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Development of a new measurement system to detect selectively volatile organic compounds derived from the human body*

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Abstract— **A new concept expired gas measurement system used double cold-trap method was developed. The system could detect selectively volatile organic compound (VOC) derived from the human body. The gas chromatography (GC) profiles of healthy volunteer's expired gas collected by our system were analyzed. As a result, 60 VOCs were detected from the healthy volunteer's expired gas. We examined 14 VOCs among them further, which could be converted to the concentration from the GC profiles. The concentration of almost VOCs decreased when the subjects inspired purified air compared with the atmosphere. On the other hand, isoprene was almost the same. It was strongly suggested that these VOCs were derived from the human body because the concentration of these VOCs in the atmosphere were nearly zero. Expired gas of two sleep apnea syndrome (SAS) patients were analyzed as preliminary study. As a result of the study, the concentration of some VOCs contained in the expired gas of the SAS patients showed higher value than a healthy controls.**

I. Introduction

Since Pauling et al. reported that many substances were contained in human expiration gas and urine in 1971 [1], the diagnosis of volatile organic compounds (VOCs) in human expired gas where the sample can be taken with low invasion compared with blood is highly hoped as biomarker for some respiratory diseases. More than 200 kinds of VOCs are contained in human expired gas [2]. Some papers reported the VOCs in human expired gas are useful for the diagnostics of lung cancer [3-5], pulmonary tuberculosis [6] and breast cancer [7]. However, to detect VOCs derived from the human is very difficult because the concentration of VOCs in human expired gas is almost the same as that in the atmosphere [8-13]. Therefore, in order to measure correctly the concentration of VOCs in human expired gas, it is necessary to concentrate expired gas. Some papers have reported the technique of concentrating expired gas by using cold-trap method [14-26]. We developed a new concept measurement system that overcame these problems by double cold-trap method [27]. In this paper, the result of analyzing VOCs in human expired gas by using our system is reported.

Furthermore, preliminary clinical test for verifying whether SAS patient can be determined from expired gas was carried out. The confirmed diagnosis of SAS requires a polysomnography (PSG) inspection. Special clinical technologist and large-sized equipments are also needed for the inspection. Therefore, the inspection will make a burden heavy for a patient and a medical institution. The expectation for the simple biomarker which can reduce the inspection is high. Cysteine[28], CRP[29] and KL-6[30] have been reported as a biomarker candidate in blood. However, the expectation for the biomarker contained in expired gas measurable by low invasion is also high. We thought that VOC peculiar to SAS was contained in expired gas of SAS patient. VOC contained in expired gas of two SAS patients were analyzed by our measurement system. The analysis result of the difference in VOC contained in the expired gas of SAS patients and healthy controls is also reported in this paper.

II. Methods

New system was constructed for this research. Double cold-trap method was used to remove the influence of the VOCs in the atmosphere and extract only the VOCs derived from the human body. The outline of our system is shown in Figure.1. The external view of the equipment is shown in Figure.2. The system procedure is divided roughly into four steps (air purification, collection of expired gas, concentrating of expired gas and analysis by gas chromatography). The system captures VOCs in expired gas by using quite the same method as removing VOCs in the atmosphere to make purified air. We try the explanation of this feature by using Figure 1. It is assumed that three VOCs, A, B and C, are in the atmosphere (1). It is assumed that only two impurities, A and B, are captured with the air purification tube (2), and one impurity, C, is not captured (3). The subject inspires the purified air that contains one VOC, C. It is assumed that three VOCs, B, D and E, are included in subject's expired gas (4). So there are four VOCs, B, C, D and E in the air from the sampling unit (5). VOCs that cannot be removed with the purification tube, such as C, are not captured in the expired gas concentrating tube with the same mechanism as the purification device, and VOC E contained in subject's expired gas and not captured with the expired gas concentrating tube, is also exhausted (6). There is a fault that VOCs like E cannot be detected with our system.

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Only VOCs captured by the concentrating tube are detected by GC (7 and 8). Even if the same VOCs, such as B, is in the atmosphere and subject's expired gas, only VOCs derived from the human body are detected in our system.

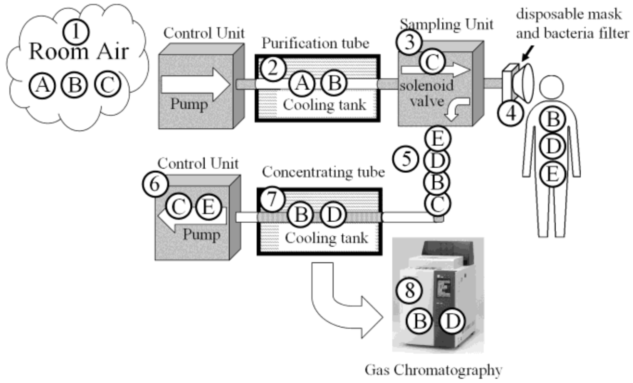

Figure 1. Outline of the system. The feature of the system is “double cold-trap”, which makes possible to detect only VOCs derived from the human body.

