harm, and severity of harm.[27] The safety requirement (light pink element <<requirement>>) that is shown in association as <<req Defence>> is described to validate long-term measures. The equipment for long-term measures associated to <<satisfy>> and the test <<test case>> associated to <<verify>> in the safety requirements can be specifically described through information sharing among the design department and the test department.

The basic countermeasures considered from malfunction behaviors are as follows: to prevent occurrence of hot spots by lowering excessive high-pressure oil, to prevent oxidation of bio-oil, and to prevent filter plugging. The specific proposals for the three long-term measures are shown below.

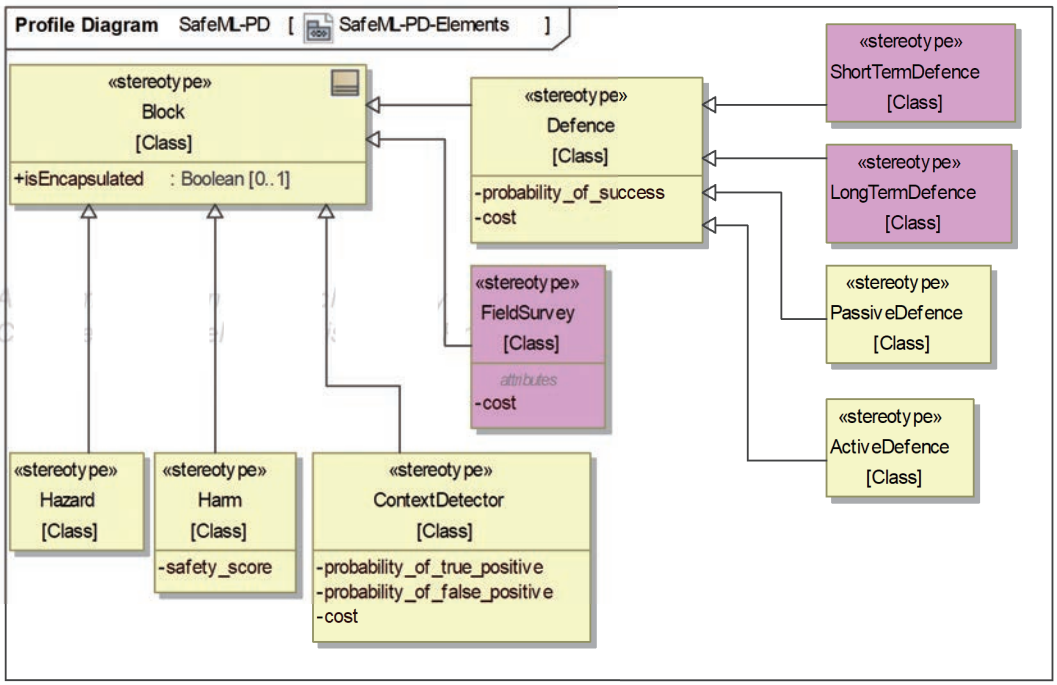

Fig. 14 Elements added to SafeML (purple element)

8.2.1 Proposal 1

The green element "(1) Auto engine controller" suppresses the release of excessive high-pressure oil from the relief valve unit of the main valve by engine control to reduce cavitation and lacquer formation. With this long-term measure proposal, a safety requirement of reducing excess oil pressure at low operating load operation (light pink element) is generated. To fulfill the requirement, it is necessary to investigate by a "Machinery Test" for the engine rotation controlling device "block, Engine Controller." The result of the countermeasure (blue element) is the ability to reduce lacquer formation by lowering the pressure of the hydraulic valve to prevent filter plugging.

8.2.2 Proposal 2

The green element "(2) Centrifugal air bubble separator" involves the removal of air bubbles in the oil using a centrifuge,[30] and the reduction of lacquer formation by adiabatic compression at the hydraulic pump. The safety requirement is the reduction of oil oxygen content to prevent oxidation of bio-oil (light pink element). For the centrifugal air bubble separator that fulfills this safety requirement, it is necessary to conduct tests for the oil feed subsystem (yellow element). The effect of the countermeasure is to prevent bypassing of oil dust by controlling the deterioration of the bio-oil (blue element).

8.2.3 Proposal 3

The green element "(3) Improved Filter" involves the prevention of plugging by lacquer by improving the filtration performance of the filter. The safety requirement is to prevent filter plugging (light pink element). For the improved filter that uses the newly improved filter media that can fulfill this requirement, it is necessary to conduct a "Filter Bench Test." The result of the countermeasure is to prevent the release of oil dust by increasing the capacity of the filter (blue element).

8.3 Technological investigation of countermeasure

Oil temperature decreases if pressure of high-pressure oil is reduced at low-load operation by automatic control of the engine, and release of excess high-pressure oil from the relief valve of the main valve is reduced. However, since cavitation occurs by other hydraulic valve operations, the effect is limited. For the deterioration of hydraulic oil, Sakama[30] clarified that the progress of oxidation reaction can be controlled by reducing the oxygen content in the hydraulic oil by centrifuging the air bubbles in oil. However, as mentioned before, antioxidants, which can sufficiently inhibit radical reactions, are not added to bio-oil, and it is thought that oxidation reaction occurs even in a state of reduced oxygen content. Therefore, sufficient results of oxidation prevention cannot be expected by the centrifuge method.

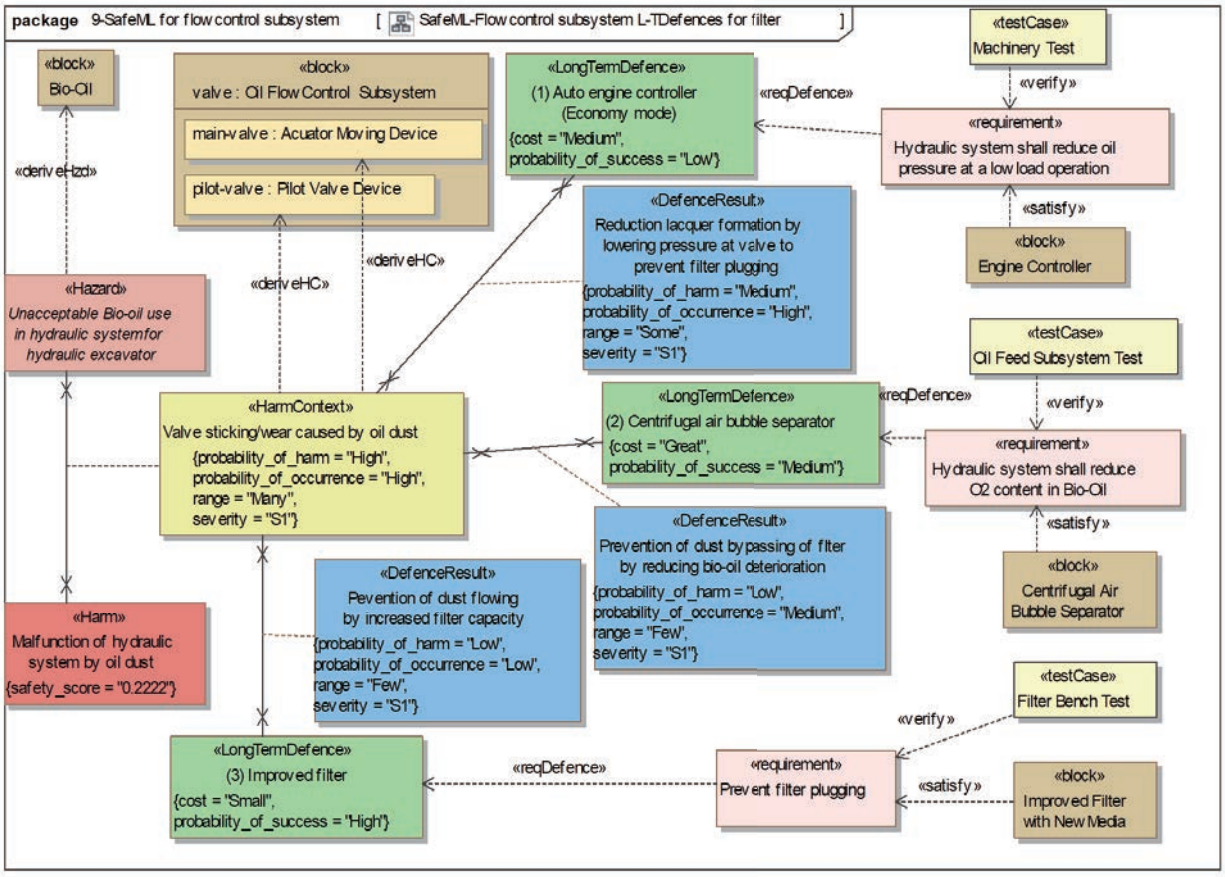

Fig. 15 SafeML diagram for three long-term measures for malfunction of hydraulic valve caused by bio-oil

Table 6. Comparison of five items tag value, safety score, and cost evaluation of cases without countermeasures and cases with three countermeasure proposals

No.	Countermeasures (defence)	Probability of defence success *S*	Probability of context occurrence of *P(Ou)* or *P(Od)*	Probability of harm occurrence *P(Hu)* or *P(Hd)*	Range of harm *Ru* or *Rd*	Severity of harm *Su* or *Sd*	Safety score *SS*	Cost evaluation
0	Undefended case	-	High (1.0000)	High (1.0000)	Many (0.7500)	S2 (0.5000)	0.3750	-
1	Automatic engine controller (economy mode)	Low (0.3333)	High (1.0000)	High (1.0000)	Some (0.5000)	S2 (0.5000)	0.3333	Small (0.5000)
2	Centrifugal air bubble separator	Medium (0.6667)	Medium (0.6667)	High (1.0000)	Few (0.2500)	S2 (0.5000)	0.0741	Great (1.0000)
3	Improved filter	High (1.0000)	Low (0.3333)	High (1.0000)	Few (0.2500)	S2 (0.5000)	0.0000	Small (0.5000)

Table 7. Occurrence of the malfunction in machinery before/after filter improvement

	Average annual occurrence of malfunction (over 2 years)
Vehicles using conventional cellulose filter	10.5
Vehicles using improved filter	Less than 1.4

For filters, improvement of filter materials has advanced recently, and the capturing performance of dust has increased and the lifespan until plugging has been extended.[52] In the measurement results of JIS hydraulic filter performance tests,[53] while a conventional cellulose filter material captures over 50 % of the dust of 20–30 μm or more, an improved filter material made by mixing glass fiber with polypropylene fiber can capture 50 % of dust of the size of 5 μm or more.[1] It is also reported that the filter lifespan using the improved filter media can be more than twice longer than the conventional material.[52] The diameter of a cellulose filter is at maximum 30 μm, while the diameter of the improved filter media is at maximum 1.0 μm. This is the reason the filter performance has improved, and it has prevented plugging of the filter by lacquer, and has enabled capture of oil dust, thus preventing sticking and abnormal wear of the hydraulic valve.

8.4 Determination of countermeasures

Table 6 shows the "Safety Score" (*SS*) and cost evaluation calculated using the equation in Appendix A, based on the tag values in the elements of Fig. 15, for a case without countermeasures (Undefended case) and cases with countermeasures. The probabilities of occurrence were categorized and quantified into three stages: low (Low: 1/3), medium (Medium: 2/3), and high (High: 1/1). The ranges of harm were set in four levels: 0.3 % or less (Few:1/4), medium level (Some: 2/4), high level (Many: 3/4), and 10 % or more (Most: 4/4). The severities of harm were set in four levels: cases that only require part replacement, cleaning, and adjustment that incur hardly any cost (S1: 1/4); cases that require replacement of parts or subsystems on site, or require disassembly and cleaning (S2: 2/4); cases that require repair of subsystems in a repair shop (S3: 3/4); and cases that require total repair of the vehicle or in which human injury has occurred (S4: 4/4). Cost evaluation was set in 4 levels: compatible and inexpensive (Minimum: 1/4), to small (Small), medium (Medium), and cases that require addition of part numbers of new subsystems and modification of the car body (Great: 4/4).

In the case of undefended case, the safety score is 0.3750, but the filter improvement plan has a safety score 0.0000 with the lowest value (highest effectiveness) and also has low cost. Therefore, we adopted the use of the improved filter. The safety score for the auto engine controller is 0.3333 and the one for the centrifugal air bubble separator is 0.0741, so the effectiveness of the countermeasures is lower than that of the improved filter. For cost evaluation, the auto engine controller was already employed in some models and its installation in all models had been decided, and the cost was evaluated as small. The centrifugal air bubble separator requires a new design of the hydraulic tank, and its installment has not been determined except in a few models,[54] and we set the cost evaluation as "great." Since the improved filter is not compatible with the conventional filter due to the strength of the filter media, the cost was set as "small" rather than "minimum."

8.5 Technical validation of countermeasures

Recently, the authors employed a filter with improved filter media to extend oil change interval. As a result, we found that there was no occurrence of malfunction of the hydraulic valve due to bio-oil in construction machinery as shown in Table 7. The effects expected by the use of the improved filter was to extend the oil change interval of petroleum hydraulic oil that had high oxidation stability and sufficient oil lifespan, by capturing and removing as much oil dust as possible.

The auto engine controller was already used in some machinery that also used the conventional cellulose filter, but it was assumed that there was hardly any effect on malfunction. The centrifugal air bubble separator for

hydraulic oil was adopted in some machinery[54] as mentioned earlier, but the effect on bio-oil has not been observed. From these results of confirmation, it was shown that the determination of countermeasures using the safety score was appropriate.

9 Discussion

The project team was able to quickly implement short-term and long-term measures because component analysis methods had been deeply rooted in the construction machinery manufacturers. With the series of countermeasures (short-term and long-term measures), a certain degree of recognition was gained from the users and service persons, but the unsolved malfunctions of the hydraulic valve caused discontent among the users and service persons.

By analyzing the unsolved failures and proposing measures through an holistic analysis method using the system model described by SysML, we were able to complete the development of the hydraulic system of the construction machinery that allowed use of bio-oil. It was found that we were able to describe the cause and mechanism of the malfunctions based on the description in SysML, and we were able to determine the countermeasures based on the description in SafeML. Through this analysis, while only lead dissolution in bearing metal and oil discoloration were conventionally thought to be the problems caused by the low oxidation stability of bio-oil, it was newly clarified that filter plugging occurred by lacquer formation in the hydraulic oil. It was found that the filter not only captured oil dust, but also had the function of preventing oxidation and deterioration of oil by capturing lacquer produced by oxidation. In the future, development looking at the whole system using this new integrated analysis method will be possible. SafeML can graphically describe hazardous situations, countermeasures (defences), and safety requirements for failures, and these can be reviewed by a multidisciplinary team including those who are not engineers. The selection of countermeasures becomes possible from easy-to-understand indices of safety scores and cost. While the component analysis method has the disadvantage against problems that cover the whole complex system, it is an effective method for the analysis of failures in subsystems or parts as it can be done in a short time. The holistic analysis method using system models by SysML description has the disadvantage that it may take time to gain proficiency. Therefore, as shown in Fig. 4, it is recommended that the component analysis be used for the development of subsystems, while the holistic analysis be used for system development and problem solving.

10 Conclusion

In this paper, we attempted cause investigation and considered countermeasures for some problems of biodegradable hydraulic oil in construction machinery as promoted in Europe, utilizing a holistic analysis method using system models.

As a result, through the activities of the project team for bio-oil using the component analysis method, the following countermeasures were implemented.

1) As a short-term measure, a manual of bio-oil was created within half a year. This was distributed to the users through service personnel. This manual was effective until the completion of the long-term measures.

2) For long-term measures, hydraulic subsystems were improved in 4 to 5 years as scheduled, and were installed gradually to the construction machinery.

However, the cause and mechanism (behavior) of the unsolved malfunctions by bio-oil in construction machinery were not clarified since the occurrence of malfunctions ceased and no countermeasures were taken for failures.

Therefore, by analyzing the malfunctions using the new holistic analysis method and implementing countermeasures, the following results were obtained.

3) The cause and behavior of the malfunctions were clarified as follows using a system model described by SysML.
3-1 Lacquer is formed in bio-oil by adiabatic compression of air bubbles in the hydraulic pump and cavitation at the main valve.
3-2 The formed lacquer gradually accumulates and plugs the filter. Oil dust that flows without being filtered causes sticking and abnormal wear of the hydraulic valve, and this causes the malfunction of the hydraulic valve.

Based on these analysis results, countermeasures (long-term measures) were investigated using SafeML, and from evaluations of safety scores and cost, we were able to derive a countermeasure by an improved filter that is most effective and of low cost.

4) Through this analysis, we were able to complete the development of construction machinery that is compatible with bio-oil.

In the future, for development and failures that are large enough to affect business, it is expected that this holistic analysis method can be used for the cause analysis and countermeasure planning, in addition to the conventional component analysis method.

Finally, we are grateful for the advice given on SafeML by Dr. Geoffrey Biggs of AIST.

Appendix Equation for the safety score[27]

$$SS = Qu(1-P(S)) + QdP(S) \quad (1)$$

$$Qu = P(Ou)P(Hu)RuSu \quad (2)$$

$$Qd = P(Od)P(Hd)RdSd \quad (3)$$

provided

SS is safety score,

Qu is the preliminary safety score for undefended (no countermeasure) case,

Qd is the preliminary safety score for defence case,

P(S) is the probability of success of defence,

P(Ou) is the probability of context occurrence for undefended case,

P(Hu) is the probability of harm occurrence for undefended case,

Ru is the range of harm occurrence for undefended case,

Su is the severity of harm undefended case,

P(Od) is the probability of context occurrence for defence case,

P(Hd) is the probability of harm occurrence for defence case,

Rd is the range of harm for defence case, and

Sd is the severity of harm for defence case.

References

[1] Japan Society of Tribologists (S. Ohkawa ed.): *Sangyo-yo Sharyo No Junkatsu* (Lubrication for Industrial Vehicles), Yokendo, 4, 20–26, 63–69, 79–87, 107–108, 190–195 (2012) (in Japanese).

[2] S. Ohkawa: Seibunkaisei sadoyu no genjo to kadai (Current situation and issues of biodegradable hydraulic oil), *Japan Fluid Power System Society Winter Seminar*, 15–27 (1997) (in Japanese).

[3] A. Hirosawa and S. Ohkawa: Kankyo fuka ni taisuru seibunkaisei sadoyu no saiyo (Use of biodegradable hydraulic oil against environmental load), *Yukuatsu Gijutsu* (Hydraulics and Pneumatics), 49 (8), 25–32 (2010) (in Japanese).

[4] Wasserhaushaltsgesetz (Federal Water Act), 12 November 1996 (Federal Law Gazette I, 1695) (1996).

[5] United Nation Sustainable Development Goals: Freshwater Country Profile Germany (2011), http://www.un.org/esa/agenda21/natlinfo/countr/germany/germany_freshwater.pdf, accessed 2018-10-24.

[6] Verband Deutscher Maschinen- und Anlagenbau (VDMA) Standard VDMA24-568: 1994, Biologisch schnell abbaubare Druckflssigkeiten.

[7] ISO 15380: 2016, Lubricants, industrial oils and related products (class L)—Family H (Hydraulic systems)—Specifications for hydraulic fluids in categories HETG, HEPG, HEES and HEPR.

[8] European Commission: Commission Decision of 24 June 2011 on establishing the ecological criteria for the award of the EU Ecolabel to lubricants, *Official Journal of the European Union*, L169/28–39 (2011).

[9] A. Negishi: The relationship between EU Competition Law and market integration in the internal market, *EU Studies in Japan*, (32), 18–28 (2012) (in Japanese).

[10] T. Takei: Environmental acceptability and biodegradability of chemical substances, *Journal of Oleo Science*, 2 (7), 403–409 (2002) (in Japanese).

[11] Ministry of Health, Labour and Welfare: Kankyo ni taisuru yugaisei (Hazard against environment), UN Document on Globally Harmonized System Part 4, https://www.mhlw.go.jp/bunya/roudoukijun/anzeneisei07/pdf/05-03.pdf, accessed 2018-10-27 (in Japanese).

[12] M. Kaneko: High pressure rheology of lubricants (Part 1), *Tribologist*, 62 (10), 654–666 (2017) (in Japanese).

[13] J. Hirano: Recent trends of polyol ester lubricant, *Yukagaku* (Journal of Japan Oil Chemists' Society), 29 (9), 627–635 (1980) (in Japanese).

[14] A. Suzuki, M. Masuko and H-Y. Zhang: EHD film forming capability and boundary lubrication characteristics of hindered polyol esters, *Tribologist*, 47 (8), 671–674 (2002) (in Japanese).

[15] A. Konishi, S. Ohkawa, N. Nakamoto, M. Nanba and T. Yoshida: Development of a high performance biodegradable hydraulic oil for construction equipment, *SAE Transactions*, 971632 (1997).

[16] R. Eguchi, Y. Ohtake, S. Ohkawa, M. Iwamura and A. Konishi: Compatibility of hydraulic seal elastomer with biodegradable oils, *SAE Transactions*, 960210 (1996).

[17] S. Ohkawa and A. Konishi: Biodegradable hydraulic oil for heavy-duty construction equipment, *1st Internationales Fluidtechnisches Kolloquium*, (1), 207–214 (1998).

[18] Japan Industrial Standards: Analysis techniques for system reliability—Fault tree analysis (FTA), Dependability Management Part 4-4, JIS C 5750-4-4 (2011) (in Japanese).

[19] Japan Industrial Standards: Analysis techniques for system reliability—Procedure for failure mode and effects analysis (FMEA), Dependability Management Part 4-3, JIS C 5750-4-3 (2011) (in Japanese).

[20] S. Friedenthal, A. Moore and R. Streiner: *A Practical Guide to SysML: The Systems Modeling Language*, Morgan Kaufman (2011) [H. Nishimura, trans.: *System Modeling Gengo SysML*, Tokyo Denki University Press (2012) (in Japanese)].

[21] D. Walden, K. J. Dorsberg, R. D. Hamelin and T. M. Shortell: Model-based systems engineering, *INCOSE Systems Engineering Handbook—A Guide for System Life Cycle Processes and Activities*, Wiley, 11, 189 (2015).

[22] S. Ohkawa, A. Konishi, H. Hatano, K. Ishihama, K. Tanaka and M. Iwamura: Oxidation and corrosion characteristics of vegetable-base biodegradable hydraulic oils, *SAE Technical Paper*, 951038 (1995).

[23] N. Hamasaka, H. Saito, K. Ishikawa, S. Ohkawa and A. Konishi: Shodo zairyo (Sliding material), Japanese Unexamined Patent Application Publication No. Hei 9-67630, 1995-08-29 (in Japanese).

[24] P. Wilkinson, M. Novak and A. Mavin: Integrating safety into system design with SysML, *Journal of Safety and Reliability Society*, 29 (4), 79–93 (2009).

[25] F. Mhenni, J. Y. Choley and N. Ngyuyen: SysML extensions for safety-critical mechatronic systems design, *2015 IEEE International Symposium on Systems Engineering* (ISSE), 242–247 (2015).

[26] E. Villhauer and J. Brian: An Integrated model-based approach to system safety and aircraft system architecture development, *25th Annual INCOSE International Symposium*, 25 (1), 1373–1387 (2015).

[27] G. Biggs, T. Sakamoto and T. Kotoku: A profile and tool for modeling safety information with design information in SysML, *Software & Systems Modeling*, 15 (1), 147–178 (2016).

[28] G. Biggs, T. Sakamoto and T. Kotoku: 2A2-I06 SafeML—

A model-based tool for communicating safety information (Robotics with Safety and Reliability), *Proceedings of JSME Annual Conference on Robotics and Mechatronics*, 2A2-I06 1–2A2-I06 4 (2013) (in Japanese).
[29] T. Kazama and T. Tsuruno: Thermal lubrication characteristics of swash-plate type axial piston pumps (Temperature measurement of swash-plate and cylinder-block), *Transactions of the Japan Society of Mechanical Engineers C*, 74 (738), 425–430 (2008) (in Japanese).
[30] S. Sakama: Research on bubble separation and elimination for hydraulic system, Thesis, Graduate School of Engineering and Design, Hosei University, 11–12 (2014) (in Japanese).
[31] S. Okabe: *Yuatsu Shovel Taizen* (Encyclopedia of Hydraulic Power Shovels), Nihon Kogyo Shuppan, 60–61 (2007) (in Japanese).
[32] JXTG Nippon Oil & Energy Corporation: Sho-energy-gata yuatsu sadoyu (Energy-saving hydraulic oil), https://www.noe.jxtg-group.co.jp/company/rd/intro/lubricants/shoene.html, accessed 2018-03-27 (in Japanese).
[33] Japan Fluid Power System Society: *Jitsuyo Yuatsu Pocketbook* (Practical Hydraulic Pocketbook), 244–248, 319 (2008) (in Japanese).
[34] E. Imanishi, T. Nanjo and A. Tsutsui: Yuatsu shovel no teinenpi wo sasaeru simulation gijutsu (Simulation technology to support low energy consumption of hydraulic power shovel), *R&D Kobe Steel Engineering Reports*, 62 (1), 32–36 (2012) (in Japanese).
[35] R. Katoh: Decomposition of organic liquids by ultrasound, *Review of High Pressure Science and Technology*, 6 (3), 159–166 (1997) (in Japanese).
[36] K. S. Suslick, J. J. Gawlenowski, P. F. Schubert and H. H. Wang: Alkane sonochemistry, *Journal of Physical Chemistry*, 87 (13), 2299–2301 (1983).
[37] S. Koda: Sonochemistry towa nanika? (What is sonochemistry?), *Journal of the Acoustical Society of Japan*, 57 (5), 345–350 (2001) (in Japanese).
[38] G. E. Totten (ed.): *Handbook of Hydraulic Fluid Technology, Marcel Dekker*, 461-463, 630–647 (2000).
[39] Yamashin Filter Corp R&D Division: Air breather kyuhaiki tokusei sokutei-sochi gaiyo (Outline of the device for measuring intake-exhaust property of air breather), Yamashin Technical Report, http://www.yamashin-filter.co.jp/ja/technology/development/main/02/teaserItems1/01/linkList/0/link/20160115.pdf, accessed 2018-04-25 (in Japanese).
[40] S. Ohkawa and H. Hamaguchi: Progress of a new hydraulic fluid specification HX-1 for construction equipment, *SAE Asia Colloquia*, 11 (2003).
[41] K. Iizuka: Jido sanka hanno ni chumoku shita junkatsuyu kanri hoho no kento (Investigation of the lubricant management in consideration of auto-oxidation reaction), Experiment Report, Kochi University of Technology (2001), http://www.kochi-tech.ac.jp/library/ron/2000/env/1010001.pdf, accessed 2018-03-26 (in Japanese).
[42] International Union of Pure and Applied Chemistry: Radical (free radical), IUPAC Gold Book, http://goldbook.iupac.org/html/R/R05066.htm, accessed 2018-04-10.
[43] J. R. J. Smith, E. Nagatomi and D. J. Waddington: The autoxidation of simple esters—Towards an understanding of the chemistry of degradation of polyol esters used as lubricants, *Journal of the Japan Petroleum Institute*, 46 (1), 1–14 (2003).
[44] Japan Industrial Standards: Lubricating oils—Determination of oxidation stability, Part 1: Oxidation stability of internal combustion engine oils, JIS K 2514-1, (2013) (in Japanese).
[45] Japan Society of Tribologists ed.: *Maintenance Tribology*, Yokendo, 117 (2006) (in Japanese).
[46] H. Minato: Chemistry of peroxyesters, *Journal of Synthetic Organic Chemistry*, 23 (1), 12–22 (1965) (in Japanese).
[47] Iowa University: Lipid oxidation - an overview, http://www.public.iastate.edu/~duahn/teaching/Lipid%20oxidation/free%20radicals.pdf, accessed 2018-11-15.
[48] K. Iijima, S. Ohkawa and A. Konishi: Seibunkaisei sadoyu no field ni okeru rekka ni tsuite (On the degradation of biodegradable hydraulic oil in the field), *Journal of Japan Fluid Power System Society*, 29 (5), 63–67 (1998) (in Japanese).
[49] T. Sakurai (ed.): *Sekiyu Seihin Tenkazai* (Petroleum Product Additives), Saiwai Shobo, 263 (1979) (in Japanese).
[50] C. Kempermann and H. Murrenhoff: Reduction of water content in biodegradable and other hydraulic fluids, *SAE Technical Paper*, 981497 (1998)
[51] K. Iwakata, Y. Onodera, K. Mihara and S. Ohkawa: Nitrooxidation of lubricating oil in heavy-duty diesel engine, *SAE Technical Paper*, 932839 (1993).
[52] Y. Kagami: Contamination control in earthmoving machines—Hybrid filter elements versus by-pass filters—Influence of biodegradable oil on filter life, *SAE Technical Paper*, 981501 (1998).
[53] Japan Industrial Standards: Hydraulic fluid power filters, Part 8: Multi-pass method for evaluating filtration performance of a filter element, JIS B 8356-8 (2002) (in Japanese).
[54] A. Konishi, S. Ishii and T. Nohara: Sadoyu tank no kogataka (Down-sizing of hydraulic oil tank), *Journal of Japan Fluid Power System Society*, 39 (5), 272–276 (2008) (in Japanese).

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Discussions with Reviewers

1 Overall

Comment (AKAMATSU Motoyuki, AIST and KOBAYASHI Naoto, Waseda University)

This paper explores the cause of failures that occur when bio-oil is used in the hydraulic system of construction machinery, and explains cases in which SysML and SafeML are used as methods to create countermeasures. Since the mechanism of failure occurrence by bio-oil is complex, analysis using accident analysis methods such as FTA is difficult in reaching a countermeasure. However, this paper shows that countermeasures can be determined using SysML that allows the description of interactions among subsystems. The paper presents a description of a scenario on how countermeasures were implemented in employing bio-oil, and this study is an example of a synthetic approach. This paper is appropriate for publication in *Synthesiology* in the points that it provides a solution to the practical issue of applying biodegradable hydraulic oil to the hydraulic system of construction machinery, contributes to the development of the hydraulic system, and shows the effectiveness of the integrated analysis method to solve complex problems through SysML and SafeML.

2 Overall picture of countermeasures

Comment (AKAMATSU Motoyuki)

I think this paper will become more appropriate for *Synthesiology* if you provide an overview showing the mutual positioning of countermeasures, short-term measures, and long-term measures for the subsystems, as well as the appeals to stakeholders (for example, a manual). I think the readers will be interested in knowing whether the countermeasures for the filter made the conventional measures no longer necessary, or the conventional measures still play important roles.

Answer (OHKAWA Satoshi)

To enhance understandability, we made corrections and additions to the diagram for the positioning of countermeasures, short-term measures, and long-term measures for the whole system, as well as measures for the subsystems. The definitions of short-term and long-term measures are explained in Chapter 1.

3 Effect on environment

Question (KOBAYASHI Naoto)

You mention that the motivation for using biodegradable hydraulic oil in the hydraulic system of construction machinery is to reduce the effect of environmental pollution when oil leakage occurs, but in reality, how often and in what amount have oil leakages occurred?

Answer (OHKAWA Satoshi)

A 30-ton class hydraulic excavator runs for an average 2,000 hours per year, and there is the possibility of about 40 L leakage during the year. Recently, instructions have been given to prevent oil leakage during work (such as placing plastic sheets when service persons provide services or removing oil with oil absorbing agents), and I think the amount of leakage has reduced. One-liter oil leakage contaminates 1 m^3 (2 ton) of soil, and the processing cost is 100,000 yen/ton. It is calculated that the processing cost of contaminated soil is 8 million yen a year. On the other hand, cases for which immediate measures must be taken are when a hydraulic hose hits a rock or reinforcing steel

in concrete, the hose is broken, and oil spews out. In such cases a 350 L hydraulic tank will become empty in a minute.

4 Difference from conventional methods

Question (KOBAYASHI Naoto)

You clearly state the difference between the conventional component analysis methods (FTA and FMEA) and the newly employed integrated analysis methods (SysML and SafeML), but in practice, the boundary between them is not necessarily clear. Therefore, can you provide examples for the following: (1) what are your views on using different methods in various situations, such as using the new method in places where it didn't go well with the conventional method; and (2) what are the systems to which this method (SysML and SafeML) is highly applicable?

Answer (OHKAWA Satoshi)

All analyses can be done by holistic analysis methods using the system model described by SysML, without using component analysis. In fact, the author attempted cause analysis and countermeasure selection only by holistic analysis, and was able to find causes and mechanisms that could not be found with component analysis only. However, while this may depend on the skill level of the authors, holistic analysis (particularly, describing by SysML) required several times more time compared to component analysis.

(1) Since component analysis is simple and quick, it should be used on all systems. As shown in this paper, holistic analysis should be applied only to complex problems that cannot be clarified by component analysis.

(2) The holistic analysis method can be meaningfully applied to computer-controlled machine systems, machine systems affected by human behavior, and machine systems affected by complex chemical reaction.

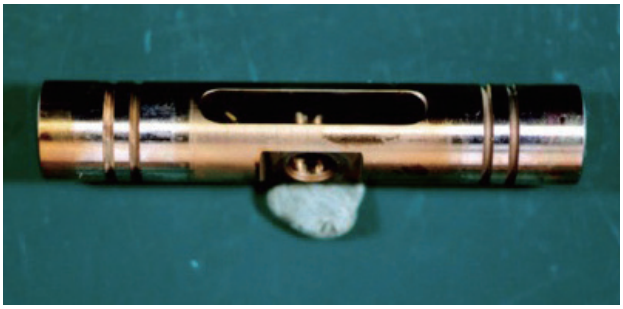
5 Lacquer formation

Question (KOBAYASHI Naoto)

You state that the major causes of lacquer formation, or oxidation condensate, are (1) adiabatic compression of air bubbles in the hydraulic pump and (2) cavitation at the main valve. Please provide explanation of the formation mechanism using supporting data and photographs. (It can be data from your published papers or other papers.) Lacquer has important effect on inducing plugging of the filter, but please provide typical chemical structures or names of the substance. How about other oxidation products?

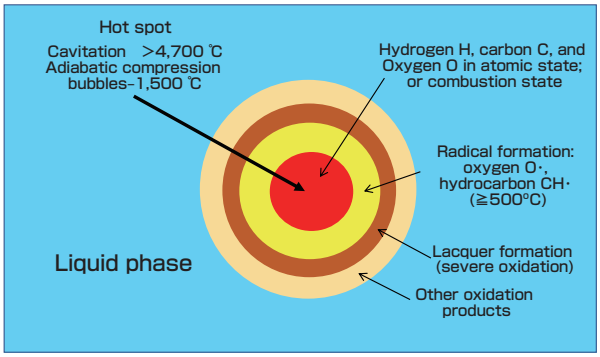
Answer (OHKAWA Satoshi)

As an example of lacquer, we added a photograph of the interior surface of a hydraulic pump part. It is assumed that the adhering lacquer was produced by adiabatic compression in the hydraulic pump. The lubricant oxidation test (JIS K 2514-1, 2018) of JIS standard provides a measurement method of the degree of lacquer formation by oxidation. The author has published a paper on a subject in which this oxidation test was conducted for biodegradable hydraulic oil [S. Ohkawa *et al.*: Oxidation and corrosion characteristics of vegetable-base biodegradable hydraulic oils, *SAE Paper*, 951038 (1995)], but did not mention lacquer as I was oblivious to its importance at the time.



Lacquer coat (brown part) on servo valve surface of hydraulic pump tester produced by commercial hydraulic oil (photo by authors).

When random oxidation is set off by radicals, the biodegradable hydraulic oil increases in viscosity (molecular weight 200–8,000; 100,000 in very high cases), as oxidation products containing alcohol (-OH), aldehydes (-CO), and acids (COOH) with complex composition and various molecular weights are formed. With severe oxidation polymerization by radicals, adhesive-like resin, or lacquer, is formed. Since lacquer is produced by such uncontrolled oxidation, there is no set chemical structure, and analysis is difficult. "Other oxidation products" are also difficult to analyze, and there is no prior research on such analysis. The author imagines that the formation of lacquer and "other oxidation products" progress as shown in the following figure.



Imagined state of oxidation of biodegradable hydraulic oil caused by cavitation and adiabatic compression bubbles (figure drawn by authors).

Development of a bovine sperm selection procedure for improvement of livestock fertility

Nagata MariaPortia B. and Yamashita Kenichi*

[Translation from *Synthesiology*, Vol.12, No.2, p.75–83 (2019)]

Improving the reproductive performance of livestock has wide-ranging significance that includes promotion of local industry, bioeconomy, and stabilization of food supply. Our research focused on sperm manipulation to improve the reproductive performance of cattle. Our experiments were based on previous studies on infertility treatment for humans by relying on the advantages of motile spermatozoa, i.e. spermatozoa that are capable of swimming against the flow of solutions, which is regarded as an attribute of healthy and physiologically functional spermatozoa. For the first time, we succeeded in collecting a number of spermatozoa that can be used for artificial insemination and obtained good conception results in a field trial. In addition, the field trial clarifies the advantageous relationship between sperm trajectory and conception.

Keywords : Chemical engineering, fluidics, livestock, breeding, spermatozoa

1 Introduction

The increase in world population is projected to continue for some time in the coming years, and with the changes in diet accompanying economic growth, in addition to quantitative food demand, changes in qualitative demand such as for animal-based protein are expected. Therefore, there is high technological demand for increased production in livestock industry. In addition, sustainable development of agriculture including livestock has wide-ranging significance, such as contribution to local food culture, and promotion of local and regional industrial recovery. Recently, social demands have incorporated new concepts such as bioeconomy, sustainable development goals (SDGs), and animal welfare, thus emphasizing the necessity of conducting R&D wherein various concepts from different disciplines are included. Particularly, bioeconomy is expected to become one of the main axes of technological innovation in the near future. For example, the Ministry of Economy, Trade and Industry has published Reference [1] "Toward a new bioeconomy society through bio x digital," and the market size is expected to reach 1.6 trillion dollars in 2030.

In Japan, livestock holds about 35 % of the agricultural production, and occupies the number one position among agricultural products leaving behind rice, vegetables, and fruits.[2] On the other hand, since it involves animal husbandry, constant work is necessary, and it is an archetypal example of industry in which "one cannot take time off."[3] It is also particularly hard hit by a shrinking agricultural workforce and aging population. Among livestock, "cattle" is the most influential in terms of market size and environmental impact. In this study, the focus is placed on the stage of "breeding" rather than "fattening" which is a stage where the cattle are raised to a large size. A cow gives birth only once a year even in ideal breeding situations, and unlike hogs, it produces only one calf per birth. Since the price per animal is high and since the animal is large, the cost of feed is high, and the success and failure of breeding greatly affects the farm business. Sperms collected from a bull that has excellent economic traits and reproductive ability are diluted, divided into 0.5 mL amounts, sealed and frozen in straw-like containers, and are distributed commercially. Breeding by artificial insemination (AI) is done by thawing the sperms and then introducing them into the cows' reproductive organs. That is, a natural mating program utilizing service live bulls does not occur in cattle breeding under the AI program. Currently, most cattle breeding (90 % or more) is done by AI. Although *in vitro* fertilization and transplantation of fertilized eggs have been put to practice as next-generation technologies, they have not diffused widely, since AI is simple and there is plenty of practical experience gained in such a method.[4] The rate of breeding success (conception rate) by AI in Japan is in a long-term downward trend, and currently it is 50–60 % for beef cattle and 40–50 % for dairy cattle.[5] To increase the reproductive capacity, studies from both the bull side (spermatozoa and sperms) and cow side (eggs and reproductive organs) are being conducted. Many involve selective breeding, clinical veterinary medicine from the cow side, and estrus monitoring using ICT in recent years, but the production method, for example, of frozen sperms has not really changed since the 1950s,[6] and

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not much research has been conducted on the bull side.

On the other hand, in the field of human infertility treatment, against a background of progress in analytical devices and embryo culturing technology, the importance of spermatozoa factors is becoming clear. For example, it is reported that healthy spermatozoa have significantly higher advantage in the rate of implantation, conception, and abortion.[7] The "healthiness" of spermatozoa here specifically means the integrity of elements and functions as reproductive cells such as "having little fragmentation of DNA." Moreover, it has been reported that spermatozoa with high motility[8] and those with good morphology[9] are relatively healthy and functional. It is also reported that high motility is advantageous in traveling through female reproductive organs and this is important in increasing the pregnancy rate.[10] It is also reported that about one-fourth of infertility in cattle actually involves extremely early miscarriages of which one may be even unaware of conception.[11] It is indicated that improvement of the health of spermatozoa is important, from the perspective of ensuring the healthiness of the embryo which does not stop developing after fertilization. That is, to improve the pregnancy rate of cattle and to increase productivity, thereby reducing workload in the Japanese cattle industry, we considered the prospect of improvement in the male factor and started R&D for improvement of semen quality by selecting high quality spermatozoa, a rather unexplored area.

There are complex reasons for the long-term downward trend of the pregnancy rate. They include complex combinations of genetic degradation (that leads to inbreeding depression or inability to survive and reproduce) occurred as a result of inbreeding in pursuit of traits with economic value, as well as aging of personnel in charge of estrus monitoring which is dependent on tacit knowledge. It is apparent that various kinds of research are necessary in such areas. As the importance of the spermatozoa became known in recent years in the field of human infertility treatment, we decided to work on the improvement of frozen sperms on which hardly any studies have so far been done.

2 Design of research plan

Figure 1 summarizes the process of investigation from organization of social demand to building of specific research.

For the final goal which is transfer of technology, it was necessary not only to obtain highly motile spermatozoa but also to conduct field trials at farms, and this was an issue

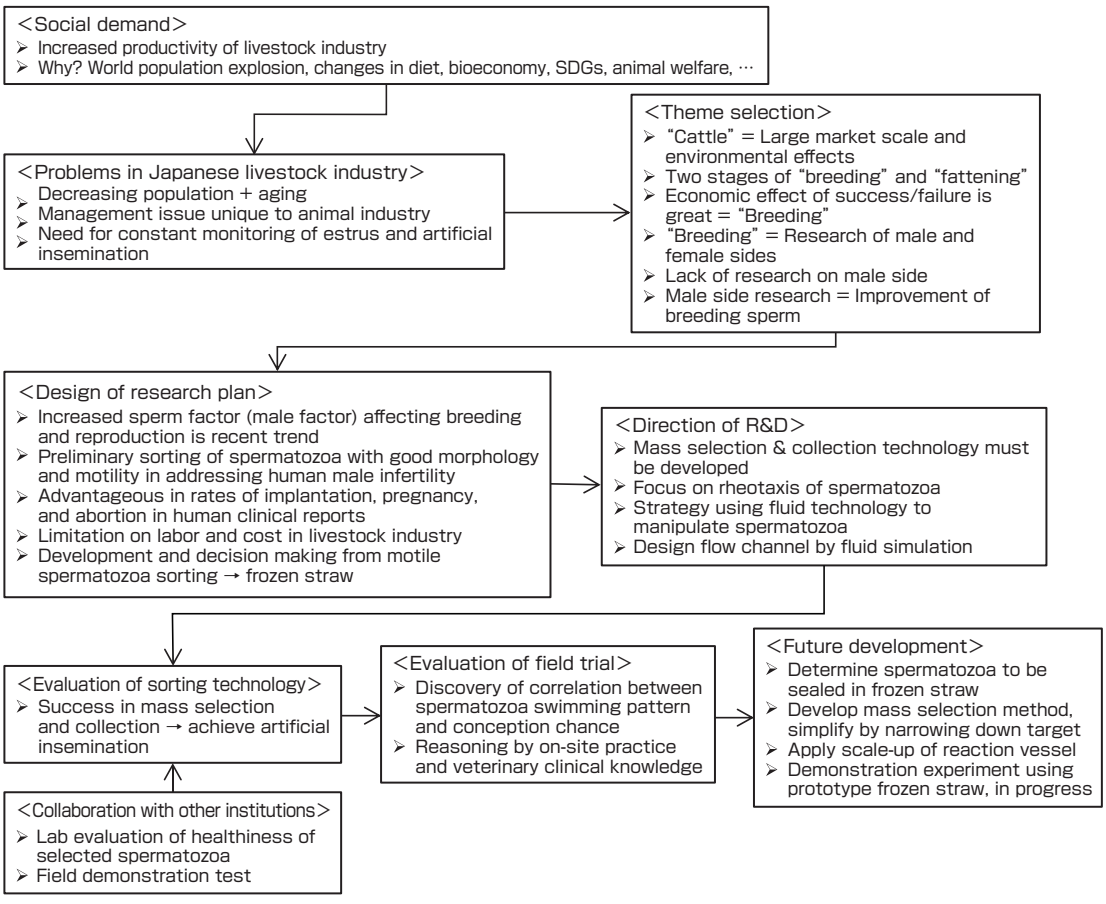

Fig. 1 Conceptual diagram of concept flow where research plan based on social demand is broken down into specific actions

that could not be dealt with by AIST alone. Meaning, an interdisciplinary fusion was necessary, and considering that the sites at which research could be jointly conducted are dispersed in the regional farmland areas (due to the nature of the livestock industry), a regional collaborative effort was called for in order for the project to materialize. In general, the points that need attention in the interdisciplinary fusion (and thus, crossing knowledge boundaries) include differences in premises that were considered common knowledge, and lack of common understanding for expected outputs, and for heights of barriers that need to be overcome. These were also apparent in this research, and, for example, although good results were obtained in the field as a result of sorting highly motile spermatozoa, in order to achieve social implementation, there was a hurdle faced in the area of chemical engineering in terms of scaling-up of sperm sorting. The essence of sperm sorting could not be judged beforehand, faced with the obstacle presented by scale-up, and vice versa, for scale-up to be realized, the necessity of determining specific conditions of sperm sorting. Moreover, from the AIST side, we had no idea which work would incur a specific workload and in what level, and other institutions have seen the research as not realistic. Therefore, in conducting this research, we only set an outline regarding research policies and division of roles, and each institution was asked to come up with specific experimental methods. By assessing technical superiority based on the data obtained, we gradually clarified the topics that had to be tackled next. That is, there was no specific work plan prepared from the beginning. When conducting such research, testing opportunities are limited because trials involve large animals like cattle, and satisfactory trial plans cannot be drawn. Therefore, it was necessary to repeatedly scrutinize the data and plan the next trial.

In conducting improvement of semen by sperm selection for livestock breeding, R&D was planned under the assumption that unlike human male infertility, a large-scale facility, manpower, and cost could not be obtained. In human infertility treatment, conventional methods involved obtaining healthy spermatozoa by selecting spermatozoa with high motility. However, only a small quantity of spermatozoa can be collected by this method, and it was mainly used for micro-fertilization. On the other hand, cattle breeding is primarily done by artificial insemination, and it was necessary to increase the number of selected spermatozoa to several hundreds of thousand times which was normally obtained by conventional methods. We thought that this should be conducted mainly by engineering methods, and AIST became in charge. Since it would eventually become necessary to conduct field trials wherein work must be done by closely observing cattle, it should not involve complex work procedures. On the other hand, the institutions at which field trials were conducted evaluated the properties of spermatozoa every time prior to artificial insemination, and performed reproductive health checks of the cows. They also kept records of the time when estrous behavior was observed, time at which insemination was performed, sizes of the follicles, and estimated time of ovulation. Universities conducted cellular biological analysis of the spermatozoa, to evaluate the adequacy and efficiency of AIST's sperm sorting technology, and to investigate the cause-and-effect relationship with conception.

It was also agreed that the "final product will be frozen sperm straws" in organizing future concepts. This meant that the work of sealing livestock breeding spermatozoa into straw-shaped containers and providing the product in this standard format, must be routine work without incurring any additional burden to the cattle farmer. This means without the requirement of learning new techniques or installing new facilities. It was clarified and identified what type of spermatozoa should be sealed, and once the method to select and collect large amounts of such spermatozoa was developed, as the next step, a common understanding that the procedure to create frozen straws would be conducted was organized. The main role of AIST was to develop the process technology of developing an inexpensive and simple manufacturing facility that was highly compatible with the current sperm straw manufacturing process. However, in the structure of interdisciplinary fusion, expectation ran high, and it was necessary to constantly check the awareness on the height of the obstacle of each phase.

3 Selection of research elements by considering specific role of participating institutions

The general goal to improve livestock breeding through the improvement of breeding sperms involves three factors of research: to develop technology to select and collect large amounts of highly motile spermatozoa; to check the isolated spermatozoa for their healthiness as reproductive cells; and to conduct field trials by using the sorted spermatozoa in the breeding process at farms. AIST was mainly in charge of the development of technology to select spermatozoa with high motility. Analyses of selected and isolated spermatozoa were conducted mainly by the National Agriculture and Food Research Organization, Saga University, University of Toyama, and Toyama Prefectural Agricultural, Forestry and Fisheries Research Center. Field trials at farms were done by the National Livestock Breeding Center, Saga Prefectural Livestock Experiment Station, and Morinaga Rakunou Co., Ltd. AIST approached these institutions, shared the understanding that improvement of spermatozoa was important to increase livestock breeding, obtained agreement to select spermatozoa with high motility as a specific method, and asked cooperation considering work content and facilities of the institutions.

There are several conventional technologies and methods

for sorting highly motile spermatozoa that AIST was in charge. The simplest method is routine work of capturing highly motile spermatozoa with a pipette when they gather at the laminar interface. The swim-up method is a method in which sperms are centrifuged and allowed to settle, and then highly motile spermatozoa that swim upward are collected. This method is conducted widely, and there are tools used to perform this procedure easily and plainly. There are also methods that use Percoll, a solution that creates density gradients during centrifugation.[12] Such conventional methods can be distinguished from our concept, if considered from the perspective that the conventional methods are designed to remove spermatozoa that died or lost motility, rather than gathering the ones with high motility. Such pretreatments are commonly done in bovine *in vitro* fertilization and human infertility treatment.

On the other hand, the number and quality of spermatozoa gathered by such conventional methods could not be maintained at the required levels simultaneously. If qualitative homogeneity was to be maintained from the perspective of healthiness, the number of spermatozoa necessary was in units of several hundreds or thousands. On the other hand, it is difficult to maintain quality when a high number of spermatozoa is desired. For AI application which is the main approach to cattle reproduction, a large number approximately approaching several to tens of millions spermatozoa is necessary. With the conventional methods, it is difficult to gather large amounts of highly qualitatively healthy spermatozoa, and it is unknown how many spermatozoa of such high quality is necessary to conduct a successful AI.