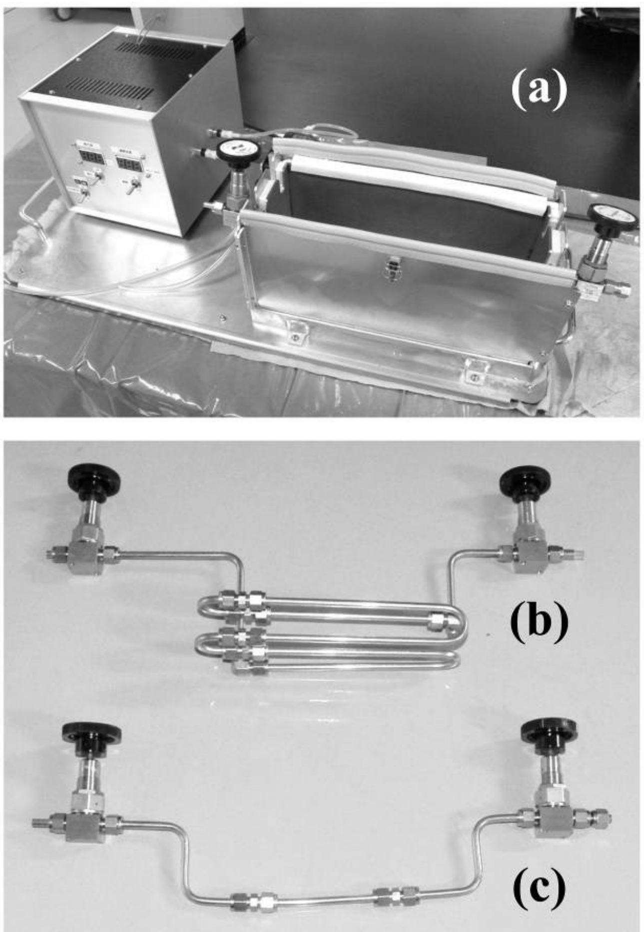

Figure 2. External view of the system. (a) Control unit, which contains pumps to inhale and exhale the gas (left) and cooling tank, which is container of purification tube, concentrating tube and dry ice (right). (b) Purification tube. Stainless wool is filed in the tube. The stainless wool captures the VOCs in the air. (c) Concentrating tube. The structure is the same as the purification tube. It concentrates VOCS in the expired gas.

III. Patients

The expired gas of 19 healthy controls who had obtained written agreement was collected by using our system. Expired gas when inspires the purified air (hereinafter referred to as “purified breath”) or the atmosphere (hereinafter referred to as “atmosphere breath”) was collected, respectively. This experiment was executed according to the protocol having been approved by Tokai University Ethics Committee. All healthy controls had neither the history nor the symptom of the respiratory diseases. Many of these healthy controls were medics. The healthy controls were divided into two groups, one group that inspires the purification air, and the other group that inspires the atmosphere, at random.

Furthermore, VOC contained in expired gas of SAS patients were measured by using our system. This experiment was also executed according to the protocol having been approved by Tokai University Ethics Committee.

The expiration gas of two patients who receives a medical examination in our hospital on suspicion of SAS were collected by our system. The SAS patient with a different stage was selected. The stage of SAS was judged by Apnea Hypopnea Index (AHI). AHI means the rate whose patient was in the state of the apnea or hypopnea, and the patient to whom AHI exceeds 30 is judged to be severe SAS. One mild SAS (AHI = 29.5) patient and one severe SAS (AHI = 83.7) patient were conducted to our study. The clinical information of the mild SAS patient is as follows. Male, Age: 37, Height: 173 cm and Weight: 91.9 kg. The clinical information of the severe SAS patient is as follows. Male, Age: 71, Height: 161 cm and Weight: 62 kg.

IV. Results

An example of the GC profile of the expired gas from a healthy volunteer is shown in Figure 3. Approximately 60 VOCs were detected from atmosphere breath and approximately 50 VOCs were detected from purified breath. VOCs contained in healthy volunteer's expired gas which can be converted into concentration are summarized in Table I. Except one VOC, the concentration of VOCs in purified breath decreased than that in atmosphere breath. We also compared the concentration of the VOCs with the past reports [31-33]; almost the same result was obtained.

GC profile of SAS patients and typical healthy control is shown in Figure.4. Compared with the healthy control, many VOCs were detected by SAS patients. Averaged VOC concentration of SAS patients are as follows. Isoprene: 150.86 ppb, hexane: 0.47 ppb, Ethyl benzene: 0.67 ppb and nonane: 4.82 ppb. Compared with VOC concentration contained in expired gas of healthy controls shown in Table I, some VOC concentration of SAS patients showed the high value.

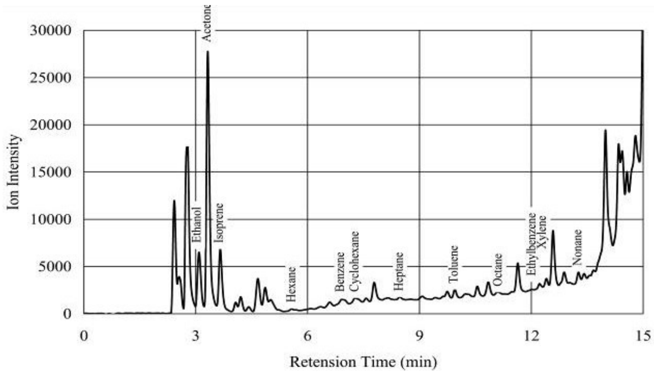

Figure 3. An example of GC profile of healthy volunteer's purified breath. Approximately 50 peaks of VOC were found. The names of 14 VOC peaks which can be converted into the concentration were also shown in this figure.