Based on previous studies, a microfluidic approach is the most appropriate platform to ensure the quality of the selected spermatozoa. The relationship of the size of spermatozoa (several ten micrometer unit) and the size of microchannels (several hundred micrometer unit) is suitable for effective spermatozoa sorting, apart from the sieve method. Various devices have been developed, and several reports present investigation on the qualitative property of the sorted spermatozoa.[13] However, as the size of the microchannel relative to the size of spermatozoa to maintain swimming against the flow has a limit, the number of spermatozoa that could be selected at a specific time is also limited, thus, challenging the microfluidic approach in terms of surpassing such limits. Looking at the property of spermatozoa motility, the phenomenon of "rheotaxis" is well-known. Rheotaxis is described as the property of "swimming against the flow." The phenomenon itself has been known for quite some time, but recently, detailed reports have been provided on the physics of the actual behavior.[14] This phenomenon was considered for the possibility of guiding the navigation of spermatozoa with the flow, and this led to the technological idea of "having the spermatozoa gather by themselves." This initially seemed to be a strange idea for researchers who have been involved in conventional livestock breeding.

On the other hand, in the field trials conducted by institutions other than AIST, the experiments could not be similarly planned as the organized condition in a laboratory, and therefore, technologies such as machine learning were applied for data organization. Details will be explained later. While AI is conducted based on the observation of estrous behavior, at actual sites, it can be missed depending on the scale and form of the feeding facility or the number of available personnel. There were two feeding forms at the institutions involved in this study: one in which cattle were kept in individual sections of a barn; and another in which cattle moved from pasture to barn at regular intervals. In the latter case, it was difficult to observe estrous behavior or conduct AI while cattle were in the pasture. When the cattle were kept in a barn, stickers could be placed on their backs to record estrous behavior or frequent monitoring could be done after observing follicles by echo. These ensured the detection of estrous behavior, and it was also possible to check ovulation by echo AI.

4 R&D of each element

To realize the basic research structure for using the flow to gather large amounts of highly motile spermatozoa and to guide the spermatozoa to self-gather for collection, we investigated specific methods to be used.[15][16]

To "guide the spermatozoa" by "flow" or to link the gap between two elements of fluid manipulation technology and spermatozoa motility, investigation was done using fluid simulation technology. Here, the parameters set included the property of sperm movement (the rate is several tens to hundred micrometers per second) or the size of the microchannel of 100 μm that could be realistically fabricated.

The flow in the microchannel was a slow "laminar flow," and the central part of the channel had a faster flow rate than alongside the wall. It was calculated that the number of spermatozoa that could be transported with just one microchannel was far from enough. That is, unless the difference between the parts where the flow was fast and where the flow was slow could be minimized as much as possible, the separation efficiency of spermatozoa would not increase. The solution to this issue was simple; a partition board was placed in the channel. At the same time, the size of spermatozoa and the size of the wall that could be realistically fabricated were set as prerequisites of simulation. For example, if the walls were made too thin to increase the number of spermatozoa, the partition would not stand on its own, and might break when it was removed from the mold. However, the number of spermatozoa that could be selected

was still insufficient.

Pressed by the necessity to gather spermatozoa more aggressively, we decided to set a layered crescent-shaped channel as shown in Fig. 2. Spermatozoa are sucked in and gathered at the edge of the crescent regardless of motility, and then transported to an area near the entrance of the microchannel. Of the selected spermatozoa, those with high motility will swim up as they sense the flow in the microchannel, while those without motility will be pushed along with the flow. Although the point of using a crescent-shaped flow channel as a structure that allows continuous separation may seem to be a breakthrough, anyone with knowledge of mechanical engineering that looks at the viscous force of fluids will more likely consider that given a flow suddenly entering a wide space, a flow that pulls in the surrounding fluid is formed. A flow that repeats such separation and a flow channel that realizes this action were designed by fluid simulation, and the overall design of the device was determined considering the ease of use at farms and the actual amount of sperms that would be treated. Following the overall design, a device was fabricated by a mold cutting process and transferred to silicon rubber. The sorting device for highly motile spermatozoa fabricated by this technology is simple, as shown in the schematic diagram of Fig. 2 and the photograph of Fig. 3. Fluids are delivered by the height difference of the liquid surface of the three fluid reservoirs of the device, and no exterior mechanism such as pumps is required.

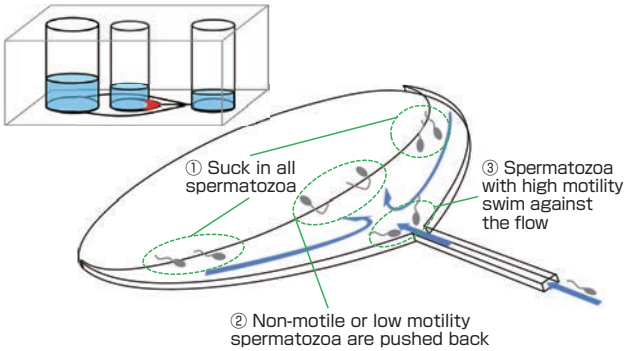

Fig. 2 Overall conceptual diagram of device for motile spermatozoa sorting, positioning of crescent-shaped layered flow channel (red part), and concept of flow of solution and motion of spermatozoa in crescent layered channel

Fig. 3 Photograph of device for sorting motile spermatozoa

The actual selection of highly motile spermatozoa was conducted using this device, and it was confirmed that, for example, about 1 million to 10 million highly motile spermatozoa could be obtained in a sorting process of about 30 minutes, using one typical straw of frozen sperms (number of contained spermatozoa 30–60 million, 0.5 mL fluid). The actual number of spermatozoa obtained is dependent on the concentration and quality of the spermatozoa in the original sperm. In the case of cattle, this number of spermatozoa fulfills the number of spermatozoa required for AI.

In addition, by adjusting the rate of fluid flow, it is possible to sort according to the manner of movement of spermatozoa, such as “straight swimming” or “zigzag swimming” (Fig. 4), and not merely whether they are motile. Spermatozoa do not possess fertilization ability from the beginning even if they become mature sperm cells. It is known that there is capacitation, or changes into fertilizable form through various biochemical reactions and increased motility as they travel through the female reproductive organ, and that the swimming forms change accordingly. That is, selecting spermatozoa based on the characteristic motility provides a means of selection of spermatozoa at a specific stage of change or a “capacitation event,” and this was the first time that we realized a method to study the characteristics of spermatozoa that is advantageous to AI.

5 Evaluation of results

The correlation between highly motile spermatozoa and qualitative healthiness had been known, but in this study, evaluation was done for the spermatozoa that were sorted using the developed technology. When the DNA

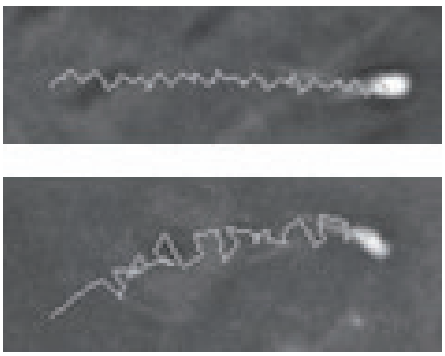

Fig. 4 Motion of spermatozoa is not uniform, and some may swim straight while others may swim zigzag. The swimming pattern is one of the indices that represent the condition of spermatozoa.

fragmentation rate of the selected motile spermatozoa was investigated, it was 7 % before treatment and 0.4 % after treatment, demonstrating and confirming a remarkable improvement in terms of DNA. Low DNA fragmentation, in other words, means that the integrity of DNA carried by the spermatozoa is high. Considering the fact that the DNA fragmentation rate of commercial breeding sperms is around 5 %,[17] the value obtained is sufficient. The sperm after treatment has high mitochondrial activity, and it was also found that the high activity was maintained over a long time. For example, the mitochondrial activity of untreated sperms decreased to about 20 %, but the treated sperms had about 60 % activity six hours after treatment.

Since the technology that we developed was for livestock breeding, it was necessary to conduct AI at farms and investigate the performance. The aforementioned devices were distributed to a number of farms, and when cows were observed to be in estrus, the thawed sperms were treated using the device, and selected sperms were used in AI. The determination of pregnancy or non-pregnancy was conducted 40–50 days later, and correlation of the test results on the motility of the selected sperms and pregnancy test results was established. One frozen straw containing cattle breeding sperms used in ordinary artificial insemination contains about 20 million to 30 million spermatozoa. In this test, AI was performed with about one million selected highly motile spermatozoa. Upon comparison, the pregnancy rate of cows using this remarkably lower number of selected spermatozoa was the same as the pregnancy rate of the past few years of the subject area (test farm). The progress of pregnancy and the calves born were normal.

Since field trials were done at a number of farms, there were differences on how work was handled at different farms, and the environmental conditions could not be kept completely uniform; a reasonable reality of field trials. Under such situation in which conditions could not be kept uniform, we attempted excavating new findings, and various statistical methods and machine learning were used for data analysis. As a result, we found that there was a correlation among the results of pregnancy and infertility, the motility of spermatozoa used in artificial insemination, and the timing of artificial insemination. Specifically, the pregnancy rate was better in spermatozoa that swam zigzag compared to the ones that swam straight. This tendency was more apparent when timing of AI was later from the discovery of estrus.

As mentioned earlier, spermatozoa do not possess fertilization ability from the beginning, but change to a state in which they can fertilize after undergoing various biochemical reactions and increased motility in the female reproductive organ, and the swimming form changes during this process. While a commercial spermatozoa motility analyzer can measure the number of vibrations of the head of the spermatozoa and travel speed such as linear and curve velocity (spermatozoa may travel arc-like while waving their heads, and there are several ways of representing the speed according to the interpretation of the track), there was no index for representing the "swimming form." Therefore, we originally defined SMI index as the index to represent the "swimming form," and conducted an evaluation. The SMI index was defined as the value in which the product of the linear velocity and the number of head vibrations was divided by the curve speed. The larger SMI indexes indicate linear swimming, while the smaller indexes show zigzag forward motion.

Figure 5 shows the results of pregnancy and infertility after AI. The time from the discovery of estrous behavior in cows to performing AI is shown in the horizontal axis, while the vertical axis shows the SMI index of the spermatozoa used in AI. The pregnancy and non-pregnancy plots are indicated by different colors. These results demonstrate that the pregnancy rate is higher for zigzag-swimming spermatozoa for the time span of 8 to 24 hours that is generally used for AI. For AIs conducted earlier than the above timing, the same or at the least not less pregnancy rate was observed in the straight-swimming spermatozoa. Such results suggest an existing relationship between the time required for the spermatozoa to travel in the female reproductive organ and the timing of ovulation. Moreover, these results clarify the property of spermatozoa, and identify the sperm population types that should be supplied and contained in the livestock breeding straw. In addition, we were able to discern and classify spermatozoa according to the swimming form or motility pattern. On the other hand, the plots representing non-pregnancy (infertility) show that SMI remains almost constant in a horizontal line against time, suggesting infertility to be ascribed to other factors such as the physical conditions of the cows.

6 Future development

To achieve technological implementation and realize the benefit of this work in terms of increasing reproduction, the following development has been considered while taking into account both the obtained scientific findings and the actual practice on site.

For AI of cattle, the "AM-PM method" had become the standard, in which insemination is done in the afternoon of the day when one finds the cow in estrus in the morning, and on the morning of the following day when estrus is observed in the afternoon. By this method, the interval between ovulation and AI is roughly controlled. Understanding and analysis of Fig. 5 will be facilitated better with the AM-PM method in mind. For the time frame starting from estrus discovery to AI, as shown in the horizontal axis, rather than the short time, the long time frame matches the timing of AI based on the AM-PM rule. Looking at the distribution of

pregnancy and fertility in the long time frame, the zigzag-swimming spermatozoa lead to higher pregnancy. With the AI purpose in mind, it is concluded that incorporating higher percentage of zigzag -swimming spermatozoa in the sealed frozen straws will yield more efficient reproduction in cows. This is based on the concept of fine-tuning the timing of the encounter of the ova and spermatozoa in the female reproductive organ.

Currently, these selected "spermatozoa appropriate for AI" are manufactured as frozen straws, and their efficiency in increasing conception rate is being conducted at farms. To produce frozen straws, it is necessary to scale up the selected and isolated highly motile spermatozoa while considering the manufacturing facility for mass production. This requires a review of the cryopreservation process.

Scaling up of the selection of highly motile spermatozoa is investigated based on the concept of the microfluidic device. By narrowing the target to zigzag-swimming spermatozoa, we are working to enable easy operation during on-site implementation, including simplifying the setting conditions of actual sperm selection maneuvers. On the other hand, for the cryopreservation process, the method of adding a cryoprotectant to the spermatozoa solution and spermatozoa concentration must be simultaneously investigated. Egg yolk and glycerol are used as cryoprotectants. The technology for sorting highly motile spermatozoa involves the selection of traveling spermatozoa, and the presence of granular substances like egg yolk in amounts beyond the limit tolerable by a specific number of spermatozoa must be avoided, as such substances may damage the spermatozoa and consequently inhibit forward motility and sperm function. Cryopreservation takes the course in which temperature is gradually decreased to 4 ºC and then rapid freezing is done, and the components of the cryopreservation solution needed to protect the spermatozoa at each stage are fixed. Therefore, each component of the cryoprotectant solution must be considered and investigation must be performed on what specific component must be added at which particular stage of cryopreservation.

Field trials will be done by creating frozen straws under a combination of various conditions, checking the motion of spermatozoa after thawing, and after achieving the most appropriate frozen straws verifying its efficiency by investigating the pregnancy rate by performing AI at farms. This is a time-consuming trial but we are making progress with the cooperation of farmers, and in the near future, we expect to release a new livestock breeding sperm product.

7 Summary

This research was aimed to increase the livestock reproductivity, particularly cattle by improving the frozen semen used in AI thru healthy and functional sperm selection. The specific procedures mainly depends on reports and literature on human infertility treatment. Table 1 presents the reports in the field of human infertility treatment, things expected to be applicable to livestock breeding by analogy, and the findings obtained in this research. In addition, our study developed the technology to select highly motile spermatozoa by several millions or more in number, and the relationship between the swimming form and pregnancy rate

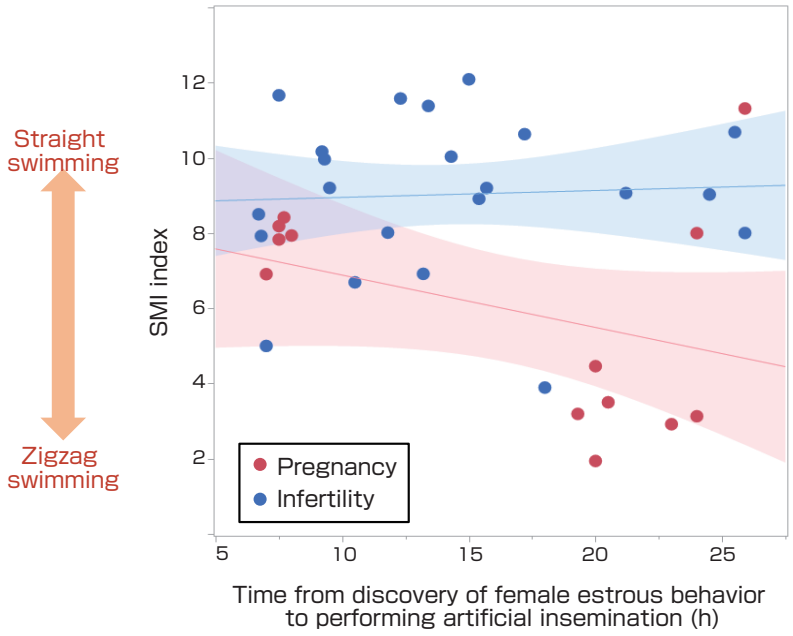

Fig. 5 Relationship between swimming pattern of spermatozoa and artificial insemination rate. Shaded area represents 95 % confidence interval 95 % for pregnancy/infertility.

Table 1. Reports in the field of human infertility treatment and the effectiveness of sorting technology for highly motile spermatozoa in cattle breeding

Perspective	Findings in human infertility treatment	What is expected in livestock breeding	What became clear from the study
Reason for improving sperm factor (rather than female factor)	Significance of sperm factor is becoming evident	Effect of improvement in breeding sperm exceeds expectation	Obtained almost same conception rate as conventional method by artificial insemination with about one-tenth the number of spermatozoa, by conducting motile spermatozoa selection
Qualitative healthiness of spermatozoa as a gamete	Sperm with good morphology and motility has good quality such as low DNA fragmentation	Strategy of selecting highly motile spermatozoa to improve livestock breeding is effective	Spermatozoa collected by this original sorting technology had greatly reduced DNA fragmentation (7 % → 0.4 %)
Clinical performance	High implantation rate, high conception rate, and low miscarriage rate	Increased conception rate and reduced embryonic death (very early miscarriage)	Conception rate was maintained even with less number of spermatozoa
Direction of improvement in breeding sperm	(Not applicable)	Clarify direction of improvement of livestock breeding sperm Possibility of achieving frozen straw containing spermatozoa capable of increasing conception	Relationship between swimming pattern of spermatozoa and conception rate in artificial insemination
Actualization of mass selection by ripple effect	(Not applicable)	Increase efficiency of breeding by in vitro fertilization and fertilized egg implantation	Succeeded in artificial insemination by establishing mass collection technology by selecting motile sperm

was clarified from the trial results using this technology. We believe on the potential of these findings to be extended to human infertility treatment.

These are the points that make this research original.

- We used fluidics to guide highly motile spermatozoa. Selection of appropriate number of spermatozoa was achieved using this technology, and for the first time in the world has succeeded in artificial insemination by pretreatment of spermatozoa and without incurring damage to this gamete.
- The characteristic of spermatozoa appropriate for conception was identified by their swimming form. We achieved a method for isolating a homogenous group of healthy and functional spermatozoa based on their swimming form.

Currently, research is being conducted targeting artificial insemination that dominates most of the breeding practice, but we hope to apply the findings obtained to *in vitro* fertilization in the future.

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References

[1] Bio-Industry Division, Ministry of Economy, Trade and Industry: Bio x digital ni yoru aratana keizai shakai (bioeconomy) ni mukete (Toward a new bioeconomy society through bio x digital), https://www.nedo.go.jp/content/100870410.pdf, accessed 2019-05-08 (in Japanese).

[2] Ministry of Agriculture, Forestry and Fisheries: Seisan nogyo shotoku tokei (Statistics of agricultural income produced), https://www.e-stat.go.jp/stat-search/files/data?sinfid=000031813108&ext=pdf, accessed 2019-05-08 (in Japanese).

[3] Stress-Off Alliance: Stress-off hakusho 2018~2019 (Stress-off whitepaper 2018~2019), https://prtimes.jp/main/html/rd/p/000000002.000038683.html, accessed 2019-05-08 (in Japanese).

[4] Livestock Production and Feed Division, Livestock Industry Department, Agricultural Production Bureau, Ministry of Agriculture, Forestry and Fisheries: Gyu Juseiran ishoku jicchi jokyo (Situation of execution of bovine fertilized egg implantation), http://www.maff.go.jp/j/chikusan/sinko/lin/l_katiku/attach/pdf/index-10.pdf, accessed 2019-05-08 (in Japanese).

[5] Maebashi Institute of Animal Science, Livestock Improvement Association of Japan, Inc.: Jutai chosa seiseki (Pregnancy test performance), http://liaj.or.jp/giken/hanshoku/jyutai.html, accessed 2019-05-08 (in Japanese).

[6] E. M. Walters, J. D. Benson, E. J. Woods and J. K. Critser: The history of sperm cryopreservation, *Sperm Banking: Theory and Practice* (A. A. Pacey and M. J. Tomlinson (eds.)), Cambridge University Press, 1–10 (2009).

[7] A. Hazout, M. Dumont-Hassan, A. M. Junca, P. C. Bacrie and J. Tesarik: High-magnification ICSI overcomes paternal effect resistant to conventional ICSI, *Reproductive BioMedicine Online*, 12 (1), 19–25 (2006).
[8] K. Shirota, F. Yotsumoto, H. Itoh, H. Obama, N. Hidaka, K. Nakajima and S. Miyamoto: Separation efficiency of a microfluidic sperm sorter to minimize sperm DNA damage, *Fertility and Sterility*, 105 (2), 315–321 (2016).
[9] A. Berkovitz, F. Eltes, S. Yaari, N. Katz, I. Barr, A. Fishman and B. Bartoov: The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm, *Human Reproduction*, 20 (1), 185–190 (2005).
[10] M. Zaferani, G. D. Palermo and A. Abbaspourrad: Strictures of a microchannel impose fierce competition to select for highly motile sperm, *Science Advances*, 5 (2), eaav2111 (2019).
[11] P. Humblot: Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants, *Theriogenology*, 56 (9), 1417–1433 (2001).
[12] S. Kaneko: Human sperm processing in assisted reproduction technology, *Journal of Mammalian Ova Research*, 22(1), 24–27 (2005) (in Japanese).
[13] S. M. Knowlton, M. Sadasivam and S. Tasoglu: Microfluidics for sperm research, *Trends in Biotechnology*, 33 (4), 221–229 (2015).
[14] V. Kantsler, J. Dunkel, M. Blayney and R. E. Goldstein: Rheotaxis facilitates upstream navigation of mammalian sperm cells, *eLife*, 3, e02403 (2014).
[15] M. P. B. Nagata, K. Endo, K. Ogata, K. Yamanaka, J. Egashira, N. Katafuchi, T. Yamanouchi, H. Matsuda, Y. Goto, M. Sakatani, T. Hojo, H. Nishizono, K. Yotsushima, N. Takenouchi, Y. Hashiyada and K. Yamashita: Live births from artificial insemination of microfluidic-sorted bovine spermatozoa characterized by trajectories correlated with fertility, *Proceedings of the National Academy of Sciences of the United States of America*, 115 (14), E3087–E3096 (2018).
[16] "Jutai ni yuri na seishi wo oyogi-kata de erande hoshu suru gijutsu (Technology to sort and select the spermatozoa advantageous for fertilization based on swimming form), AIST Press Release, March 20 (2018) (in Japanese).
[17] K. Takeda, K. Uchiyama, M. Kinukawa, T. Tagami, M. Kaneda and S. Watanabe: Evaluation of sperm DNA damage in bulls by TUNEL assay as a parameter of semen quality, *Journal of Reproduction and Development*, 61 (3), 185–190 (2015).

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Discussions with Reviewers

1 Overall

Comment (YUMOTO Noboru, National Cerebral and Cardiovascular Center)

This is an interesting paper in which technology was developed to sort motile spermatozoa from frozen cattle sperms using fluidics, and collecting a sufficient number of spermatozoa that can be used directly for artificial insemination. It also demonstrated in actual cattle breeding that the technology was advantageous for conception. I evaluate the paper as appropriate for publication in *Synthesiology*.

Comment (IKEGAMI Keiichi, AIST)

Using engineering methods, the authors developed sorting technology of spermatozoa that may contribute greatly to increased productivity of livestock, through collaboration with bioscience university labs and institutions that engage in field trials at farms. In this R&D, a premise was set from the planning stage that the developed technology must be applicable at sites of livestock production, where conditions such as individual animals and environments cannot be controlled. Synthetic R&D was conducted, and the case study is very thought-provoking for the readers of *Synthesiology*.

2 Building of collaborative framework

Comment (IKEGAMI Keiichi)

It will be great if you could provide explanation from the perspective of regional collaboration, not just interdisciplinary collaboration.

Comment (YUMOTO Noboru)

I believe there were many hardships in building the collaboration, but how did you overcome them? Building of a collaborative framework is an important point in the scenario of Synthesiology, so can you please add descriptions as much as you can?

Answer (YAMASHITA Kenichi)

The most important point you are asking, I think, is "why these members?" I added descriptions to Chapter 3 "Selection of the research element considering work of participating institutions." The flow of collaboration building was that AIST made the approach and got the cooperation of those that agreed to the theme of research and had facilities where actual experiments could be conducted. Rather than in the building of collaboration, the hardship was in how to process the trial results since trial conditions could not be unified among the institutions, and there was an accumulation of trial results at each institution in its area of specialty. The solution to this issue is explained in the final

paragraph of Chapter 3.

3 Comparison between research for human infertility and cattle breeding

Comment (YUMOTO Noboru)

I think you should create a table that lays out in an easy to understand manner what is known, not known, and what was found in the authors' research, concerning in vitro fertilization in human infertility treatment and artificial insemination for cattle breeding.

Answer (YAMASHITA Kenichi)

We added a summary which is presented in Table 1, and likewise added descriptions in Chapter 7 "Summary." Our research has accomplished what was not known or could not be accomplished in human infertility treatment, and therefore, we addressed the possibility of feedback to human infertility treatment in the text.

4 Relationship between spermatozoa motility and pregnancy rate

Comment (IKEGAMI Keiichi)

Can you explain so the non-experts can understand, how the number of non-motile spermatozoa sealed in the same straw affects the pregnancy rate, assuming that there are a million motile spermatozoa? I understand that spermatozoa with damaged DNA can have adverse effects, but I think you should provide a clearer explanation. For example, does inclusion of some poor spermatozoa make no difference because non-motile spermatozoa have low probability of reaching the egg? Those are the points that are unclear to those who have no expertise in the field.

Answer (YAMASHITA Kenichi)

There are a number of phases in which spermatozoa travel inside the female reproductive organ, arrive in the proximity of an egg, and cooperate together as a group to form a pathway to the egg. Then, fertilization occurs, and the process progresses to cell division and development. It has been reported earlier that active oxygen generated by dead spermatozoa may be harmful, and there have been similar recent reports. Dead spermatozoa (however, non-motile spermatozoa are not necessarily dead) may have adverse effects, and the transfer of damaged DNA may also have ill effects, but I think it is difficult to clearly and quantitatively state what factors affected how much at which stage.

Efforts toward commercialization of antifreeze proteins

ISHII Hirotaka* and INOUE Toshifumi

[Translation from *Synthesiology*, Vol.12, No.2, p.84–91 (2019)]

Antifreeze proteins adsorb to ice crystals and have the function of suppressing their growth. To apply antifreeze proteins to frozen foods where the coarsening of ice crystals leads to the deterioration of quality, we advance mass production technology. However, there were not many cases where the quality of frozen foods was improved by adding the antifreeze protein we developed. So, we changed the direction of development and conducted market research. Our market research revealed new possibilities for utilizing antifreeze proteins. We promoted the development of products that meet the needs of users and began sales as research reagents in 2016. Currently, we are working to address user problems, while manufacturing and selling research reagents.

Keywords : Antifreeze protein, frozen food, ice crystal, reagent, fish, mass

1 Introduction

1.1 History of food freezing

Freezing is defined as a process of removing heat from an object by creating low temperature using physical and chemical changes in substances. Nakahara Kota built the first refrigerator in Japan, and he froze fish in Yonago, Tottori Prefecture in 1899. In 1920, Kuzuhara Ihei, who had visited the United States and Europe, built a refrigerator in Mori, Hokkaido, and started production of frozen fish. This was the start of food freezing business in Japan. The place (currently, the site of Mori Plant, Nichirei Foods Inc., a subsidiary of Nichirei Corporation) is considered to be the birthplace of frozen food business in Japan.

Nichirei Corporation was originally established in 1942 during World War II as Teikoku Marine Products Control Company, a government-controlled entity based on the "Fisheries Control Ordinance." After the devastation of the war, the executives of the company realized that their mission was to rebuild the food economy that at the time was almost completely destroyed. The company restarted as a private company Nippon Reizo Co. Ltd. in December 1945. Nippon Reizo focused on frozen fish, ice-making, and refrigeration business, and embarked to fulfill its responsibility to help increase food production through "cooling and freezing abilities" that were essential in fisheries and food distribution. It saw potential in frozen food that allowed the food quality to be maintained for a long time without using preservatives, and became the pioneer of frozen food business.

1.2 Frozen food

Frozen foods that have significant relevance to Nichirei are products tolerable to long-term preservation. The voluntary standards of the Japan Frozen Food Association determined that frozen foods are to be stored and distributed at minus 18 ºC or less. Frozen conditions stop and/or delay the actions of microorganisms and enzymes, enabling long-term preservation. Simultaneously, ice crystals form in water in the foods during freezing, thereby degenerating tissues and proteins and degrading food quality. Particularly, ice crystals become larger when the cooling speed is slow, and therefore, freezing is normally completed by rapid freezing methods in which heat transfer coefficients are increased. Development is underway for new freezing devices to further control quality degradation during freezing, but there is a limit to the approach from the device side due to energy load, facility cost, and operation issues. Therefore, processing technology prior to freezing has drawn attention. In general, the studies include control of water content in foods or decreasing free water in foods. Other attempts include addition of cryoprotectants into food to avoid the effect of ice crystals. While such processing is effective in many processed foods, there is a major issue that taste and texture change greatly, and that is a problem when one wishes to maintain the original conditions of food materials such as vegetables, fish, and meat.

1.3 Antifreeze proteins[1][2]

Antifreeze proteins (AFPs) are proteins that have the function of adsorbing to ice crystals and inhibiting their growth. They are stored in the bodies of various organisms that can live in cold regions, and they evolved to enable the organisms to survive in low temperatures that freeze the body. One of the AFPs was first discovered by Dr. Arthur DeVries of the University of Illinois in 1969, from blood serum of fish living in the Antarctic Ocean. This discovery ignited academic interest, and more AFPs have been found in

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Table 1. Division of roles in joint research

Role of AIST	Role of Nichirei
Basic research	Application research
Elucidation of molecular function of AFP	Investigation of effect on food
Genetic and molecular structure analyses of AFP	Establishment of mass production method of AFP
Elucidation of biochemical and physicochemical properties	Usage development, exploration of new usage

fish, insects, plants, and fungi so far. In general, the growth inhibition of ice crystals increases with increase of AFP concentration, which leads to create an unusual frozen state of water composed of millions of tiny ice crystals. There are several types of AFPs depending on the organism with some minor differences in amino acid compositions and three-dimensional structures, whose target ice surfaces are also different. Therefore, the size and morphology of ice crystals formed in an AFP solution are diverse according to the concentration and type of the AFPs.

1.4 Joint research for industrial application of AFP

The effect of ice growth inhibition by AFP is extremely high, and since there was no other substance that had similar performance, it was expected from the beginning to be used in ice cream, frozen confectioneries, frozen foods, and freeze-dried foods, in which coarseness of ice crystals affected the quality. Nichirei has been engaged in the technological development for inhibiting quality change during freezing and freeze storage in the manufacturing process of frozen foods, and was interested in AFPs that might be effective in such processes, and wanted to study their effectiveness.

Although AFPs were discovered almost half a century ago, their rarity prevented them from being put to practical use. Particularly for fish, there was a semi-stereotype idea that AFPs could be found only in fish from Antarctica. This was supported by the fact that an American company started selling AFP from Antarctic fish at a high price ($10 per mg) from the late 1990s. Dr. Tsuda Sakae of AIST Hokkaido broke this stereotype. Tsuda *et al*. have been studying AFP from the 1990s, and discovered various new AFP species from fishes, plants, and fungi living in the Hokkaido region. It was thought that AFP could only be collected in small amounts from the blood of organisms in the polar region, but Tsuda extracted AFPs from muscle homogenates of regional fish, and this paved the way to collecting AFP in larger amounts that allowed investigation of effects in foods. We noticed the potential of AFPs for practical application in foods, and started joint research with the National Institute of Advanced Industrial Science and Technology (AIST) for industrial application of AFP.

2 History of development

2.1 Division of roles in joint research with AIST

As mentioned earlier, the reason that the practical use of AFPs has not progressed was due to their rarity that made usage development unfeasible. Although AFP was discovered in the organisms of cold regions, the amount and performance in individual organisms were varied, and it was difficult to obtain large amounts of organism-derived raw materials (for example, insects) that contained highly effective AFP for inhibiting ice crystal growth. There were also concerns in production that it might be difficult to obtain from natural products, due to degradation of protein in the extraction and purification processes, as well as overall difficulty of separation and refinement. Investigations of manufacturing AFP by chemical synthesis and genetic engineering have been conducted from a long time ago, rather than using natural substances. However, it appeared that the performance of the synthesized substances was significantly lower compared to the ones obtained from natural sources, and a product with effects that match the cost was not synthesized.

AIST was already working on the following: 1) search and functional analysis of AFPs that could be practically applied, and 2) development of a method to purify AFP in amounts necessary for practical use.[3] Preparation of AFPs from natural sources, genetic expression and chemical synthesis were progressed simultaneously, but our initial priority was set to fish and fungi-derived AFPs that could provide excellent performance in gram amounts.

In light of this situation, we decided to work jointly with AIST in the search, the mass production, and the effective use of AFPs that were expected to preserve good quality of foods after freezing and defrosting. The division of role was AIST conducted basic research such as elucidation of molecular functions of AFP, and Nichirei worked on application research such as verification of effects on food, usage development, and establishment of a mass production method (Table 1).

Figure 1 shows the scenario toward industrial application.

AIST had already checked the effect in over 100 species of organisms, and it was confirmed that several dozen organisms possessed AFP. AIST embarked on the structural analysis and functional clarification, and Nichirei decided to search for AFPs that might be effective in foods and to investigate their concentration.

For mass production, AIST had already established the technology to purify AFP in gram amounts. Therefore, Nichirei aimed for the establishment of a mass production method by constructing a facility at a pilot scale where several hundred grams to several ten kilograms of product could be produced, the search of raw materials that enabled commercial scale production of several tons or more, and the investigation of an efficient purification method for scale up. Moreover, while the usage development of AFP was conducted in various foods, investigations were also performed on additive methods, risks of activity loss in the processing procedure, and persistence of the effect, looking at actual manufacturing and distribution.

We created a scenario that by advancing such efforts, a production system for AFP would be established and AFP would be used in many frozen foods in the future.

2.2 Verification of effects in foods

In the beginning, we verified the effects in foods using fish AFP that could be easily purified at lab scale. Previous research papers mainly reported experiments at extremely low concentration of several μg/ml, and our verification experiments were conducted based on these reports. Agar gel, a familiar food, was used for verification. Agar gel is a representative food that is less tolerable to freezing. Once it is frozen, the gel structure is destroyed by coarse ice crystals, and when it is thawed, the polymer network cannot be maintained since water is not retained. Based on this property, we checked the effect of AFP on how frozen and thawed agar gel maintained its form. Following an initial test, it was found that the effect was not appreciable with the addition of several μg/ml. We hence increased the AFP concentration to several hundred μg/ml, by which the gel structure was still more greatly destroyed, which proved the negative effect.

The above described tests were conducted according to the method described in the papers. We observed differences in form and size of the ice crystals at several μg/ml concentration, suggested from the observation of ice crystals in the presence of AFP on a photomicroscope system. However, it appeared that a sufficient amount of AFPs had to be present in the food to exert the preservation effect. That is, we conducted the agar gel experiments by increasing the AFP concentration to 1,000 times, and found that the preservation of the gel texture was achieved with such a high concentration; i.e., the quality of agar gel after freezing and thawing significantly increased. Note that it was necessary to spend one year for this verification. If we just followed the published information, we might have determined the effect of AFP on food as "none." We became aware of the dangers of blindly following prior research results. In our case, it was simply a matter of increasing the concentration if the effect was not seen at an indicated concentration. The situation might have been different if we had AFP in large amounts rather than in grams. A lack of a sufficient AFP preparation method at Nichirei was perhaps another reason for spending one year on that simple experiment.

2.3 Investigation for mass production

We started investigation for technology of mass preparation of AFP using fish as raw materials, as we recognized the effect of increased freezing tolerance when added to agar gel, as well as its effectiveness of inhibiting ice crystal growth.

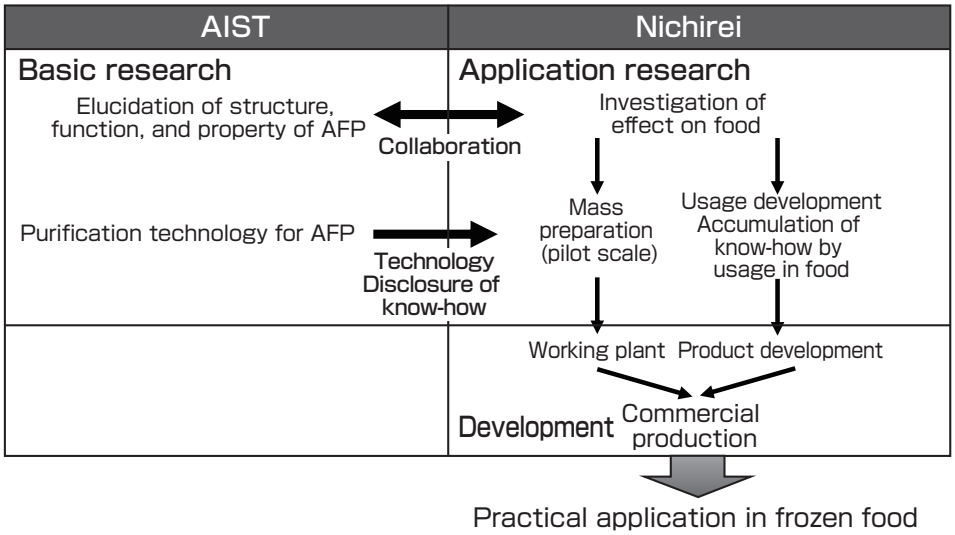

Fig. 1 Scenario for industrial application of AFP (realization in frozen foods)

Because fish could be obtained as raw materials in kilo units and yielded highly functional AFPs, we started investigation primarily with fish.

2.3.1 Selection of raw materials

AFPs in fish are roughly categorized into four types (AFP type I, type II, type III, and antifreeze glycoprotein), and all have the effects of inhibiting ice crystal growth, but their biochemical properties are varied. As indices of raw material selection, we placed importance on high yield and amount, ease of extraction and purification, and performance of purified AFP. We also considered raw material cost, sustainability of procurement, location of fishing, and fishing seasons. Since we were investigating with consideration on obtaining much raw material possible including fish or residues from fisheries processing whether domestic or overseas, as well as differences of fishing seasons, we spent a longer period of time on raw material selection. If it were not fish, there might have been only small quality change caused by location or season, but since importance was placed on being able to obtain high-performance AFP in large amounts, we reached the current results. We were able to obtain knowledge of the best raw material for practical use by investigating a large number of raw materials.

2.3.2 Investigation of purification method

Initially, we worked on the establishment of an efficient purification method at pilot scale using a raw material of type III AFP that had high effect of inhibiting ice crystal growth. However, type III AFP was not resistant to heat, and was not appropriate for manufacture for food that needed sterilization and heating. Therefore, considering heat resistance, we investigated a purification method using raw materials of type I AFP and antifreeze glycoproteins. The optimal conditions for crushing, extraction, separation, fractionation, concentration, and drying were investigated. The processes of crushing, separation, and fractionation were performed at low temperature to avoid decomposition by self-digestion and increase in viable count. However, fish contained fatty acids and various proteins, and precipitation of fatty acids during filtration and clogging of the membrane due to aggregation of protein occurred frequently. The yield from raw materials was low at 1 % or less, and there were large amounts of residual substances. Therefore, the main technical point was to select a membrane having an optimal pore-size, which allowed efficient collection of AFPs at high yield without leaving AFPs in the residues. Since there was hardly any budget for facilities, we somehow managed to develop a method for manufacturing at pilot scale, although it was not an optimal solution.

2.4 Accumulation of knowledge in food use for usage development

By increasing the additive concentration, effects were seen in many foods such as agar gel, ice cream, boiled eggs, and tofu. However, they had weak freezing tolerance, and hardly any effect was seen in vegetables, fish, and meat that were often used in frozen food. In the beginning, we thought the problem was permeation to the inner parts. That was because we confirmed by microscope that ice crystal growth was inhibited by increasing the immersion time in boiled eggs and tofu. However, a major difference was not seen for the aforementioned foods even when immersion time was increased or permeation was increased using pressure.

At the time, cellular level investigation was not conducted, and we did not have knowledge of cell preservation. Therefore, it was thought that cell membranes and cell walls inhibited permeability, but investigation had not been done. Later, the reason for not obtaining the effect became clear, as our knowledge of cell preservation increased and the fact that AFPs adsorbed to the cell membranes became apparent. AFPs did not show effect unless they were adsorbed well to water before freezing. It became clear that in foods with cells, good effects were not seen unless AFPs were distributed thoroughly to tissues and cells. Therefore, it was thought the freezing tolerance of vegetables, fish, and meat did not increase simply with the immersion process.

3 Activity toward product realization

We attempted practical application to frozen foods and worked on usage development and mass production of AFPs, but it was difficult to add freeze-tolerance to vegetables, fish, and meat, and there was no product which we wanted to differentiate by adding purified AFP among inexpensive frozen processed foods. Therefore, the initial scenario had to be changed. Following discussions, we decided to provide AFPs that were produced at pilot scale to organizations outside our company to seek different usage. The scenario for industrial application considering the provision of AFP outside the company is shown in Fig. 2. Since realistic effects were obtained if the additive AFP concentration was increased, we estimated that there would be applications to high additional value products. We created a scenario that if we provided more AFPs for such usage, there would be cost merit of mass production, and use at Nichirei would also increase.

3.1 Efforts for AFP samples

As mentioned earlier, there are limited types of foods in which freeze-tolerance can be increased by AFP. Since freeze-tolerance is increased by controlling the water content in the conventional frozen processed foods, dramatic quality improvement cannot be expected by the addition of AFP. Also, there are several inexpensive materials that provide freezing tolerance such as some glycosides, and purified AFP will not be adopted unless there is a special superior effect. Against such a background, it was necessary to extend usage development widely to items other than foods, and we

started providing AFP samples to organizations outside the company. In practice, we announced the provision of AFP samples on the Nichirei's website in 2009, had participants sign a material transfer agreement (MTA), and provided crudely purified products. There were dozens of companies to which the samples were supplied. Through this effort, we started to see several areas possessing high potential for AFP application. On the other hand, there were areas in which investigation for practical use did not advance by crudely purified products, and it was necessary to prepare more samples.

3.2 Toward product realization of AFP reagent

In the effort of sample provision, we received requests for provision of highly purified AFP and provision of inexpensive samples that could be used at production scale. Since AIST had already established a method for high purity refinement, production was basically performed according to that method. However, there was an issue that although the same protocol was employed, highly purified AFP could not be obtained similarly. For product realization as reagents, the minimum standards were that the product had to clear a certain degree of quality, and there were no variations in performance. Since the products were proteins, we conducted electrophoresis and protein quantification at each purification phase, and checked the yield and quality while changing the purification condition little by little. Finally, we were able to obtain a stable supply of highly purified AFP using a slightly improved method developed by the collaboration research with AIST. The aforementioned variation was resulted from slight differences in the devices and reagents used, and this gave a good opportunity to learn the difficulty of reproducing the results using the same method in a different place, for both Nichirei and AIST.

We received several questions especially about solubility and heat stability of the AFP samples, so we monitored changes and performance of the samples during storage.

To commercialize the product, there was absolutely no established know-how, and we had to find the way. There was neither previous examples nor useful information to commercialize this kind of products within the company, so nothing went forward unless we moved. We had to do everything ourselves, including checking risks from legal and intellectual property aspects, clearing the matters of quality assurance, and writing the text for websites and catalogs. Check of related laws to sell the reagent could not be done solely within the company, and we prepared for imagined risks by consulting experts outside our company.

4 Product realization

4.1 Product realization of fish AFPs

We started the sales of research reagents (Fig. 3) in September 2016 from the Nichirei's website. A press conference "Announcement of the sales of AFPs" was held on the day of launch, and some media covered the event.

A list of current AFP products is shown in Table 2. There are four types of fish AFP: antifreeze glycoprotein, type I AFP, type II AFP, and type III AFP. They are all extracted and purified from fish that live in the low temperature regions (antifreeze glycoprotein = Gadidae, type I AFP = Pleuronectidae, type II AFP = Cottoidei, and type III AFP = Zoarcidae). There are two types of products: "highly purified product" in which the purity is 90 % or higher, and "crudely purified product" that contains foreign substances consisting of other proteins that are derived from raw materials. The highly purified product is sold in 100 mg units, and the crude

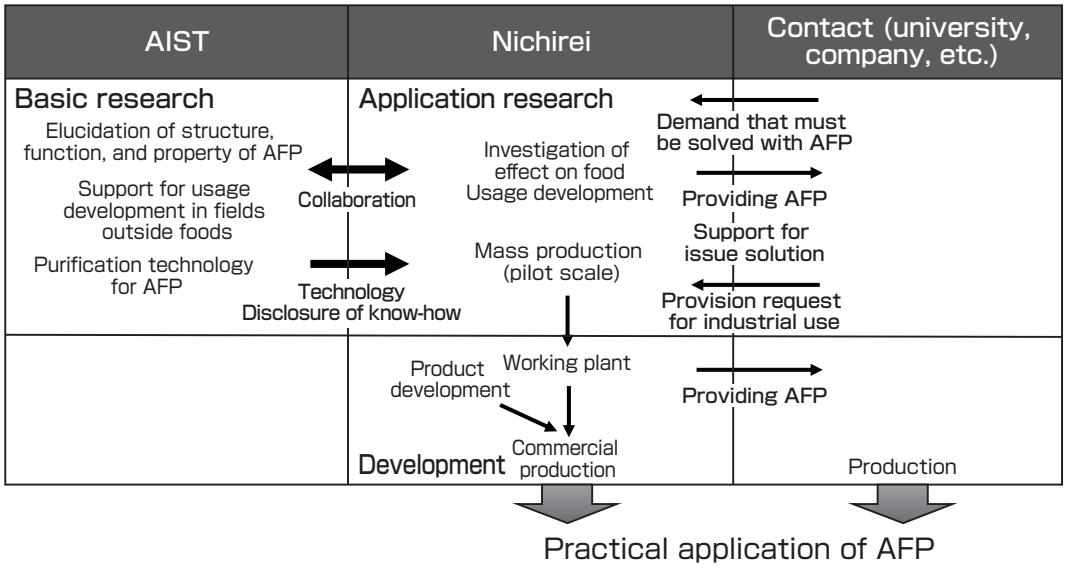

Fig. 2 Scenario for industrial application through provision of AFP to outside organizations

Table 2. List of AFP products sold (as of May 2019)

Product name	Sales unit
Highly purified fish-derived antifreeze glycoprotein	100 mg
Highly purified fish-derived type I AFP	100 mg
Highly purified fish-derived type II AFP	100 mg
Highly purified fish-derived type III AFP	100 mg
Crudely purified fish-derived antifreeze glycoprotein	1 g
Crudely purified fish-derived type I AFP	1 g
Crudely purified fish-derived type II AFP	1 g
Crudely purified fish-derived type III AFP	1 g

product is available in 1 g units. The use of highly purified products is expected to be for basic research at universities and research institutions as well as researchers of cell preservation, while the crudely purified products are targeted mainly at researchers of companies and universities that conduct application research.

4.2 Achievements in sales of reagents

Inquiries for AFPs have reached several hundred cases as of May 2019, and the sales performance is over 100 cases. The contents of inquiries are varied concerning food, medical care, industry, and research. We also received inquiries from abroad.

Through the sales of reagents, we have had opportunities to meet many people outside our company. There, we listened to the issues in freezing and related matters, and have had discussions on new technological information. Many topics cannot be solved by the AFPs that are being sold now. However, even in the fields different from food, the topics that people wish to solve are often similar, and we believe that a solution can be offered in the future, by utilizing other freezing technologies that Nichirei possesses.

Through the efforts on AFPs, Nichirei was able to increase the knowledge of freezing technology and freshness keeping technology. In addition, we received the "Life Nanotechnology Award" at the International Nanotechnology Exhibition and Conference in 2017. These accomplishments helped Nichirei's developmental efforts on freezing technology to be recognized widely both inside and outside the company.

5 Future issues and prospects

The sales of fish AFP products were started in 2016, and many people have realized their effect. We also feel that the awareness of AFP since the launch of the reagent has increased gradually. On the other hand, a little less than three years have passed since the launch, but there has been no major achievement that has led to practical application.

However, we believe that AFPs will be widely put to practical use in the future. Currently, in the field of cell preservation, evaluations are being performed using various cells, jointly with universities and research institutions. Various efforts are being made with several companies on the issues of freezing control in cold regions and freezing control during product manufacturing. In foods, effects are seen in foods that were not investigated at Nichirei, and some companies are saying that they would like to use it immediately if it can be used in foods. For raw materials and manufacturing methods, those that were excluded since this product was initially for food are being reconsidered now. As a result, AFPs exhibiting

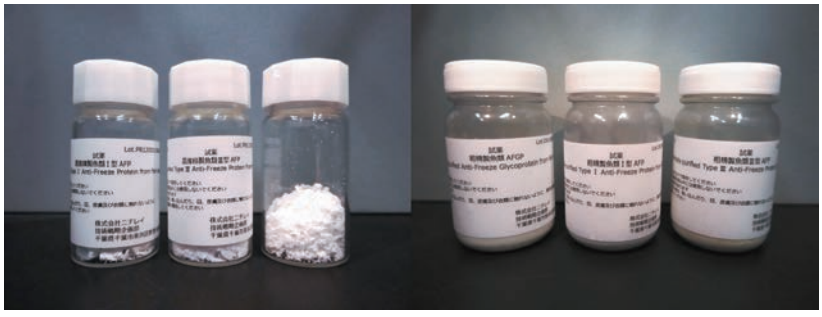

Fig. 3 Appearance of AFP reagent on the market

the performances that were not identified before are being discovered, and we are working on the method for their mass production. By clarifying the working mechanism of high-performance AFPs, it may become possible to artificially produce more effective AFP. If that becomes possible, I believe the issues of production cost will be cleared. The action of AFP can be seen at ppm order, and good effects can be obtained with a very small amount if it is reacted with water.

We are aware that there are issues in each field when utilizing AFPs in a wide range of fields. For example, for foods, it is necessary to manufacture a product that has minimum effect on taste and flavor caused by foreign substances. For medicine, it is necessary to establish a manufacture method that fulfills the standard for animal-derived raw materials. Since Nichirei has succeeded in reagent sales, the R&D for AFP will be continued. In the future, we wish to strengthen collaboration among companies, not limited to joint research with universities and research institutions. We hope to offer solutions to issues of the world through the development of AFPs.

Acknowledgement

We shall use this opportunity to thank Dr. Sakae Tsuda of AIST who has been cooperating with us in this research and throughout the joint research.