TABLE I. CONCENTRATION OF 14 VOCS IN ATMOSPHERE BREATH AND PURIFIED BREATH.

	Atomosphere Breath	Purified Breath	Reported Concentration in breath
Isoprene	112.08	111.79	11.1 - 295.5
Acetone	141.17	55.33	80.9 - 2653.7
Ethanol	12.22	8.10	4.5 - 1520.1
Decane	1.69	0.79	
Toluene	0.35	0.16	9.2 - 179.4
Phenylacetic acid	0.83	0.15	----
Nonane	0.06	0.13	----
Heptane	0.19	0.11	----
Benzene	0.12	0.09	0 - 19.0
Hexane	0.11	0.08	2.5 - 76.1
Octane	0.07	0.06	----
Ethylbenzene	0.22	0.05	4.6 - 89.3
Xylene	0.14	N.D.	0 - 95.2
Cyclohexane	0.11	N.D.	----

V. DISCUSSION

The concentration of almost all VOCs contained in purified breath decreased compared with atmosphere breath. This would mean that VOC accumulated in the human body was exhaled promptly by purified air. Some reports [34, 35] say that if the concentration of VOC in the atmosphere, the concentration of the same VOC in expired gas promptly. The same tendency was also shown by our system.

Isoprene was hardly detected in the atmosphere and the purified air, but was detected at a high concentration from expired gas. Therefore, it is thought that isoprene was biosynthesized in humans. It has been reported that the isoprene concentration in expired gas and a blood cholesterol level have correlation [36]. It is expected that isoprene will serve as a leading biomarker candidate substance.

Although there were still very few SAS patients, some VOC concentration rose by the SAS patient compared with the healthy controls. There is a report that the state of SAS and BMI have correlation, and many other parameters which should be taken into consideration exist. It is thought that it becomes possible to identify VOC peculiar to SAS patient clearly by carrying out larger-scale study.

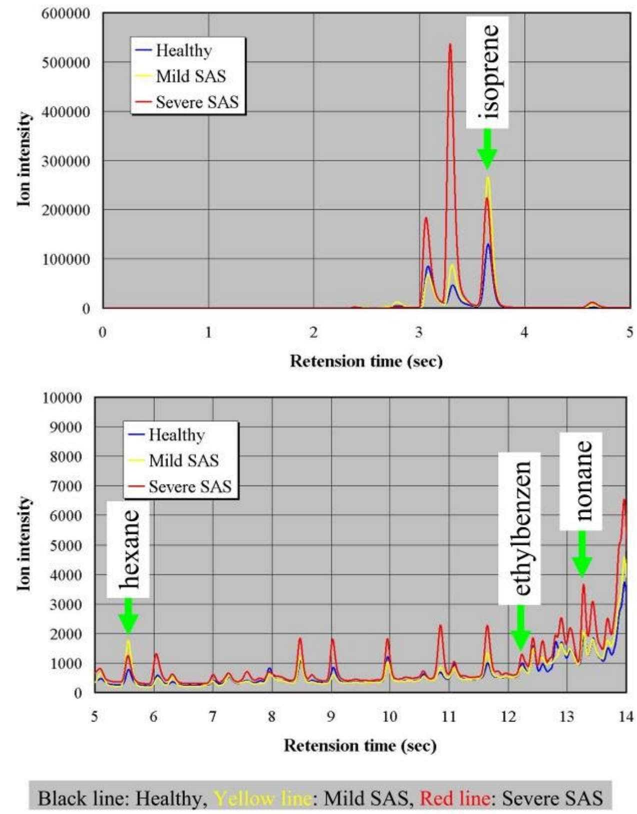

Figure 4. Typical result of GC profile in healthy person, mild SAS patient and severe SAS patient.

VI. CONCLUSION

New concept expired gas measurement system that used double cold-trap method was described in this paper. It became possible to detect only VOCs derived from the human body by air purification and concentrating of expired gas that used double cold-trap method. As a result of the study, there was a significant difference in the analytical result of the expired gas between inspiration of atmosphere and purified air. However, even if our system is used, it is difficult to remove the influence of VOC that exists in the atmosphere completely. Most of the healthy control in this study is a medic who works for the same hospital. The possibility that living in almost the same atmosphere influences the analytical result is not possible to deny. Larger scale study might be needed for the healthy control.

Because the disease of the respiratory system is increasing, the achievement of the early diagnostics and the early treatment is a pressing issue. Our system is scheduled to be able to be used more easily by developing a special detector that can reduce the cost of the entire system. As shown in this paper, it is clear that a lot of useful information for the diagnosis is contained in expired gas. Further progress of this research is requested.

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