References

[1] S. Tanaka, Y. Kobashigawa, K. Miura, Y. Nishimiya, A Miura and S. Tsuda: Antifreeze protein, *Seibutsu Butsuri*, 43 (3), 130–135 (2003) (in Japanese).
[2] Y. Nishimiya, H. Kondo, M. Sakashita, A. Miura and S. Tsuda: Antifreeze protein: Functions and application, *Kagaku To Seibutsu*, 48 (6), 381–388 (2010) (in Japanese).
[3] Y. Nishimiya, Y. Mie, Y. Hirano, H. Kondo, A. Miura and S. Tsuda: Mass preparation and technological development of antifreeze protein—Toward a practical use of biomolecules, *Synthesiology*, 1 (1), 7–14 (2008) (in Japanese) [*Synthesiology English edition*, 1 (1), 7–14 (2008)].

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Basic Research Group, Technological Strategy Planning Division, Nichirei Corporation. Graduated from the Graduate School of Pharmacology, Meiji Pharmaceutical University. Joined Nichirei in 2004. Has engaged in search of AFP, establishment of mass production method, and usage development. Worked hard on product realization of AFP reagents, and has been in charge from their production to sales from the initial launch of the product. Currently, working on development of new products and practical applications. In this paper, was in charge of verification of effect on foods, effort in product realization of the reagents, and writing of this paper.

Inoue Toshifumi

Basic Research Group, Technological Strategy Planning Division, Nichirei Corporation. Graduated with specialty in Food Process Engineering, Graduate School of Agriculture, Kyushu University. Joined Nichirei in 1995. Found potential in AFP and has been involved in AFP from its beginning. Worked mainly on verification of effect, search of raw materials, mass production, and usage development. In this paper, was in charge of verification of effect on food, investigation for mass production, and efforts on sample provision.

Discussions with Reviewers

1 Overall

Comment (Yumoto Noboru, National Cerebral and Cardiovascular Center)

This is an interesting paper that describes in detail the transition of the scenario, as industrial application and practical use of antifreeze proteins in frozen food shifted to application in other fields. Although this paper was submitted as a "report," I see the authors' originality in the scenario and the synthetic components (selection and integration) and I think it fulfills the requirements as a "research paper" of *Synthesiology*. Therefore, I recommend that this be published as a research paper.

Comment (Goto Masanori, AIST)

This paper is a summary of the basic research on antifreeze protein by Nichirei and AIST, the joint research by these two organizations toward practical use, and the actual product realization. They work on issues such as mass production and application to actual foods that are topics that cannot be tackled by academic researchers. I think it should be read by researchers who are engaging in joint research with companies, and I recommend its publication.

"The danger of blindly following prior research results" that you mention in Subchapter 2.2 is a matter that one must consider, regardless of basic or practical research. Also, in Subchapter 2.4, you found the reason why freezing tolerance of vegetables, fish, and meat cannot be increased, how this allowed you to see limits at the time, and gave you the opportunity to change direction. I think this illustrates the importance of basic research in product realization. On the other hand, Subchapter 3.2 describes the story of hardship due to factors that depart from your main business. This sheds light to the internal situation of a company that we don't get to see often, and I thoroughly enjoyed reading it.

2 Changes in the scenario

Comment (Goto Masanori, AIST)

I think what triggered success was the change in your scenario. I think there are many companies that currently take open innovation strategies, but was there any resistance, at the time, in providing information and materials to organizations outside your company?

Answer (Inoue Toshifumi)

Since we were working on this project for application to our company's product, there were voices of opposition to provision outside the company. However, we could not see enough effect in

the prospective food groups, and we were pretty much lost. In the first effort for sample provision, we gained company consensus by having clients enter their objectives of use in the MTA, and putting in a clause stating that they must engage in discussions if they obtain results or inventions.

3 Future prospect

Comment (Yumoto Noboru, National Cerebral and Cardiovascular Center)

I think you are in the process of considering to which fields you shall apply AFPs in the future, but can you give us a little more specific prospect (for example, like in the field of cell preservation) and describe the issues in that field?

Comment (Goto Masanori, AIST)

I think the most important part is future prospect. Although you succeeded in product development as reagents, the development of application is the issue if one wishes to have major business. Particularly, I hope you succeed in the utilization in cells that seem to have stalled in the middle of the study, and I hope this technology is applied to the medical field, not just foods.

Answer (Ishii Hirotaka)

I added a brief explanation of the examples of work in various fields for practical application of AFPs and the issues in foods and medical fields in Chapter 5. Dr. Tsuda Sakae of AIST has consistently studied the effect of AFP on cell protection. The fact that better usage methods and usage places can be discovered with long and persistent efforts can be said for all usage development, not only in the cell preservation field. Cell protection effect of AFP is thought to be operating through a mechanism different from the existing cell protectants. As we clarify the mechanism, advancement of practical use can be expected. Nichirei is advancing development of AFPs that are expected to be effective, and I think clarification of their action mechanism can be done through collaboration with outside institutions.

Measurement of mass of aerosol particles

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[Translation from *Synthesiology*, Vol.12, No.2, p.92–106 (2019)]

An aerosol particle mass analyzer (APM) which classifies aerosol particles according to their mass has been developed. Mass distributions of aerosol particles are measured by the APM combined with a particle counting device. Particle masses can be measured in the range 3×10^{-18} g to 2×10^{-12} g, which partially fills the mass range that has not been covered by existing mass measuring instruments such as mass spectrometers and conventional balances. The invention of the APM has led to a variety of new techniques for evaluating aerosol particle properties such as effective density, material density, porosity, fractal dimension, and mass concentration of suspended particulates, among others. This article describes the principle of the APM, its features differentiating it from other instruments for classifying aerosol particles, and its applications to characterization of aerosol particles. The significance of measurement of particle mass in aerosol science, and the historical process that has led to commercialization of the APM are also described from the viewpoint of "synthesiology."

Keywords : Aerosol particles, particle mass, mass distribution measurement, aerosol particle mass analyzer

1 Introduction

A two-phase system consisting of suspended solid or liquid particles and the surrounding gas, typically air, is called an aerosol. Aerosols have attracted much attention from a variety of fields, because of their involvements in a broad range of phenomena such as potential damage to health and the environment by airborne nanoparticles, transport of radioactive elements through the air, global warming, and particle contamination in cleanrooms, among others. Recent interests in aerosols are due not only to these undesirable effects, but also to their possible useful applications such as aerosol processes for creation of novel materials.

In order to understand phenomena in which aerosol particles are involved in some way or other, measurement of various properties of the aerosol particles is needed.[1] Among these properties, particle size (particle diameter, if the particle in question is spherical) is an especially important quantity to be measured, because it significantly affects many of the aerosol-related phenomena. Aerosol particles are, however, often non-spherical, and a geometric diameter cannot be defined unequivocally for them; instead, several kinds of effective diameters are defined and measured. On the other hand, particle mass is a quantity inherent to each individual particle: it is uniquely defined even for a non-spherical particle. It is a direct measure of the amount of substance contained in an individual particle, and has crucial effects on particle motion and other physical phenomena; hence it should be no less important than the particle size. Until recently, however, there has been no established method for measuring mass of aerosol particles. In the present article, the aerosol particle mass analyzer (APM) that has enabled measurement of mass of aerosol particles† is described from the viewpoint of "synthesiology."

2 Significance of particle mass and its measurement

In this section, the relationship of particle mass with some other particle properties is described first in Subsection 2.1. Then the significance of knowing mass of aerosol particles in understanding their impacts on various human activities is discussed in Subsection 2.2. Finally in Subsection 2.3, how we became interested in their measurement is briefly reviewed.

2.1 Particle mass and equivalent diameters

The electron microscope images of particles in Fig. 1 show a variety of shapes particles can exhibit depending on how they are produced. Particle mass is a physical quantity that can be unambiguously defined for particles of any shapes, but particle size is not. For non-spherical particles, such as shown in Figs. 1(b) to 1(d), an equivalent diameter is used instead of the geometric diameter. An equivalent diameter is defined as the diameter of a spherical particle that has the same value of a certain physical quantity as the particle under consideration. Among representative equivalent diameters are: kinematic equivalent diameters including the mobility equivalent diameter and the aerodynamic diameter; geometric equivalent diameters including the volume equivalent diameter and the surface area equivalent diameter; and optical equivalent diameters including the light

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scattering equivalent diameter.[1] In the following, kinematic equivalent diameters are described in some detail, because they are closely related to the particle mass.

Mechanical mobility†† is the ratio of the velocity of a particle in motion under the influence of a certain external force to that force. Mobility equivalent diameter, D_B, is defined as the diameter of a spherical particle having the same mechanical mobility, B, as the particle in question. The mechanical mobility of a spherical particle with diameter D can be expressed as

$$B = \frac{C(D)}{3\pi\eta D}, \tag{1}$$

where η is the viscosity of the surrounding air, and $C(D)$ is the slip correction coefficient (a numerical factor expressing the deviation of drag force experienced by a particle from Stokes' law). The mobility equivalent diameter, D_B, of a non-spherical particle with mobility B is given by solving equation (1) for D. Because the drag force depends only on the spatial extension of the particle and not on its mass nor density, so do D_B and B. The product qB of a particle of charge q and mechanical mobility B is known as electrical mobility ($z = qB$), and it represents the velocity of a charged particle in an electrostatic field of a unit strength. Because methods to measure electrical mobility are well developed compared to methods to measure mechanical mobility (see Subsection 3.2), the electrical mobility is often measured in place of the mechanical mobility. In the following, the term "mobility" is used for simplicity to indicate either the electrical mobility or the mechanical mobility, as far as there is no fear of confusion.

Aerodynamic diameter D_A is another kinematic equivalent diameter; it is the diameter of a standard-density (ρ_0 = 1 g/cm^3) sphere (i.e., a spherical water droplet) which has the same terminal velocity as the particle in question when it settles under gravity. The mass m of a spherical water droplet with diameter D is $\rho_0\pi D^3/6$, and its terminal settling velocity v_T is given by mBg. (g is the gravitational acceleration). Hence the product mB in this expression can be rewritten as

$$mB = \frac{C(D)D^2\rho_0}{18\eta}. \tag{2}$$

If we measure v_T in the gravitational field (or in some acceleration field of a known magnitude), the quantity mB can be determined as v_T/g. Once the value of mB is known in this way, the aerodynamic diameter, D_A, is obtained by solving equation (2) for D regardless of its particle shape. Particles with various shapes migrate at the same terminal velocity in a given acceleration field, as far as they have the same value of D_A. This is the major reason why we want to know the value of D_A.

Equations (1) and (2) indicate that if we know two of the three quantities, m, D_B, and D_A, the rest can be known from them. For example, the value of D_A of a particle with a known m can be derived from a measurement of D_B, and vice versa. Recent studies show that, in general, if simultaneous measurement of particle mass m and some other particle property is conducted, various properties of the particle can be derived from them; a variety of such examples are given in Section 4.

2.2 Endpoint effects of aerosols

We denote an impact that aerosol particles ultimately have on human activities, whether desirable or not, as an endpoint effect. To control an endpoint effect of aerosols, it is crucial to evaluate its magnitude. However, it is often not easy nor useful to directly evaluate the endpoint effects of aerosols.

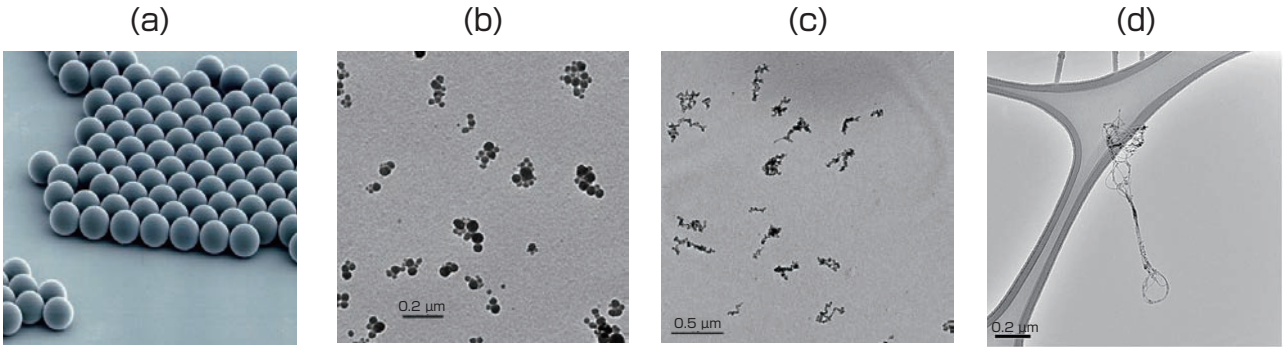

Fig. 1 Examples of the shape of various particle types: (a) polystyrene latex particles, (b) Al_2O_3 particles, (c) diesel exhaust particles, (d) single-walled carbon nanotubes. Photograph (a) is by courtesy of JSR corporation. Photographs (b) and (c) are reproduced from reference [2], and photograph [d] from reference [3], both with permission.

† Though we use the phrase "measurement of mass" in this article for simplicity, it would be more adequate to use the phrase "measurement of mass distribution," because in aerosol measurements, we are almost always interested in a property of the particles contained in an aerosol as a whole, and not of one specific particle. Also, note that "mass of aerosol particles" here does not imply the total mass of particles collected, for example, on a particulate filter, but implies mass of individual aerosol particles.

† † The particle mobility B is in general a tensor, but is treated here as a scalar for simplicity.

Table 1. Forces employed and particle properties classified in representative instruments for classifying aerosol particles.

Type of force	Electrostatic	Diffusion	Inertial	Centrifugal	Gravitational	Drag	Property to be classified
Quantity involved / Instrument	Charge (q)	[note 1]	Mass (m)	Mass (m)	Mass (m)	Mobility (B)	
Differential mobility analyzer	✓					✓	Mobility equivalent diameter
Diffusion battery		✓				✓	Mobility equivalent diameter
Impactor			✓			✓	Aerodynamic diameter
Centrifugal classifier [note 2]				✓		✓	Aerodynamic diameter
Elutriator					✓	✓	Aerodynamic diameter

[note 1] The diffusion force is expressed as $-kT(\nabla n)/n$, where n is the particle number concentration, k the Boltzmann constant, and T the thermodynamic temperature, and does not depend on any properties of individual particles.
[note 2] Examples of centrifugal classifiers include a Stöber centrifuge, a Goets spectrometer, and a cylindrical aerosol spectrometer.

For example, in the case of potential health hazards of nanoparticles that has attracted recent concern with respect to the emerging nanotechnology, the endpoint effect would be a long-term deterioration of health of people who have inhaled the nanoparticles. However, it is impossible to conduct an experiment to quantify such an effect with humans as experimental objects. Moreover, for the purpose of preventing the hazardous effect, evaluation of the endpoint effect after a person's health is damaged is useless. What we should do instead is to first characterize the nanoparticles with respect to their various physical and chemical properties, and then clarify the relationship of each particle property with biological toxicity of the particles through, for example, inhalation studies using experimental animals or desirably through *in vitro* analyses. Control of an endpoint effect of aerosols will be only possible on the basis of our knowledge of such relationships, combined with measurements of relevant properties of the particles concerned.

In general, it is *a priori* unknown which particle property is relevant to a given endpoint effect of an aerosol. For example, before we conduct inhalation exposure tests, we do not know which property of nanoparticles is involved in their suspected biological toxicity; size,† mass, shape, surface area, chemical component, or some other property. Hence, many methods for measuring as many kinds of particle properties as possible should be available when quantification of the endpoint effect of some specific aerosol is required. In particular, methods to measure the mass of aerosol particles are essential.

2.3 Motivation for starting research on particle mass measurement

Before our research and development efforts commenced, there was no practical method to measure aerosol particle mass. Further, the research community had not vigorously promoted the development of such a method. Hence, primarily on our own initiative, we developed a new fundamental measurement method for aerosol science and technology. From the beginning, we anticipated that such a method would enable new particle characterization techniques since mass is a very basic quantity. In our view, this expectation has been realized as described later in Section 4.

3 Aerosol particle mass analyzer (APM)

3.1 Existing particle classification instruments

A typical method for measuring the distribution of a specific particle property is to use an instrument that can extract only particles with property values in a narrow interval centered on an adjustable central value. The distribution of the property can be obtained by quantifying the extracted particles for various values of the property. Such quantification can be done, for example, with a single-particle counting device such as a condensation particle counter (CPC) or a light scattering airborne particle counter (LSAPC); an aerosol electrometer, which detects the electric current carried by charged particles to determine the particle number concentration; or a microbalance to weigh particles collected onto a particulate filter.

Table 1 shows the forces employed and the particle properties classified by representative existing instruments for classification of aerosol particles.[4] When a particle moves at velocity v relative to the surrounding air, it experiences a drag force equal to $-v/B$. We can regard particle classification by the instruments listed in Table 1 as being realized by balancing a

† The term "size" is used in this article to indicate a rough measure of the spatial extent of a generally non-spherical particle.

specific force externally applied to the particle with the drag force. Take the differential mobility analyzer as an example. A particle with charge q placed between electrodes experiences an electrostatic force, and migrates at a certain velocity. The condition that the electrostatic force (proportional to q) equals the drag force (inversely proportional to B) is satisfied at a specific value of electrical mobility qB. Only particles whose electrical mobility is in a narrow interval about this value are separated out (see Subsection 3.2). Similarly, for instruments that employ a force proportional to particle mass m, particle classification is realized by balancing that force with the drag force. For this type of instrument, the particle property to be classified turns out to be the product mB, or equivalently the aerodynamic diameter.

Thus the drag force, being dependent on particle mobility, plays an essential role in the existing instruments for classification of aerosol particles. As a result, the property according to which particles are classified inevitably has a dependence on mobility, and thus classification according to particle mass cannot be realized. In the case of the APM, the drag force does not play an essential role, which enables classification by mass. Further details of the APM is described in the following.

3.2 Principle of the APM

In this subsection, the operating principle of the APM is described while contrasting it to that of the differential mobility analyzer (DMA).[5] The main part of the DMA consists of coaxial cylindrical electrodes as shown in Fig. 2. A sample aerosol is introduced through the slit at the upper part of the outer electrode into clean air flowing parallel to the electrode axis. A charged particle in the aerosol migrates between the electrodes along a trajectory that depends on its electrical mobility, and only those particles having a certain electrical mobility are extracted through the slit at the lower part of the inner electrode. Using a CPC to count the particles exiting the DMA, we can measure the distribution of mobility equivalent diameter of the particles. The combined DMA-CPC system is extensively used in this way for the purpose of particle-size distribution measurement.

The APM similarly consists of coaxial cylindrical electrodes, but is constructed so that the electrodes, both the inner and outer ones, rotate at the same angular velocity (see Fig. 3). A charged particle introduced into the gap of the rotating electrodes migrates under the influence of an inward electrostatic force and an outward centrifugal force. These two forces balance when the particle has a specific mass-to-charge ratio. When the forces on a particle achieve this balance, the particle is transmitted through the electrode gap.† Because mass-to-charge ratio is the property to be classified, this instrument was termed aerosol particle mass analyzer.[6]

The existing classification instruments such as listed in Table 1 employ only one type of external force (note that drag force is not an external force), and make use of particle motion caused by that force. The operating principles of these instruments can be categorized as the so-called deflection method, in which difference in travel distance between particles having different values of the property is utilized for classification of that property. On the other hand, the APM employs two forces, electrostatic and centrifugal, which allows particles having a specific mass-to-ratio not to migrate relative to the surrounding air.†† This feature of the

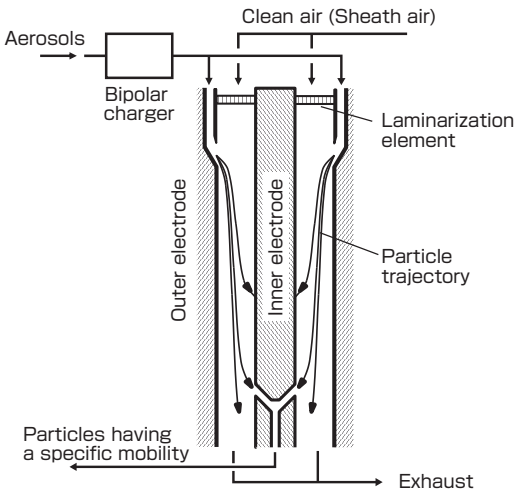

Fig. 2 Principle of the differential mobility analyzer (DMA).

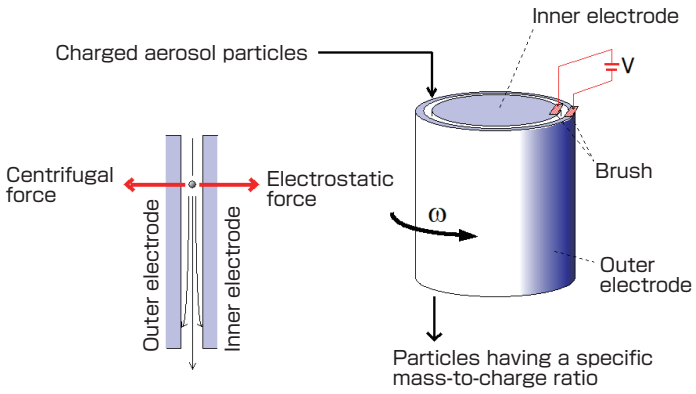

Fig. 3 Principle of the aerosol particle mass analyzer (APM).

† For simplicity, the APM is treated in this article as a classifier of particle mass, though strictly it is a classifier of mass-to-charge ratio. We can often set up a measurement system so that particle charge is specified, and in such cases the particle mass can be readily inferred from the mass-to-charge ratio.

†† To be strict, particles that penetrate the electrode gap may have migrated in the radial direction of the cylindrical electrodes over a distance equals to the gap length at the maximum. Such particle migration in the radial direction determines the resolution in mass classification, but will not be discussed in this article.

APM makes mass classification possible. The principle of the APM is referred to as the so-called zero method, where a force balance reduces particle motion to zero.

The classification performance of the APM is characterized by the APM transfer function† $\Omega(m; V)$.[6] The transfer function is the ratio of an exiting number flux to a penetrating (incident) number flux of particles having a specific value of mass m. It depends on the voltage applied between the electrodes V, and the angular velocity of the electrodes ω. The symbol $\Omega(m; V)$ indicates that it is a function of m with V being a parameter (the ω dependence is not explicitly indicated for simplicity). The transfer function can be theoretically calculated by solving the equation of motion of particles within the electrode gap.

When aerosol particles with a mass distribution $f(m)$, where $f(m)\mathrm{d}m$ represents the number concentration of particles within a range (m, m+dm), are drawn to the APM operated at voltage V, the number concentration of particles exiting the APM is given by††

$$n(V)=\int_0^\infty f(m)\Omega(m;V)\mathrm{d}m. \tag{3}$$

The concentration $n(V)$ normalized by the concentration of the particles entering the APM ($\int_0^\infty f(m)\mathrm{d}m$) defines the particle penetration rate. The concentration $n(V)$, or the particle penetration rate, as a function of V is called an APM spectrum. Based on measurements of $n(V_i)$ at various values of the applied voltage V_i, and a theoretical model for the mass distribution $f(m)$, one can estimate the parameters of the theoretical model by the method of least squares fitting.[7]

3.3 Studies on the APM by other groups

Since we publicized the principle of the APM, studies on the performance of the APM as well as on analysis of APM data have been conducted by several research groups. These studies include experimental evaluation[8] and theoretical analysis[9] of the APM transfer function, investigation of the effects of Brownian diffusion on the APM performance,[10] and handling of the inverse problem for reconstruction of particle mass distributions.[11] We will not go into the details of these studies.

As already described, the APM was designed so that the inner and outer electrodes rotate at the same angular velocity. As a result of this feature, the point of equilibrium between the electrostatic and centrifugal forces for a particle having a given mass-to-charge ratio corresponds to a slightly unstable equilibrium, meaning that the potential energy experienced by the particle is the maximum, not minimum, at the equilibrium point. Olfert and Collings, then at Cambridge University, developed an instrument that they termed a Couette centrifugal particle mass analyzer (CPMA).[12][13] This instrument is similar in design to the APM except that the inner electrode rotates faster than the outer electrode, so that the point of equilibrium between the two forces corresponds to the minimum of the potential energy. Due to this feature, if the APM and CPMA are operated under conditions realizing the same resolution in classification, the CPMA can extract, in theory, more particles than the APM does for a given number flux of incident particles. This property is often advantageous in experiment, because quantification of extracted particles is easier when more particles are available. Unfortunately, however, probably due to difficulty in establishing the fluid-dynamically ideal Couette flow††† between the coaxial cylindrical electrodes, a CPMA that can achieve a theoretically expected performance has not yet been realized.[13] In what follows, the CPMA is not discriminated from the APM, and treated as one type of the APM.

3.4 The APM as an instrument for mass measurement

Figure 4 shows mass measurement ranges covered by representative instruments on a log-scale. The smallest change in mass detectable by high-resolution microbalances is about 0.1 μg. A tapered element oscillating microbalances (TEOM) collects a small amount of particulate matters suspended in the air such as PM2.5 on an oscillating element, and determines its mass from the change in the oscillation frequency. The TEOM covers the mass range of

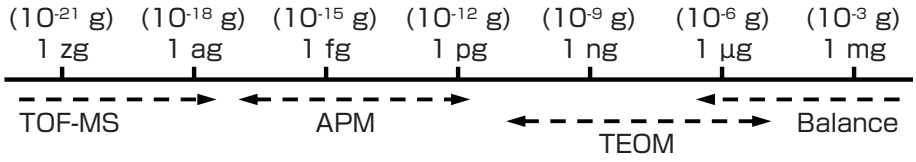

Fig. 4 Mass ranges covered by representative mass measuring instruments (TOF-MS: time of flight mass spectrometer, TEOM: tapered element oscillating microbalance).

† The term "transfer function" is adopted after the similar function that has been used extensively in analyses of DMA data.

†† It is assumed here that all particles that enter the APM carry a single unit of charge. Such a condition can be approximately realized by installing a DMA upstream of the APM.

††† Couette flow is a flow of a viscous fluid between two surfaces, such as two flat plates or two cylindrical pipes, that have a finite relative velocity in their tangential directions. The components of the flow velocity parallel to the surfaces have a gradient perpendicular to the surfaces in the Couette flow.

approximately 10^{-11} g to 10^{-5} g.[14] On the other hand, mass of an atom or a molecule is measured by mass spectrometers, and a time-of-flight mass spectrometer (TOF-MS) among other types of mass spectrometers has a relatively broad measurement range, covering approximately 1.7×10^{-24} g to 1.7×10^{-18} g (1 D_a to 10^6 D_a).

The mass range of approximately 10^{-18} g to 10^{-11} g is not covered by existing instruments. The APM can partly fill this blank region, covering approximately 3×10^{-18} g to 2×10^{-12} g.[7] It should be noted, however, that the mode of measurement is different between the instruments. While the balance can measure the mass of a given object, the TEOM measures the mass of collected particles as a whole, and the APM and TOF-MS measure the mass distribution of a collection of particles.

4 Applications of the APM

Since the APM became available for practical use, several new methods of characterization of aerosol particles have emerged. Because they are considered direct outcomes of the invention of the APM, we describe them in some detail in the following.

4.1 Effective density[15]–[20]

The distribution of effective density of aerosol particles can be measured by connecting a DMA and an APM in series, and counting the number of separated particles by a CPC. The effective density is defined here as the mass of a particle, m, divided by the volume of a spherical particle, $\pi D_B^3/6$, that has the same mobility equivalent diameter D_B as the particle under consideration. If the particle is spherical, the effective density reduces to the particle density. Because the effective density reflects constituent materials and morphology of the particle, it carries information not available only through measurement of the particle size. The method of measuring the effective density using a combined DMA-APM system was first proposed by P. H. McMurry of the University of Minnesota and his colleagues.[15] Using this method in the city of Atlanta, they showed for the first time that airborne particles in urban areas are sometimes composed of two rather distinct components, one with a relatively large effective density and the other with a small effective density. Since the publication of their study, measurements of effective density for atmospheric particles in various places, as well as for various types of particles generated in laboratories have been conducted by a significant number of groups.[16]–[20]

4.2 Material density and particle density[2][21][22]

The density of an aerosol particle can be determined from its mass and volume; the former obtained with the APM, and the latter by some other means, typically by electron microscopy.[2] The density determined in this way is the so-called particle density, if the volume used in this procedure includes that of the voids within the particle, while it is the material density, if not. Using this method, S. H. Kim and his colleagues of the University of Maryland succeeded in determining the density of multi-walled carbon nanotubes; in this case, the particle density including contribution from the hollow of the nanotubes.[21] By similar methods, the densities of diesel exhaust particles, metal particles, and metal oxide particles among other types of particles have been measured by several groups.[2][22]

4.3 Mass and volume[23]–[32]

The mass fraction of volatile materials contained in particles can be determined from the change in the mass upon heating the particles. Sakurai H., then at the University of Minnesota, and his colleagues used this technique to study the size dependence of the mass fraction of volatile materials in diesel exhaust particles, where an APM combined with a tandem DMA system (two DMAs connected in series) was utilized.[23]

When the material density of a particle is known, the volume of the particle can be determined from its mass. A. A. Lall of the University of California, Los Angeles, and his colleagues used an APM to experimentally determine the mass and volume of aggregated particles for the purpose of testing the validity of their "idealized aggregate theory," which is to predict the volume and number of primary particles in an aggregate particle from its mobility and the diameter of primary particles constituting it.[25]

Moteki N. and Kondo Y. of the University of Tokyo used an APM in their performance evaluation study of the single particle soot photometer (SP2), which uses the laser induced incandescence technique to measure the mass and mixing state of individual elementary-carbon particles in the atmosphere, which are considered to have significant impacts on global warming.[24]

4.4 Mass concentration[33][34]

The mass concentration of atmospheric particles with aerodynamic diameter smaller than a certain stipulated threshold (i.e., the total mass of such particles per unit volume of air) is an indicator of particulate pollution of the atmosphere. Well-known indicators include PM2.5, with the threshold equal to 2.5 μm, and suspended particulate matter (SPM), with the threshold equal to 10 μm. The reference method to measure the mass concentration is based on gravimetric measurement of the mass of the particulate matter collected onto an air filter. This method is considered accurate overall, but it also has drawbacks such that evaporation of volatile components and/or adsorption of foreign substances may occur during the collection process, impairing the reliability of measurement, and that a long collection time is often needed to collect enough amount of particles that can be measured with a desired accuracy.

K. Park of the University of Minnesota and his colleagues proposed an alternative way of measuring the mass

concentration of atmospheric particles.[33] In their method, the mean mass $\bar{m}(D_B)$ of the particles having a specific value of mobility equivalent diameter D_B is determined prior to the measurement by means of a combined DMA-APM-CPC system. The measurement is conducted for the number distribution of mobility equivalent diameter $\mathrm{d}N/\mathrm{d}D_B$ using a combined DMA-CPC system. The mass concentration M can be obtained from calculating

$$M=\int \bar{m}(D_B)\frac{\mathrm{d}N}{\mathrm{d}D_B}\mathrm{d}D_B\,. \tag{4}$$

This method has an advantage that no measurement bias associated with the evaporation/adsorption phenomena during the particle collection process occurs, because neither measurement of $\bar{m}(D_B)$ nor that of $\mathrm{d}N/\mathrm{d}D_B$ requires particle collection. Another advantage is that as far as the mean particle mass as a function of the mobility equivalent diameter, $\bar{m}(D_B)$, remains unchanged, the mass concentration can be determined solely from $\mathrm{d}N/\mathrm{d}D_B$, resulting in a relatively short measurement time even when the particle number concentration is significantly low.

4.5 Fractal dimension[35]–[49]

Aggregate particles, such as shown in Figs. 1(b) and 1(c), grow through coagulation of relatively small primary particles. It is known[50] that under certain conditions, the growth of such particles can be described by the following scaling law between the particle mass m and the mobility equivalent diameter D_B,

$$m \propto {D_B}^{d_\mathrm{f}}. \tag{5}$$

The exponent d_f characterizes the growth process and the resultant morphology of aggregate particles, and is regarded as a kind of fractal dimension.† Experimentally, the value of d_f can be determined by fitting the power law equation (5) to a set of data pairs (D_B, m) obtained for a number of aggregates at various stages of growth with a combined DMA-APM system. This method was proposed by K. Park of the University of Minnesota and his colleagues[35]: Using this method, they clarified how the fractal dimension of diesel exhaust particles varies as the engine load of diesel vehicles is increased. S. C. Kim, also of the University of Minnesota, and his colleagues showed that as the sintering temperature was increased from 20 ºC to 600 ºC, the fractal dimension of silver nano-particle aggregates varied from 2.07 to 2.95.[40] It is reasonable that the latter value is close to the fractal dimension of spherical particles of 3.

4.6 Dynamic shape factor[51][52]

The dynamic shape factor of a non-spherical particle, χ, is defined as the ratio of its mobility B to the mobility B_ve of a spherical particle having the same volume as the particle under consideration. As the particle shape deviates more from the sphere, its dynamic shape factor, which is unity for a spherical particle, increases; hence it is used as a quantitative measure representing non-sphericity of the particle. The value of B_ve can be determined if its volume is inferred, for example, by the method mentioned in Subsection 4.3, while the mobility B of the particle can be conveniently measured with a DMA, and thus the value of χ can be derived from them.

J. Beranik of the Pacific Northwest National Laboratory, USA, and his colleagues presented a particle characterization method in which multiple particle properties including the dynamic shape factor are simultaneously measured with a combined system of an APM, a DMA, and a SPLAT (an instrument that analyzes chemical composition of individual aerosol particles).[51] L. Hillemann of the Technical University of Dresden and his colleagues experimentally investigated the change in the dynamic shape factor of particles produced at different sintering temperature.[52]

4.7 Porosity[53][54]

Porosity of a particle is defined as the ratio of the total volume of voids existent within or at the surface of the particle, V_v, to the volume surrounded by the envelope surface of the particle, V_e. If the envelope is approximately spherical, V_e can be derived from the mobility equivalent diameter. In addition, if the material of the particle is known, the particle volume excluding V_v can be derived from a measurement of the particle mass with an APM. The porosity can then be calculated as $V_\mathrm{v}/V_\mathrm{e}$. S. Y. Lee of Hiroshima University and his colleagues used this technique to investigate differences in the porosity of silica particles produced by several different methods.[53]

4.8 Specific surface area of individual particles[3][55]

It is known that if morphology and size of a particle satisfy certain loose conditions, the projected-area equivalent diameter of the particle can be approximated rather well by the mobility equivalent diameter.[56] This implies that the surface area of the particle should well correlate with the mobility equivalent diameter. A. D. Maynard, then at the Wilson Center, USA, and his colleagues proposed a new index of particle property based on this consideration, which is defined as

$$\Gamma=\pi {D_B}^2/\tilde{m}\,, \tag{6}$$

where $\tilde{m}$ is the mode of the mass distribution of particles that have a given value of mobility equivalent diameter

† Because actual particles cannot have an exact self-similarity as defined mathematically, equation (5) holds true only approximately in limited ranges of m and D_B. The exponent d_f should rather be called mass-mobility exponent or fractal-like dimension. In the present article, however, we simply call it fractal dimension, for simplicity.

D_B.[3] Experimentally, the index Γ can be measured with a combined DMA and APM system.

Specific surface of powder represents the ratio of the total surface area to the total mass of a sample of powder as a whole, whereas the index Γ represents the ratio of the surface area to the mass of an individual particle having a given D_B. They demonstrated experimentally that the distribution of Γ and hence the physicochemical properties of single-walled carbon nanotubes (SWCNTs) vary significantly depending on the manufacturing process and production lot of the SWCNTs. They assert, on the basis of this observation, that such variations of particle properties should be taken into consideration when evaluating the potential hazards of nanoparticles.

5 From a concept to commercial products

Figure 5 shows an outline of the historical development leading to commercial products of the APM, divided crudely into three phases: the feasibility study phase, the problem solving phase, and the instrument development phase. The experimental part of the first phase research became possible only when it was funded by the Environment Agency of Japan (currently, the Ministry of the Environment) through a grant-in-aid for pollution protection that lasted from 1994 to 1998. When the prototype instrument constructed in this project was found to work roughly as we expected, we anticipated it would not be so difficult to develop an instrument with a sufficient practical performance. It turned out, however, that a couple of critical problems existed that hindered the instrument from working as we expected. Most of our efforts in the second phase research were devoted to solving these problems. For that, we received a grant from the New Energy and Industrial Technology Organization (NEDO), Japan, through the "Project on Basic Technologies for Nanomaterial Metrology" which lasted from 2001 to 2007. In what follows, development of each phase of the research is described from the standpoint of "synthesiology."

5.1 Feasibility study phase

The principle of the APM was devised by one of the authors of this article, Ehara K., while he was a guest researcher at the Statistical Engineering Division (SED) of the National Institute of Standards and Technology (NIST), USA, from 1991 to 1992. While at NIST, he conducted statistically-designed experiments to evaluate the performance of a DMA. This work was supported by G. W. Mulholland at the Building and Fire Research Laboratory, NIST. A part of the findings obtained in this study was later published in a paper on the analysis of DMA data.[57]

In the course of this study, he became aware of the lack of a technique to measure mass of individual aerosol particles. Aerosol particles are in general non-spherical, and mobility of a non-spherical particle is a tensor depending on its orientation relative to the velocity. Unlike mobility, mass is an unambiguously defined physical property even for non-spherical particles, and is a strictly inherent property of the particle. The mass of aerosol particles should be worth measuring. The mass of atoms and molecules can be measured by the mass spectrometry, but if we try to apply its principle to aerosol particles, the instrument would become intolerably large in size, because the mass of typical aerosol particles is much larger than that of atoms and molecules. Further, a mass spectrometer operates only in a vacuum. If aerosol particles are brought into a vacuum, volatile materials in particles could produce change in their properties. Also, the concentration of particles when dispersed in a vacuum could become so low to detect the particles. Another principle which allowed us to measure mass of particles as they were suspended in the air was considered necessary. The principle described earlier in Subsection 3.2 was thus devised.

There were two good fortunes for Ehara, while he was staying at NIST. One was that the division he stayed was a group of scientists having expertise in mathematical statistics and probability theory. It is expected that when particles as small as 20 nm or below are to be measured, their Brownian motion can have non-negligible effects on measurement. He asked C. Hagwood and K. J. Coakley of the SED, NIST, to investigate its effects. A theoretical APM transfer function in

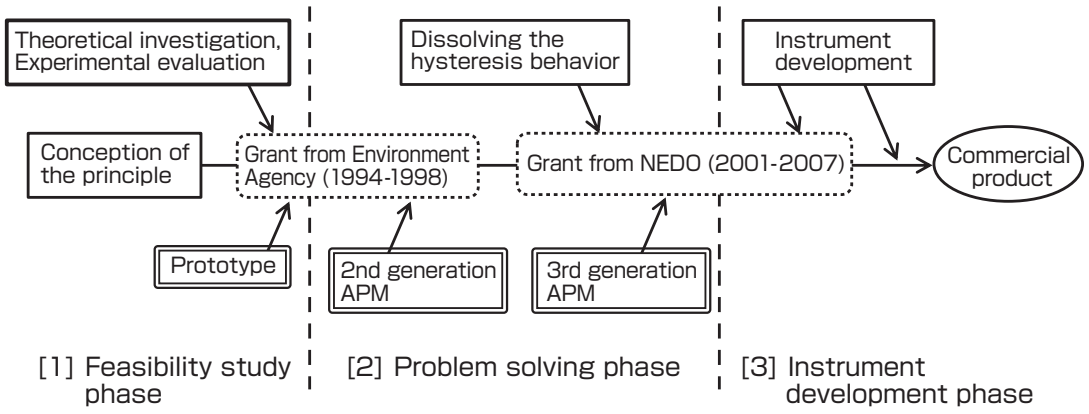

Fig. 5 Processes leading to commercial products of the APM.

which Brownian diffusion of the particles moving between the APM electrodes was taken into account was obtained in 1995, and this significant result led to the first paper regarding the APM.[58]

The other good fortune was that Fukushima N. of Kanomax Japan Inc., who was on a trip to the United States, had a visit to NIST. His visit was just by chance, but Ehara took this opportunity to consult with him about the feasibility of constructing an instrument based on the principle mentioned earlier. Fukushima had expertise in aerosol science as well as in engineering. He could grasp the significance of measurement of particle mass in aerosol science and technology, and also anticipate the difficulties in developing such an instrument.

Joint research to develop an APM started in 1994 between the National Research Laboratory of Metrology (NRLM; currently the National Metrology Institute of Japan, AIST) and Kanomax Japan, and a prototype instrument of APM was built in 1995. The mass distribution of monodisperse polystyrene latex (PSL) particles with a known diameter and density was measured, and a distinct peak in the APM spectrum corresponding to the PSL particles was successfully observed near the mass location expected theoretically.[6] This result convinced us of the feasibility of developing a practical instrument of the APM.

5.2 Problem solving phase

In designing the prototype, we recognized that it had some problems such as leakage of sample aerosols through the bearings. These problems were resolved in the improved second-generation instrument that was designed and built in 1996. The improved instrument had better resolution due to its higher maximum electrode rotational velocity. With this instrument, the peak locations in APM spectra observed for monodisperse PSL particles agreed well with those predicted from theory. However, in the course of extensive tests of its performance, two unexpected phenomena were occasionally observed on the particle penetration rate. One was unexpectedly low reproducibility in particle penetration rates that could hardly be ascribed to statistical errors, and the other was peak heights in spectra significantly lower than those theoretically predicted. Both features were observed not consistently but on a rather unpredictable manner. Ehara and Fukushima tried for about a year to find the causes for such unexpected behavior of the APM, but did not succeed. In 1999, K. Worachotekamjorn of Prince of Songkla University, Thailand, stayed at AIST as a guest researcher for a year. During his stay, he took part in a more detailed experimental investigation on the unusual behavior of the APM. Also in 1999, Coakley of NIST had a short visit at AIST, and was engaged in a theoretical analysis of particle motion in the APM.

Experimentally, transient behavior of the particle penetration rate for monodisperse PSL particles was investigated. It revealed that the time required for the penetration rate to settle at a stationary value when the applied voltage was changed from a certain value to another was much longer than predicted theoretically. Strangely, the direction in which the penetration rate approaches a stationary value, i.e., whether the penetration rate increases or decreases during the transition time, was observed to be dependent on the previous value of the applied voltage. The experimental data indicated as “2nd generation” in Fig. 6(A) show transient behavior of the normalized penetration rate at the peak voltage of an APM spectrum, when the previous value of the voltage was lower than the peak voltage, while those in Fig. 6(B) show the transient behavior when the previous voltage was higher than the peak voltage.[59]

Theoretically, transient motion of particles within the APM electrodes when the applied voltage was changed stepwise was analyzed. Time-dependent stochastic differential equations were solved numerically, and the temporal variation of particle penetration rate was investigated. We were particularly concerned with the effect of slowly-moving particles present in the vicinity of the instrument walls,

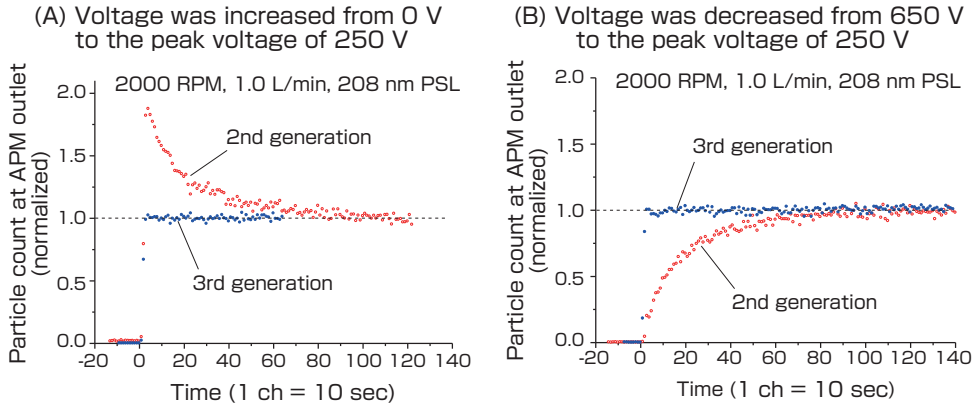

Fig. 6 Hysteresis observed in the temporal response of the second-generation APM, and that of the improved third generation.

as well as the effect of Brownian motion on the temporal variation of the particle penetration rate. Unfortunately, it turned out that neither of these effects explained the unexpected behavior of the penetration rate. The results obtained in this analysis, however, gave us clues narrowing down the causes for the unexpected phenomena, and helped us eventually reach the solution.

The attempt to find the cause continued for about two years in vain. However, in 2002, a plausible model that could explain the observed phenomena emerged. Particles that penetrate through the electrode gap carry charges of the same electrical polarity determined by the electrode configuration: When the outer electrode is set as the anode, the particles are positively charged, which we assume in the following scenario. The particles coming out of the electrodes enter a disk-shaped space leading to the outlet of the APM, which, in the second-generation APM, is surrounded by electrical insulators for isolation of the two electrodes. As shown in Fig. 7, when no voltage is applied, the particles pass through this space virtually without loss. When a certain voltage is applied, the particles experience an electrostatic field normal to the insulator surfaces, and thus some particles deposit on the surface of the insulator covering the cathode. As the number of deposited particles increases, Coulomb repulson by them increases accordingly and starts to push back the incoming particles. It takes a certain transient time for the surface density of the deposited particles and the number flux of the particles penetrating the space to converge to their equilibrium values. Note that these equilibrium values depend on the strength of the applied voltage. This implies that when the applied voltage is varied abruptly from one value to another, the direction of convergence depends on the previous voltage setting. For example, if the voltage is set at V_{peak} (the peak voltage of an APM spectrum) from 0 V, the number of penetrating particles gradually decreases and converges to an equilibrium value, but when it is set at the same V_{peak} but from some higher voltage V_{high}, it gradually increases and reaches the same equilibrium value. In this way, this model could explain both the unexpectedly long transient time and the hysteresis behavior of the particle penetration rate as shown in Fig. 6.

On the basis of this model, the method of electrical insulation of the electrodes was modified so that particles experience no electrostatic field perpendicular to their flow direction outside the electrode gap. A third-generation APM with this modification was designed and constructed in 2003. We confirmed experimentally that the particle penetration rate behaved almost exactly as we theoretically expected, which is shown as the "3rd generation" data in Fig. 6.[60] We also found that the observed values of particle penetration rate agreed quite well with those theoretically predicted. These findings finally cleared the way toward an instrument having the expected performance. From the year 1997 when the unexpected behavior in particle penetration rate was found in the second-generation APM, it took us almost six years to eliminate these problems.

5.3 Instrument development phase†

After the unexpected behavior of the particle penetration

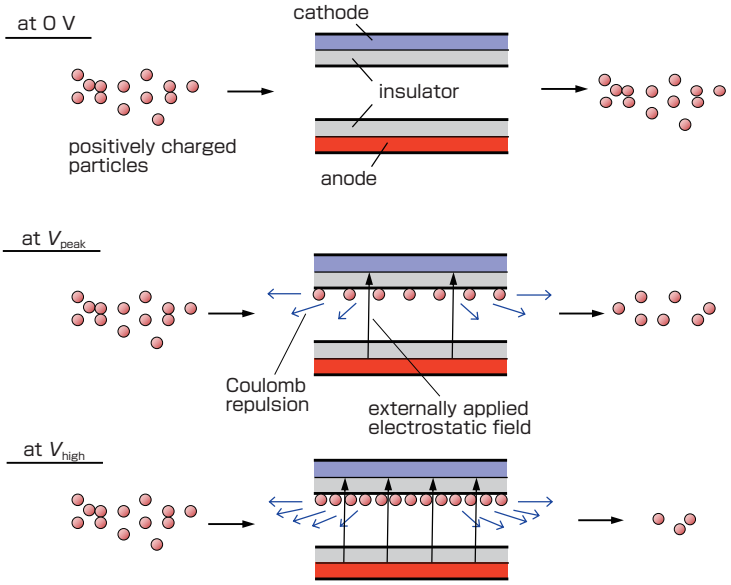

Fig. 7 A model of particle behavior in the disk-shaped space downstream of the electrode exit. Due to Coulomb repulsion from charged particles deposited on the insulator surface, the particle penetration rate through this space is lower than expected and dependent on the strength of the externally applied voltage.

† Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

rate was cleared, we focused our efforts on development of instruments with improved accuracy, performance, and usability. Also, we conducted a study to apply the APM to characterization of diesel exhaust nanoparticles.[16][34] In the course of this study, Sakurai proposed the scanning mode operation of the APM, in which the applied voltage is varied not stepwise but continuously with time. Unlike the DMA for which the scanning mode operation is well-established, applicability of the scanning mode operation to the APM is not obvious, because particles cannot move straight through the electrode gap when the electrostatic field varies with time. Sakurai found that APM spectra obtained in the scanning mode operation under certain operating conditions can give almost the same measurement accuracy as the stepping mode operation.[61] This finding has enabled us to reduce the time to acquire one typical APM spectrum from approximately forty minutes down to five minutes.

The first commercial instrument, APM Model 3600, was developed by Kanomax Japan, and has been available since the year 2008. This instrument is capable of classifying particles in the mass range from 0.01 fg to 100 fg at a sample aerosol flow rate of 1 L/min. While conducting a detailed performance evaluation of this instrument, Tajima N. together with Sakurai devised the "APM operation diagram," which enables one to graphically select an operation condition optimum for a given particle mass.[7] Later, Tajima and her colleagues theoretically analyzed in detail how the design parameters of the APM affect its classification performance,[62] and on the basis of this analysis, a compact commercial instrument, APM Model 3601, was developed in 2012. The dimensions of the main body of this instrument, shown in Fig. 8, was 430 mm (width) by 200 mm (depth) by 140 mm (height). Though its standard flow rate of sample aerosols was set relatively small at 0.3 L/min, its classification performance was almost comparable to the Model 3600 APM. A newer model, APM Model 3602, with an improved control unit has replaced the APM Model 3601 and is commercially available at present.

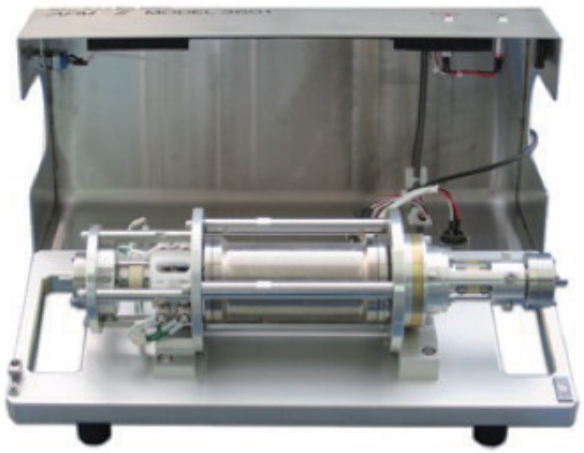

Fig. 8 Main part of the APM Model 3601.

5.4 Barrier to commercialization of an instrument

When some new technique is developed, its commercialization is essential for it to be utilized in society. However, to develop an apparently useful technique is one thing, and to render it a commercial product is another. It is usually difficult to anticipate to what extent a product will be accepted in the community relevant to the technique, especially when needs for the technique are not explicitly recognized in the community. A decision to commercialize the technique always poses some risk. There is a barrier between a seemingly useful technique and a commercial product based on it.

Just after the second-generation APM was built, P. H. McMurry of the University of Minnesota became interested in it, and introduced an instrument of the same make in his laboratory. Since then McMurry and his colleagues have published more than ten papers in which the APM was utilized in some ways. The University of Minnesota has been regarded as one of the centers of excellence in the community of aerosol science. The extensive use of the APM by McMurry and his colleagues has made the APM widely recognized in the community of aerosol science, which undoubtedly was a key factor to clear the barrier to a commercial product.

Ehara had a short visit to McMurry's laboratory in 1994, and several years later McMurry visited Ehara at the NRLM. Both visits had almost nothing to do with the APM, but Ehara had occasions to talk with McMurry about the plan and status of APM development. These unintended but fortunate occurrences played a critical role in the commercialization of the APM.

6 Conclusion

We have developed the aerosol particle mass analyzer (APM) that classifies aerosol particles according to their mass. In this article, we described, from the viewpoint of "synthesiology," the process of its development to its commercialization, as well as the work done by many other research groups to develop new techniques of aerosol particle characterization using the APM.

Expertise in aerosol instrumentation, expertise in applied mathematics to theoretically analyze the instrument performance, and generic knowledge of mechanical, electrical and fluid engineering to properly design an instrument, were all needed to develop a practically usable instrument. This naturally required involvement of many people having respective expertise. It was partly by luck that the participation of these people did occur during the process of developing the APM. Also, it was fortunate that we met a distinguished researcher who recognized the potential usefulness of the instrument while it was still under development.

There are still some issues that need to be addressed

regarding the APM. Installation of the scanning mode operation to the commercial models of the APM is yet to be completed. It might be possible to use the APM as the primary standard of particle mass in the metrological traceability system, because its working principle is the "zero method" which is usually expected to attain good measurement accuracy. Also, it is reported that below 30 nm, the diameter determined by the APM for spherical particles with a known density is consistently smaller than that determined by the DMA,[63] which needs to be investigated in more detail. In addition, a detailed performance evaluation of the CPMA, and realization of its theoretically expected performance are desired.

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References

[1] P. Kulkarni, P. A. Baron and Klaus Willeke (eds.): *Aerosol Measurement: Principles*, Techniques, and Applications, 3rd edition, Wiley, Hoboken, N.J., (2011).

[2] K. Park, D. B. Kittelson, M. R. Zachariah and P. H. McMurry: Measurement of inherent material density of nanoparticle agglomerates, *J. Nanoparticle Res*., 6 (2), 267–272 (2004).

[3] A. D. Maynard, B. K. Ku, M. Emery, M. Stolzenburg and P. H. McMurry: Measuring particle size-dependent physicochemical structure in airborne single walled carbon nanotube agglomerates, *J. Nanoparticle Res*., 9 (1), 85–92 (2007).

[4] K. Ehara: Classification of airborne nanoparticles by size and mass, *Chemical Engineering*, 73 (2), 83–86 (2009) (in Japanese).

[5] E. O. Knutson and K. T. Whitby: Aerosol classification by electric mobility: apparatus, theory, and applications, *J. Aerosol Sci*., 6 (6), 443–451 (1975).

[6] K. Ehara, C. Hagwood and K. J. Coakley: Novel method to classify aerosol particles according to their mass-to-charge ratio—Aerosol particle mass analyser, *J. Aerosol Sci*., 27 (2), 217–234 (1996).

[7] N. Tajima, N. Fukushima, K. Ehara and H. Sakurai: Mass range and optimized operation of the aerosol particle mass analyzer, *Aerosol Sci. Technol*., 45 (2), 196–214 (2011).

[8] C. Presser, X. Ma, S. Guha, G. W. Mulholland and M. R, Zachariah: Online nanoparticle mass measurement by combined aerosol particle mass analyzer and differential mobility analyzer: Comparison of theory and measurements, *Aerosol Sci. Technol*., 43 (11), 1075–1083 (2009).

[9] M. Kuwata: Particle classification by the tandem differential mobility analyzer-particle mass analyzer system, *Aerosol Sci. Technol*., 49 (7), 508–520 (2015).

[10] G.-Y. Lin, B.-X. Liao, N.-J. Tzeng, C.-W. Chen, S.-N. Uang, S-C. Chen, D. Y. Pui and C. J. Tsai: The effect of nanoparticle convection-diffusion loss on the transfer function of an aerosol particle mass analyzer, *Aerosol Sci. Technol*., 48 (6), 583–592 (2014).

[11] K. Park: In-situ measurements of physical properties of 50-500 NM particles: mass-mobility relationship, Ph. D. thesis, University of Minnesota, (2003).

[12] J. S. Olfert and N. Collings: New method for particle mass classification—the Couette centrifugal particle mass analyzer, *J. Aerosol Sci*., 36 (11), 1338–1352 (2005).

[13] J. S. Olfert, K. StJ. Reavell, M. G. Rushton and N. Collings: The experimental transfer function of the Couette centrifugal particle mass analyzer, *J. Aerosol Sci*., 37 (12), 1840–1852 (2006).

[14] E. Weingartner, H. Burtscher, C. Hüglin and K. Ehara: Semi-continuous mass measurement, in Aerosol Measurement: *Principles, Techniques, and Applications, Third Edition*, P. Kulkarni, P. A. Baron, K. Willeke eds., John Wiley & Sons, 255–268 (2011).

[15] P. H. McMurry, X. Wang, K. Park and K. Ehara: The relationship between mass and mobility for atmospheric particles: A new technique for measuring particle density, *Aerosol Sci. Technol*., 36 (2), 227–238 (2002).

[16] K. Saito, O. Shinozaki, A. Yabe, T. Seto, H. Sakurai and K. Ehara: Measuring mass emissions of diesel particulate matter by the DMA-APM method (First Report): Measurement of the effective density of diesel exhaust particles, *Transactions of Society of Automotive Engineers of Japan*, 38 (6), 113–118 (2007) (in Japanese).

[17] Q. G. J. Malloy, S. Nakao, L. Qi, R. Austin, C. Stothers, H. Hagino and D. R. Cocker III: Real-time aerosol density determination utilizing a modified scanning mobility particle sizer—Aerosol particle mass analyzer system, *Aerosol Sci. Technol*., 43 (7), 673–678 (2009).

[18] S. Nakao, M. Shrivastava, A. Nguyen, H. Jung and D. R. Cocker III: Interpretation of secondary organic aerosol formation from diesel exhaust photooxidation in an environmental chamber, *Aerosol Sci. Technol*., 45 (8), 964–972 (2011).

[19] T. L. Barone, A. A. Lall, J. M. E. Storey, G. W. Mulholland, V. Y. Prikhodko, J. H. Frankland, J. E. Parks and M. R. Zachariah: Size-resolved density measurements of particle emissions from an advanced combustion diesel engine: Effect of aggregate morphology, *Energy Fuels*, 25 (5), 1978–1988 (2011).

[20] X. Tang, D. R. Cocker III and A. Asa-Awuku: Are sesquiterpenes a good source of secondary organic cloud condensation nuclei (CCN)? Revisiting β-caryophyllene CCN, *Atmospheric Chem. Phys*., 12 (18), 8377–8388 (2012).

[21] S. H. Kim, G. W. Mulholland and M. R. Zachariah: Density measurement of size selected multiwalled carbon nanotubes by mobility-mass characterization, *Carbon*, 47 (5), 1297–1302 (2009).

[22] M. Kuwata, S. R. Zorn and S. T. Martin: Using elemental ratios to predict the density of organic material composed of carbon, hydrogen, and oxygen, *Environ. Sci. Technol*., 46 (2), 787–794 (2012).

[23] H. Sakurai, K. Park, P. H. McMurry, D. D. Zarling, D

B. Kittelson and P. J. Ziemann: Size-dependent mixing characteristics of volatile and nonvolatile components in diesel exhaust aerosols, *Environ. Sci. Technol.*, 37 (24), 5487–5495 (2003).

[24] [24] N. Moteki and Y. Kondo: Effects of mixing state on black carbon measurements by laser-induced incandescence, *Aerosol Sci. Technol.*, 41 (4), 398–417 (2007).

[25] A. A. Lall, W. Rong, L. Mädler and S. K. Friedlander: Nanoparticle aggregate volume determination by electrical mobility analysis: Test of idealized aggregate theory using aerosol particle mass analyzer measurements, *J. Aerosol Sci.* 39 (5), 403–417 (2008).

[26] M. Kuwata and Y. Kondo: Measurements of particle masses of inorganic salt particles for calibration of cloud condensation nuclei counters, *Atmospheric Chem. Phys.*, 9 (16), 5921–5932 (2009).

[27] M. Kuwata, Y. Kondo and N. Takegawa: Critical condensed mass for activation of black carbon as cloud condensation nuclei in Tokyo, *J. Geophys. Res. Atmospheres*, 114 (D20) (2009).

[28] M. Shiraiwa, Y. Kondo, T. Iwamoto and K. Kita: Amplification of light absorption of black carbon by organic coating, *Aerosol Sci. Technol.*, 44 (1), 46–54 (2010).

[29] X. Ma, A. A. Lall, G. W. Mulholland and M. R. Zachariah: Evaporation anisotropy of free nanocrystals, *J. Phys. Chem. C*, 115 (34), 16941–16946 (2011).

[30] S. Guha, X. Ma, M. J. Tarlov and M. R. Zachariah: Quantifying ligand adsorption to nanoparticles using tandem differential mobility mass analysis, *Anal. Chem.*, 84 (15), 6308–6311 (2012).

[31] M. Laborde, P. Mertes, P. Zieger, J. Dommen, U. Baltensperger and M. Gysel: Sensitivity of the single particle soot photometer to different black carbon types, *Atmospheric Meas. Tech.*, 5 (5), 1031–1043 (2012).

[32] J. G. Radney, X. Ma, K. A. Gillis, M. R. Zachariah, J. T. Hodges and C. D. Zangmeister: Direct measurements of mass-specific optical cross sections of single-component aerosol mixtures, *Anal. Chem.*, 85 (17), 8319–8325 (2013).

[33] K. Park, D. B. Kittelson and P. H. McMurry: A closure study of aerosol mass concentration measurements: Comparison of values obtained with filters and by direct measurements of mass distributions, *Atmos. Environ.*, 37 (9), 1223–1230 (2003).

[34] K. Saito, O. Shinozaki, A. Yabe, T. Seto, H. Sakurai and K. Ehara: Measuring mass emissions of diesel particulate matter by the DMA-APM method (Second Report): Comparison with the gravimetric filter method, Transactions of Society of Automotive Engineers of Japan, 39 (4), 97–102 (2008) (in Japanese).

[35] K. Park, F. Cao, D. B. Kittelson and P. H. McMurry: Relationship between particle mass and mobility for diesel exhaust particles, *Environ. Sci. Technol.*, 37 (3), 577–583 (2003).

[36] B. K. Ku, M. S. Emery, A. D. Maynard, M. R. Stolzenburg and P. H. McMurry: In situ structure characterization of airborne carbon nanofibres by a tandem mobility-mass analysis, *Nanotechnology*, 17, 3613–3621 (2006).

[37] J. S. Olfert, J. P. R. Symonds and N. Collings: The effective density and fractal dimension of particles emitted from a light-duty diesel vehicle with a diesel oxidation catalyst, *J. Aerosol Sci.*, 38, 69–82 (2007).

[38] R. Zhang, A. F. Khalizov, J. Pagels, D. Zhang, H. Xue and P. H. McMurry: Variability in morphology, hygroscopicity, and optical properties of soot aerosols during atmospheric processing, *Proc. Natl. Acad. Sci.*, 105, 10291–10296 (2008).

[39] W. G. Shin, G. W. Mulholland, S. C. Kim, J. Wang, M. S. Emery and D. Y. H. Pui: Friction coefficient and mass of silver agglomerates in the transition regime, *J. Aerosol Sci.*, 40 (7), 573–587 (2009).

[40] S. C. Kim, J. Wang, M. S. Emery, W. G. Shin, G. W. Mulholland and D. Y. H. Pui: Structural property effect of nanoparticle agglomerates on particle penetration through fibrous filter, *Aerosol Sci. Technol.*, 43 (4), 344–355 (2009).

[41] J. Pagels, A. F. Khalizov, P. H. McMurry and R. Y. Zhang: Processing of soot by controlled sulphuric acid and water condensation—Mass and mobility relationship, *Aerosol Sci. Technol.*, 43 (7), 629–640 (2009).

[42] N. Moteki, Y. Kondo, N. Takegawa and S. Nakamura: Directional dependence of thermal emission from nonspherical carbon particles, *J. Aerosol Sci.*, 40 (9), 790–801 (2009).

[43] J. H. Scheckman, P. H. McMurry and S. E. Pratsinis: Rapid characterization of agglomerate aerosols by in situ mass-mobility measurements, *Langmuir*, 25 (14), 8248–8254 (2009).

[44] W. G. Shin, G. W. Mulholland and D. Y. H. Pui: Determination of volume, scaling exponents, and particle alignment of nanoparticle agglomerates using tandem differential mobility analyzers, *J. Aerosol Sci.*, 41 (7), 665–681 (2010).

[45] J. H. Scheckman and P. H. McMurry: Deposition of silica agglomerates in a cast of human lung airways: Enhancement relative to spheres of equal mobility and aerodynamic diameter, *J. Aerosol Sci.*, 42 (8), 508–516 (2011).

[46] T. Torvela, A. Lähde, J. Mönkäre, J. Riikonene and J. Joutsensaari: Low-temperature aerosol flow reactor method for preparation of surface stabilized pharmaceutical nanocarriers, *J. Aerosol Sci.*, 42 (10), 645–656 (2011).

[47] M. L. Eggersdorfer, D. Kadau, H. J. Herrmann and S. E. Pratsinis: Aggregate morphology evolution by sintering: Number and diameter of primary particles, *J. Aerosol Sci.*, 46, 7–19 (2012).

[48] M. Shapiro, P. Vainshtein, D. Dutcher, M. Emery, M. R. Stolzenburg, D. B. Kittelson and P. H. McMurry: Characterization of agglomerates by simultaneous measurement of mobility, vacuum aerodynamic diameter and mass, *J. Aerosol Sci.*, 44, 24–45 (2012).

[49] J. Olfert and S. Rogak: Universal relations between soot effective density and primary particle size for common combustion sources, *Aerosol Sci. Technol.*, 53 (5), 485–492 (2019).

[50] A. Schmidt-Ott, U. Baltensperger, H. W. Gäggeler and D. T. Jost: Scaling behaviour of physical parameters describing agglomerates, *J. Aerosol Sci.*, 21 (6), 711–717 (1990).

[51] J. Beranek, D. Imre and A. Zelenyuk: Real-time shape-based particle separation and detailed in situ particle shape characterization, *Anal. Chem.*, 84 (3), 1459–1465 (2012).

[52] L. Hillemann, F. Babick and M. Stintz: Measurement of the dynamic shape factor using APM and SMPS in parallel, *Procedia Eng.*, 102, 1177–1182 (2015).

[53] S. Y. Lee, H. Chang, T. Ogi, T. Iskandar and K. Okuyama: Measuring the effective density, porosity, and refractive index of carbonaceous particles by tandem aerosol techniques, *Carbon*, 49 (7), 2163–2172 (2011).

[54] Q. Liu, X. Ma and M. R. Zachariah: Combined on-line differential mobility and particle mass analysis for determination of size resolved particle density and microstructure evolution, *Microporous Mesoporous Mater.*, 153, 210–216 (2012).

[55] B. K. Ku and D. E. Evans: Investigation of aerosol surface

area estimation from number and mass concentration measurements: Particle density effect, *Aerosol Sci. Technol*., 46 (4), 473–484 (2012).
[56] S. N. Rogak, R. C. Flagan and H. V. Nguyen: The Mobility and structure of aerosol agglomerates, *Aerosol Sci. Technol*., 18 (1), 25–47 (1993).
[57] K. Ehara, G. W. Mulholland and R. C. Hagwood: Determination of arbitrary moments of aerosol size distributions from measurements with a differential mobility analyzer, *Aerosol Sci. Technol*., 32 (5), 434–452 (2000).
[58] C. Hagwood, K. Coakley, A. Negiz and K. Ehara: Stochastic modeling of a new spectrometer, *Aerosol Sci. Technol*., 23 (4), 611–627 (1995).
[59] K. Worachotekamjorn and K. Ehara: Performance evaluation of the aerosol particle mass analyzer, *Proc 1st Asian Part. Technol. Symp. APT 2000 Bangk*., S-VI-1 (2000).
[60] K. Ehara, K. Fukushima, K. Worachotekamjorn and K. J. Coakley: Analysis and improvement of the temporal response of the aerosol particle mass analyzer, *J. Aerosol Sci*., 35, Supplement 1 (Abstracts of European Aerosol Conference) (2004).
[61] H. Sakurai and K. Ehara: Ryushi shitsuryo bunseki hoho (Particle mass analysis method), Unexamined Patent Application 2008-224246 (2008) (in Japanese).
[62] N. Tajima, H. Sakurai, N. Fukushima and K. Ehara: Design considerations and performance evaluation of a compact aerosol particle mass analyzer, *Aerosol Sci. Technol*., 47 (10), 1152–1162 (2013).
[63] H. Sakurai, K. Ehara, N. Tajima and N. Fukushima: Investigation on the cause for underestimation of density measured by the DMA-APM method, *Poster Presentation at 2010 Annual Conf. Am. Assoc. Aerosol Res., Portland Or.* (2010).

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transient behavior of the particle penetration rate.

TAJIMA Naoko

Tajima Naoko graduated from the Department of Chemical Engineering, Graduate School of Engineering, Kanazawa University in 1984. She joined Nippon Kagaku Kogyo K.K. (currently, Kanomax Japan Inc.) in 1984. While working there, she completed a Ph.D. program and received a Ph.D. degree in Chemical Engineering from the Department of Chemical Engineering, Graduate School of Engineering, Hiroshima University in 2013. She joined the Nanoelectronics Research Institute in the National Institute of Advanced Industrial Science and Technology (AIST) in 2014, and is currently Project Researcher at the CNT-Application Research Center, AIST. She was engaged in development and performance evaluation especially of commercial models of the APM. She conducted theoretical and experimental analyses of the APM performance that led to the compact model of the APM.

SAKURAI Hiromu

Sakurai Hiromu received his Ph.D. degree in Chemistry from the Pennsylvania State University in 1999. He conducted research at the University of Minnesota in 1999–2003, and at the National Institute of Advanced Industrial Science and Technology (AIST) in 2003, both as a post-doctoral researcher. He joined AIST in 2004, and had led the Particle Measurement Section of the National Metrology Institute of Japan (NMIJ), AIST, since 2013. He is currently the Leader of the Particle Measurement Group, NMIJ, AIST. In the research presented in this article, he proposed the scanning mode operation, as well as a graphical tool to select an optimal operating condition of the APM. He also developed methods of applying the APM for characterization of airborne nanoparticles including diesel exhaust particles and carbon nanotubes.

Discussions with Reviewers

1 Overall

Comment (FUJII Kenichi, AIST)

There are rising interests in health, environment, and safety, and the importance of evaluation of fine particles is widely recognized in the industrial field such as in clean room management. Amidst this background, I think this is a high quality paper in the points that it describes the measurement principle of the aerosol particle mass analyzer (APM) that the authors created to measure aerosol particles in micromass range that could not be measured by conventional methods, and it presents case studies in which the developed device was used for evaluation of various particle properties.

It is extremely innovative that this method enables evaluation of aerosol particle mass in the range from 3×10^{-18} g to 2×10^{-12} g that could not be covered by conventional measurement technologies, including minimum 0.1 μg for mass measurement by an electronic balance, 10^{-11} g to 10^{-5} g that is the mass measurement range of a tapered element oscillating microbalance (TEOM), and 1.7×10^{-24} g to 1.7×10^{-18} g that is the mass measurement range by time-of-flight mass spectrometry (TOF-MS).

The definition of the kilogram was revised on May 20, 2019 for the first time in 130 years, and the shift was made from a definition by an artifact to a definition by Planck constant. In the field of mass standard, there is a growing demand for the measurement in the range of 0.1 μg or less that could not be measured before. I think it is a timely paper on pioneering a new measurement range.

Comment (ICHIMURA Shingo, Waseda University)

This paper is a compact summary from the principle to device development of the aerosol particle mass analyzer (APM), overcoming of various issues in practical application and product development, and development of various particle property evaluation technologies (mainly by others) as ripple effects of APM. I think it is a paper appropriate for publication in *Synthesiology* whose objective is "to describe the goal of research and its social value, the scenario and research procedure for achieving the goal, and the process of integrating the elemental technologies."

2 Measurement principle of APM and its uncertainty

Question 1 (FUJII Kenichi)

In Subsection 3.2, you explain the principle of mass measurement of aerosol particles using APM. I understand that the particles are classified by the ratio of mass and charge of the charged particles (mass-to-charge ratio) obtained by applying voltage between the inner and outer cylinders that rotate at the same angle speed. However, there is no description about measurement parameters or derived equations needed to specifically calculate particle mass. I think the readers will be able to understand this measurement principle more deeply if you explain specifically.

Answer 1 (EHARA Kensei)

The classification performance of the APM is characterized by the APM transfer function, and an experimental spectrum obtained by the APM can be theoretically represented by an integral involving the transfer function. I have added a brief description of the transfer function in Subsection 3.2. For more details about the transfer function, please refer to the references cited.

Question 2 (FUJII Kenichi)

You explain that mass measurement by APM is not the measurement of mass of a single particle, but is measurement of mass distribution. Can you also provide some representative example? It can be an example in which you obtained the highest precision. Can you also mention your thoughts on uncertainty in the measurement of mass distribution, relative uncertainty, and causes of uncertainty?

Answer 2 (EHARA Kensei)

The electro-gravitational aerosol balance (EAB) is a method that we have developed for absolute measurement of particle mass which operates in much the same way as the APM [K. Ehara, K. Takahata, and M. Koike, *Aerosol Sci. Technol.* 40, 514–520 (2006) and *Aerosol Sci. Technol.* 40, 521–535 (2006); K. Takahata, H. Sakurai, and K. Ehara, *Aerosol Sci. Technol.* 54, *-* (2020)]. The EAB uses gravitational force instead of centrifugal force, and provides measurement accuracy better than the APM, though it lacks practicality in measurement due to its significantly long measurement time required. Because a detailed uncertainty analysis has been conducted for the EAB, we present it first: The relative expanded uncertainty with a coverage factor of two of the number average diameter of 100 nm PSL particles (approximately 0.57 fg in mass) is 0.66 % (or 1.9 % in terms of the number average mass). We have confirmed that particle mass obtained with the APM is within ±5 % of that obtained with the EAB (see Reference [7]).

Comment 3 (FUJII Kenichi)

In Subsection 3.3, there is an explanation of the Couette centrifugal particle mass analyzer (CPMA) in which the inner electrode is designed to rotate faster than the outer electrode. You use the terminology "Couette flow." I think you should provide an explanation in the footnote to enable the readers to understand the

characteristic of this measurement principle.

Answer 3 (EHARA Kensei)

We have added an explanation of the Couette flow as a footnote in Subsection 3.3.

3 Reliability of mass measurement by TEOM

Question 1 (FUJII Kenichi)

You describe in Subsection 3.4 that mass measurement using a tapered element oscillating microbalance (TEOM) covers the range from 10^{-11} g to 10^{-5} g. I think the reason it is difficult to maintain traceability with TEOM is because it utilizes the oscillation principle, which makes it difficult to calibrate the relation between mass and oscillation. Can you please briefly explain, how TEOM normally maintains its traceability to the mass standard?

Answer 1 (SAKURAI Hiromu and EHARA Kensei)

The TEOM has a disposable filter cartridge for collection of particles attached to the tip of the oscillating element. A sample aerosol is continuously drawn through the filter, and the mass of the particles collected on the filter is obtained from the change in the characteristic frequency of the oscillator. Calibration is done by measuring the characteristic frequencies when a filter cartridge with a precisely known mass is attached to and detached from the oscillating element.

Comment and question 2 (FUJII Kenichi)

In the measurement of particle mass by TEOM, the relationship of mass and natural frequency is obtained from the change of natural frequency when the filter, whose mass has been calibrated using a balance, is installed and removed. This is described in a number of documents. However, in this case, the minimum value (0.1 μg) of mass measurement by a balance will end up extrapolated to a smaller range by the oscillation principle, and I imagine that the degree to which this extrapolation is correct will become a problem. Since the particles are trapped in the filter attached to the oscillator, if there is some kind of mechanical coupling linked by spring in the system of the oscillator, filter, and particle, there may be a possibility that the mass of particles will not be correctly reflected in the natural frequency. Therefore, there is a question of whether correct extrapolation can be done to 10 pg, and in the field of mass standard, there are not infrequent doubts expressed on the reliability of mass measurement by the principle of oscillation.

On the other hand, mass measurement based on the principle of oscillation has extremely high sensitivity, and it is a fact that this principle is being actively applied in some fields, particularly in the field of MEMS.

You show the range of mass measurement by TEOM in Fig. 4, and I think the reliability will increase if one can compare the measurement results of TEOM and APM per 1 pg. Are there past cases that investigated whether the results measured matched those by methods with different principles?

Answer 2 (EHARA Kensei)

There is a paper in which a particle diameter determined from the mass measured with the EAB (see Answer 2 in Discussion 2 for the EAB) for particles with a known density was compared with diameters measured by methods based on other measurement principles [T. A. Germer *et al*., *Proc. SPIE*., 4779:60–71 (2002)]. It reports that the diameter of 100 nm PSL particles we obtained with the EAB (100.8 nm ± 0.67 nm) and that of the same particles obtained by NIST using a DMA with rigorous metrological traceability agreed quite well. Good agreement between the APM and the EAB was already mentioned in Answer 2 of Discussion 2. We believe that these results indicate that the EAB as well as the APM has measurement accuracy of the level that we expect.

It might be possible to examine the reliability of the TEOM by generating monodispersed particles, the mass of which is determined beforehand by the EAB or APM, in the atmosphere at a known concentration, and sending them to the TEOM. We are, however, not familiar with the TEOM, and do not know for sure whether such a method works as expected. Also, we were unfortunately unable to get information on how the reliability of the TEOM is ensured.

4 Treatment of intellectual property in research aiming at commercialization of a product

Question and comment 1 (ICHIMURA Shingo)

When aiming for product realization starting from the principle of a new measurement device, in general, one of the main targets is the creation of intellectual property (such as a patent). In this paper, there is no description from that perspective.

I think it will develop readers' thinking if you add how you handled intellectual property in conducting this research (if you purposefully did not consider acquiring intellectual property, can you explain why). Please consider adding something about this.

Answer 1 (EHARA Kensei)

I did not refer to the intellectual property. I have added a text regarding the patent application in Subsection 5.1.

Question and comment 2 (ICHIMURA Shingo)

I read your answer to Comment 1. As you indicated, you added a text about the creation of intellectual property in the conceptual phase (description about basic patent application in Japan and US). However, the main point of my comment was to clarify what kind of thought process there was as a public research institution, in conducting joint research with a private company to improve the device, and thereby achieving the final practical application (product realization). For example, in the following texts, there are several indications of the potential for creating intellectual property toward product realization:

Page 11

"the first prototype of APM was completed in 1995."

Page 12

"the third-generation device in which revisions were made so the problematic phenomena did not occur by devising the insulation of positive and negative electrodes was created in 2003."

Page 14

"the scanning mode operation in which the electrode voltage was continuously changed as the function of time rather than in steps"

Page 14

"'APM operation diagram,' a tool for selecting the optimal operation condition according to the particle mass"

In this case, I think it is beneficial to the reader who are engaging in *Full Research*, if you further describe what thoughts you had for the obtainment of intellectual property (in some cases, secret know-how) toward product realization, in conducting joint research. Please consider.

Answer 2 (EHARA Kensei)

I agree that treatment of intellectual property is an important subject in "synthesiology," and think that a suitable strategy is needed to handle it. However, it is hard for us to say that our research has been pursued with sufficient recognition of the importance of the intellectual property. We do not have anything to say about it in our article.

For now, I think that patent application by a public institution like us should be as restrictive as possible, for example by limiting it to a principle patent, so that the intellectual property be shared widely in society. The patent application for the APM proceeded effectively in that way. In the case of the DMA (see Subsection

3.2), on the other hand, an instrument with a design very close to the currently prevalent model was developed in the 1950s, but a patent was not filed for it. I think the lack of a patent

made the DMA easier for many researchers to choose it as a research subject, and as a result, various improvements of the instrument had advanced. It might have been a reasonable idea not to file a patent for the APM, but without a patent protection it could be difficult for a private company to proceed to its commercialization. The intellectual property must be handled carefully with due consideration to the relevant market size and various risks associated with its commercialization. Looking back the process of APM development, I still do not know what was the right decision with regard to handling the intellectual property. I would welcome any of your advice on it.

Additional question and comment (ICHIMURA Shingo)

I read your answer to my question. I think by making your statement in the text, you will give this paper synthesiological consideration. For example, I think one of the proposals is to add Subsection 5.5 "Thinking about the intellectual property in R&D," but since this will affect the structure of the paper, I shall leave the decision to the authors.

Answer (EHARA Kensei)

Please allow us to leave the discussion on intellectual property above in this "Discussion with Reviewers" section.

5 Future development

Comment 1 (FUJII Kenichi)

On May 20, 2019, the definition of the kilogram shifted to the Planck constant. The traceability of mass measurement can be achieved by tracing mass m of the object to Planck constant h. Therefore, in the "mise en pratique" for the definition of the kilogram prepared by the Consultative Committee for Mass and Related Quantities (CCM) of the International Committee of Weights and Measures (CIPM), the watt (Kibble) balance method and the X-ray crystal density method are described as representative measurement methods. In principle, the "mise en pratique" states that as long as the ratio h/m is measured with traceability, any method can be used. In the case of APM, what is the route of traceability to h? A conceptual explanation will be fine. What must be made traceable?

Also, I think there is the possibility of measuring micromass using elementary electric charge e or Boltzmann constant k that were newly defined as SI. In the case of aerosol particles, for example, please mention any elemental technology that may be useful in the future, as well as the future direction of the development of measurement principles.

Answer 1 (EHARA Kensei)

First, let me describe the traceability of the EAB (see Answer 2 in Discussion 2), because its measurement principle is significantly simpler than that of the APM. Let us assume, for simplicity, that the sample particles have a uniform mass m and are singly-charged. Then the EAB can be regarded as measurement of the voltage V applied between plate electrodes that realizes balance between electrostatic force eV/H (e is the elementary charge, and H is the electrode gap) and gravity mg (g is the gravitational acceleration) experienced by the particles. From the equation representing the force balance, the particle mass is given by

$$m = \frac{eV}{gH}. \qquad \text{(A)}$$

To be strict, the mass distribution of real particles has a finite width, and accordingly this equation as it stands is not used to determine the number average mass in the EAB method, but is still valid as far as the measurement traceability is concerned. In the current SI, which has undergone the 2019 revision, e is a defined value; V is traceable to e, h (the Planck constant), and $\Delta\nu_{Cs}$ (the transition frequency of the caesium 133 atom) via the Josephson voltage standard; and H and g are both traceable to c (the speed of light) and $\Delta\nu_{Cs}$. Accordingly, the particle mass m is ultimately traceable to the four constants, e, h, c, and $\Delta\nu_{Cs}$.

In the case of the APM, g in equation (A) is replaced with the centrifugal acceleration associated with the electrode rotation, and the strength of the electrostatic field V/H with that relevant to the cylindrical electrodes, but the traceability paths are the same.

I think we should not simply expect that the recent revision of the SI, particularly that of the definition of the kilogram, can naturally bring some benefit to measurement of mass of tiny objects such as particles. One of the reasons for this is that the mass of particles obtained with the APM or EAB already is not directly traceable to the kilogram prototype in the previous version of the SI, but is traceable to the Planck constant h and elementary charge e via the voltage standard, as explained above. The fact that these constants have zero uncertainties in the revised SI may, in principle, lead to a reduction of uncertainty in particle mass measurement, but the uncertainty components associated with these constants were already negligible in the framework of the former SI. This means that the fact the kilogram was defined as the mass of a somewhat unstable macroscopic artifact, i. e., the international kilogram prototype, did not pose any practical obstacles already in the framework of the previous version of the SI.

The recent revision of the SI has brought some obvious benefits to measurement of microscopic entities such as elementary particles. However, all such benefits are in the reduction of measurement uncertainties, and we cannot naively expect that the revision in the definition of the kilogram will bring us a new measurement technique which can cover a mass range that has not been covered by the existing techniques. I hope a new superior technique of mass measurement which relies essentially on the revised definition of the kilogram will emerge, but I think it will take a long time, possibly a very long time, for this to happen.

Comment 2 (FUJII Kenichi)

Thank you for presenting your thoughts on traceability of particle mass measurement by EAB and APM. In the SI definition revision including the kilogram, since the uncertainty of Planck constant h became zero, the uncertainty of the fine structural constant as well as the mass of atom and elementary particles certainly decreased by applying the principle of atomic interference. In the revision of SI, it is considered important to provide a definition that is applicable to future technological innovations, not just to reduce current uncertainties. As a result of defining the length by speed of light, several technological innovations were born in the field of optical frequency measurement. I hope there will be further technological innovations in the field of particles and aerosols, triggered by the development of the APM.

Editorial Policy

Synthesiology Editorial Board

Objective of the journal

The objective of *Synthesiology* is to publish papers that address the integration of scientific knowledge or how to combine individual elemental technologies and scientific findings to enable the utilization in society of research and development efforts. The authors of the papers are researchers and engineers, and the papers are documents that describe, using "scientific words," the process and the product of research which tries to introduce the results of research to society. In conventional academic journals, papers describe scientific findings and technological results as facts (i.e. factual knowledge), but in *Synthesiology*, papers are the description of "the knowledge of what ought to be done" to make use of the findings and results for society. Our aim is to establish methodology for utilizing scientific research result and to seek general principles for this activity by accumulating this knowledge in a journal form. Also, we hope that the readers of *Synthesiology* will obtain ways and directions to transfer their research results to society.

Content of paper

The content of the research paper should be the description of the result and the process of research and development aimed to be delivered to society. The paper should state the goal of research, and what values the goal will create for society (Items 1 and 2, described in the Table). Then, the process (the scenario) of how to select the elemental technologies, necessary to achieve the goal, how to integrate them, should be described. There should also be a description of what new elemental technologies are required to solve a certain social issue, and how these technologies are selected and integrated (Item 3). We expect that the contents will reveal specific knowledge only available to researchers actually involved in the research. That is, rather than describing the combination of elemental technologies as consequences, the description should include the reasons why the elemental technologies are selected, and the reasons why new methods are introduced (Item 4). For example, the reasons may be: because the manufacturing method in the laboratory was insufficient for industrial application; applicability was not broad enough to stimulate sufficient user demand rather than improved accuracy; or because there are limits due to current regulations. The academic details of the individual elemental technology should be provided by citing published papers, and only the important points can be described. There should be description of how these elemental technologies are related to each other, what are the problems that must be resolved in the integration process, and how they are solved (Item 5). Finally, there should be descriptions of how closely the goals are achieved by the products and the results obtained in research and development, and what subjects are left to be accomplished in the future (Item 6).

Subject of research and development

Since the journal aims to seek methodology for utilizing the products of research and development, there are no limitations on the field of research and development. Rather, the aim is to discover general principles regardless of field, by gathering papers on wide-ranging fields of science and technology. Therefore, it is necessary for authors to offer description that can be understood by researchers who are not specialists, but the content should be of sufficient quality that is acceptable to fellow researchers.

Research and development are not limited to those areas for which the products have already been introduced into society, but research and development conducted for the purpose of future delivery to society should also be included.

For innovations that have been introduced to society, commercial success is not a requirement. Notwithstanding there should be descriptions of the process of how the technologies are integrated taking into account the introduction to society, rather than describing merely the practical realization process.

Peer review

There shall be a peer review process for *Synthesiology*, as in other conventional academic journals. However, peer review process of *Synthesiology* is different from other journals. While conventional academic journals emphasize evidential matters such as correctness of proof or the reproducibility of results, this journal emphasizes the rationality of integration of elemental technologies, the clarity of criteria for selecting elemental technologies, and overall efficacy and adequacy (peer review criteria is described in the Table).

In general, the quality of papers published in academic journals is determined by a peer review process. The peer review of this journal evaluates whether the process and rationale necessary for introducing the product of research and development to society are described sufficiently well.

In other words, the role of the peer reviewers is to see whether the facts necessary to be known to understand the process of introducing the research finding to society are written out; peer reviewers will judge the adequacy of the description of what readers want to know as reader representatives.

In ordinary academic journals, peer reviewers are anonymous for reasons of fairness and the process is kept secret. That is because fairness is considered important in maintaining the quality in established academic journals that describe factual knowledge. On the other hand, the format, content, manner of text, and criteria have not been established for papers that describe the knowledge of "what ought to be done." Therefore, the peer review process for this journal will not be kept secret but will be open. Important discussions pertaining to the content of a paper, may arise in the process of exchanges with the peer reviewers and they will also be published. Moreover, the vision or desires of the author that cannot be included in the main text will be presented in the exchanges. The quality of the journal will be guaranteed by making the peer review process transparent and by disclosing the review process that leads to publication.

Disclosure of the peer review process is expected to indicate what points authors should focus upon when they contribute to this journal. The names of peer reviewers will be published since the papers are completed by the joint effort of the authors and reviewers in the establishment of the new paper format for *Synthesiology*.

References

As mentioned before, the description of individual elemental technology should be presented as citation of papers published in other academic journals. Also, for elemental technologies that are comprehensively combined, papers that describe advantages and disadvantages of each elemental technology can be used as references. After many papers are accumulated through this journal, authors are recommended to cite papers published in this journal that present similar procedure about the selection of elemental technologies and the introduction to society. This will contribute in establishing a general principle of methodology.

Types of articles published

Synthesiology should be composed of general overviews such as opening statements, research papers, and editorials. The Editorial Board, in principle, should commission overviews. Research papers are description of content and the process of research and development conducted by the researchers themselves, and will be published after the peer review process is complete. Editorials are expository articles for science and technology that aim to increase utilization by society, and can be any content that will be useful to readers of *Synthesiology*. Overviews and editorials will be examined by the Editorial Board as to whether their content is suitable for the journal. Entries of research papers and editorials are accepted from Japan and overseas. Manuscripts may be written in Japanese or English.

Required items and peer review criteria (January 2008)

	Item	Requirement	Peer Review Criteria
1	Research goal	Describe research goal ("product" or researcher's vision).	Research goal is described clearly.
2	Relationship of research goal and the society	Describe relationship of research goal and the society, or its value for the society.	Relationship of research goal and the society is rationally described.
3	Scenario	Describe the scenario or hypothesis to achieve research goal with "scientific words".	Scenario or hypothesis is rationally described.
4	Selection of elemental technology(ies)	Describe the elemental technology(ies) selected to achieve the research goal. Also describe why the particular elemental technology(ies) was/were selected.	Elemental technology(ies) is/are clearly described. Reason for selecting the elemental technology(ies) is rationally described.
5	Relationship and integration of elemental technologies	Describe how the selected elemental technologies are related to each other, and how the research goal was achieved by composing and integrating the elements, with "scientific words".	Mutual relationship and integration of elemental technologies are rationally described with "scientific words".
6	Evaluation of result and future development	Provide self-evaluation on the degree of achievement of research goal. Indicate future research development based on the presented research.	Degree of achievement of research goal and future research direction are objectively and rationally described.
7	Originality	Do not describe the same content published previously in other research papers.	There is no description of the same content published in other research papers.

Instructions for Authors

"Synthesiology" Editorial Board

Established December 26, 2007
Revised April 1, 2017

1 Types of articles submitted and their explanations

The articles of *Synthesiology* include the following types:
• Research papers, reports, commentaries, roundtable talks, and readers' forums
Of these, the submitted manuscripts of research papers, reports, and commentaries undergo review processes before publication. The roundtable talks are organized, prepared, and published by the Editorial Board. The readers' forums carry writings submitted by the readers, and the articles are published after the Editorial Board reviews and approves. All articles must be written so they can be readily understood by the readers from diverse research fields and technological backgrounds. The explanations of the article types are as follows.

① Research papers
A research paper rationally describes the concept and the design of R&D (this is called the scenario), whose objective is to utilize the research results in society, as well as the processes and the research results, based on the author's experiences and analyses of the R&D that was actually conducted. Although the paper requires the author's originality for its scenario and the selection and integration of elemental technologies, whether the research result has been (or is being) already implemented in society at that time is not a requirement for the submission. The submitted manuscript is reviewed by several reviewers, and the reviewers will recommend whether the manuscript should be accepted, revised, or declined. The author completes the final draft based on the discussions with the reviewers. Views may be exchanged between the reviewers and authors through direct contact (including telephone conversations, e-mails, and others), if the Editorial Board considers such exchange necessary.

② Reports
A report describes a development example of technology which has practical value as well as an example of new technology which has been put to practical use. It contains 1) the aim, 2) the process of development (the course to the goal), and 3) the outcomes. The submitted manuscript is checked by the Editorial Board. The authors will be contacted if corrections or revisions are necessary, and the authors complete the final draft based on the Board members' comments.

③ Commentaries
Commentaries describe the thoughts, statements, or trends and analyses on how to utilize or spread the results of R&D to society. Although the originality of the statements is not required, the commentaries should not be the same or similar to any articles published in the past. The submitted manuscripts will be checked by the Editorial Board. The authors will be contacted if corrections or revisions are necessary, and the authors complete the final draft based on the Board members' comments.

④ Roundtable talks
Roundtable talks are articles of the discussions or interviews that are organized by the Editorial Board. The manuscripts are written from the transcripts of statements and discussions of the roundtable participants. Supplementary comments may be added after the roundtable talks, if necessary.

⑤ Readers' forums
The readers' forums include the readers' comments or thoughts on the articles published in *Synthesiology*, or articles containing information useful to the readers in line with the intent of the journal. The forum articles may be in free format, with 1,200 Japanese characters or less. The Editorial Board will decide whether the articles will be published.

2 Qualification of contributors

There are no limitations regarding author affiliation or discipline as long as the content of the submitted article meets the editorial policy of *Synthesiology*, except authorship should be clearly stated. (It should be clearly stated that all authors have made essential contributions to the paper.)

3 Manuscripts

3.1 General

3.1.1 Articles may be submitted in Japanese or English.
Accepted articles will be published in *Synthesiology* (ISSN 1882-6229) in the language they were submitted. All articles will also be published in *Synthesiology - English edition* (ISSN 1883-0978). The English edition will be distributed throughout the world approximately four months after the original *Synthesiology* issue is published. Articles written in English will be published in English in both the original *Synthesiology* as well as the English edition. Authors who write articles for *Synthesiology* in Japanese will be asked to provide English translations for the English edition of the journal within 2 months after the original edition is published.
3.1.2 Research papers should comply with the structure and format stated below, and reports and commentaries should also comply with the same structure and format except subtitles and abstracts are unnecessary.
3.1.3 Research papers should only be original papers (new literary work).
3.1.4 Research papers should comply with various guidelines of research ethics

3.2 Structure

3.2.1 The manuscript should include a title (including subtitle), abstract, the name(s) of author(s), institution/contact, main text, and keywords (about 5 words).
3.2.2 Title, abstract, name of author(s), keywords, and institution/

contact shall be provided in Japanese and English.
3.2.3 The manuscript shall be prepared using word processors or similar devices, and printed on A4-size portrait (vertical) sheets of paper. The length of the manuscript shall be, about 6 printed pages including figures, tables, and photographs.
3.2.4 Research papers, reports, and commentaries shall have front covers and the category of the articles (research paper, report, or commentary) shall be stated clearly on the cover sheets.
3.2.5 The title should be about 10–20 Japanese characters (5–10 English words), and readily understandable for a diverse readership background. Research papers shall have subtitles of about 15–25 Japanese characters (7–15 English words) to help recognition by specialists.
3.2.6 The abstract should include the thoughts behind the integration of technological elements and the reason for their selection as well as the scenario for utilizing the research results in society.
3.2.7 The abstract should be 300 Japanese characters or less (125 English words). The Japanese abstract may be omitted in the English edition.
3.2.8 The main text should be about 9,000 Japanese characters (3,400 English words).
3.2.9 The article submitted should be accompanied by profiles of all authors, of about 200 Japanese characters (75 English words) for each author. The essential contribution of each author to the paper should also be included. Confirm that all persons who have made essential contributions to the paper are included.
3.2.10 Discussion with reviewers regarding the research paper content shall be done openly, and the Editorial Board will edit the highlights of the review process to about 3,000 Japanese characters (1,200 English words) or a maximum of 2 pages with the names of the reviewers disclosed. The edited discussion will be attached to the main body of the paper as part of the article. Regarding the reports and the commentaries, discussion with the Editorial Board members will be opened at the Board's discretion. In this case, the Editorial Board will edit the discussion to about 800 Japanese characters (less than half a page) with the names of the Board members disclosed.
3.2.11 If there are reprinted figures, graphs or citations from other papers, prior permission for citation must be obtained and should be clearly stated in the paper, and the sources should be listed in the reference list. A copy of the permission should be sent to the Publishing Secretariat. All verbatim quotations should be placed in quotation marks or marked clearly within the paper.

3.3 Format

3.3.1 The headings for chapters should be 1, 2, 3…, for subchapters, 1.1, 1.2, 1.3…, for sections, 1.1.1, 1.1.2, 1.1.3, for subsections, 1.1.1.1, 1.1.1.2, 1.1.1.3.
3.3.2 The chapters, subchapters, and sections should be enumerated. There should be one line space before each paragraph.
3.3.3 Figures, tables, and photographs should be enumerated. They should each have a title and an explanation (about 20–40 Japanese characters or 10–20 English words), and their positions in the text should be clearly indicated.
3.3.4 For figures, image files (resolution 350 dpi or higher) should be submitted. In principle, the final print will be in black and white.
3.3.5 For photographs, image files (resolution 350 dpi or higher) should be submitted. In principle, the final print will be in black and white.
3.3.6 References should be listed in order of citation in the main text.

Journal—[No.] Author(s): Title of article, Title of journal (italic), Volume(Issue), Starting page–Ending page (Year of publication).
Book—[No.] Author(s): Title of book (italic), Starting page–Ending page, Publisher, Place of Publication (Year of publication).
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Letter from the editor

Of the four papers published in this issue, two papers are on bioengineering. I was a reviewer for one of the papers, "Efforts toward commercialization of antifreeze protein." It was a submission from a private company, and it primarily discussed the difficulty of commercialization. In fact, this paper can be seen as a follow-up report to "Mass preparation and technological development of antifreeze protein—Toward a practical use of biomolecules" by Nishimiya *et al*. published in the first issue of *Synthesiology* in 2008. At that time, the paper discussed numerous fields to which the technology would be applied, and it seemed that the product would be put to practical use right away. In reality, there were many hurdles that had to be cleared, and this turned out to be a topic that highlighted the difficulty of social implementation. Ultimately, the scenario was greatly altered to reach product realization, and there may be another change in the scenario before the technology becomes major business. When that happens, I hope there will be another paper. I shall look forward to that. Incidentally, Mr. Ishii, one of the authors, responded rapidly. Perhaps this emerges from his high corporate cost consciousness or commonsense as a businessperson, but I felt I must emulate his actions.

The other bioengineering paper was "Development of a bovine sperm selection procedure for improvement of livestock fertility," and there were many universities, research institutes, and companies that participated in the analyses and demonstration experiments. The scenario described shows that the division of roles was set from the very beginning and the final form of the outcome was laid out carefully. I thought it was excellent that the format of social implementation was imagined from the beginning, and the target product was something the farmers could accept. The enthusiasm for Tokyo Olympics is rising. Behind a sports athlete, there are dozens of supporting staff, and I hear they provide backup in their respective fields of specialty. I feel it is the same when a product is launched to society, as product realization does not happen by efforts of one single researcher even if the product is excellent.

We also received a submission of "A new process to develop a hydraulic system adapted to biodegradable hydraulic oil for construction machinery" in which the main topic is a solution for biodegradable hydraulic oil in consideration of the environment, and "Measurement of mass of aerosol particles" which is related to a timely topic of the standard of mass. I will not go into details due to limitation of space, but I am pleased that we are able to publish high quality papers that encompass both the basic and application research in their respective fields.

(Gotoh Masanori, Editor)

Aim of *Synthesiology*
— Utilizing the fruits of research for social prosperity —

There is a wide gap between scientific achievement and its utilization by society. The history of modern science is replete with results that have taken life-times to reach fruition. This disparity has been called the *valley of death*, or the *nightmare stage*. Bridging this difference requires scientists and engineers who understand the potential value to society of their achievements. Despite many previous attempts, a systematic dissemination of the links between scientific achievement and social wealth has not yet been realized.

The unique aim of the journal *Synthesiology* is its focus on the utilization of knowledge for the creation of social wealth, as distinct from the accumulated facts on which that wealth is engendered. Each published paper identifies and integrates component technologies that create value to society. The methods employed and the steps taken toward implementation are also presented.

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Starting from this issue, the order of the romanization of Japanese names is “first name, last name”.

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Highlights of the Papers in *Synthesiology*

Research papers

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