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Chemiluminescent imaging of transpired ethanol from the palm for evaluation of alcohol metabolism

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ABSTRACT

A 2-dimensional imaging system was constructed and applied in measurements of gaseous ethanol emissions from the human palm. This imaging system measures gaseous ethanol concentrations as intensities of chemiluminescence by luminol reaction induced by alcohol oxidase and luminol–hydrogen peroxide–horseradish peroxidase system. Conversions of ethanol distributions and concentrations to 2-dimensional chemiluminescence were conducted on an enzyme-immobilized mesh substrate in a dark box, which contained a luminol solution. In order to visualize ethanol emissions from human palm skin, we developed highly sensitive and selective imaging system for transpired gaseous ethanol at sub ppm-levels. Thus, a mixture of a high-purity luminol solution of luminol sodium salt HG solution instead of standard luminol solution and an enhancer of eosin Y solution was adapted to refine the chemiluminescent intensity of the imaging system, and improved the detection limit to 3 ppm gaseous ethanol. The highly sensitive imaging allows us to successfully visualize the emissions dynamics of transdermal gaseous ethanol. The intensity of each site on the palm shows the reflection of ethanol concentrations distributions corresponding to the amount of alcohol metabolized upon consumption. This imaging system is significant and useful for the assessment of ethanol measurement of the palmar skin.

1. Introduction

Various volatile organic compounds (VOCs) exist, such as those the transpired by humans, breath, body odor, smell of the living environment and aroma of food (Lindinger et al., 1998; Hierlemann and Gutierrez-Osuna, 2008; Shirasu and Touhara, 2011; Zhang and Li, 2010). Certain compounds, which are an indicator of disease may also be metabolized by a body, a skin, and breath (Miekisch et al., 2004). The human body emits various non-volatile and volatile molecules, depending on a person's genetics, stress and immune status (Pandey and Kim, 2011). Human odor caused by the combined action of the skin gland and volatile organic compounds, which are regulated by human hormonal control and the bacterial population localized at skin surface (Natale et al., 2000). Metabolism of the body can be screened by analyzing a volatile human ingredient. If the volatile organic compounds of human body can be analyzed in relation to human health, a physician and a patient easily understand and evaluate the result of a diagnosis by screening human odor.

Non-invasive diagnostic methods based on analysis of gas emitted by the body have been used. These include tests for urea expiration and tests for diagnosis of halitosis (Francesco et al., 2005; Chan et al., 2009; Natale et al., 2014). There were any problems to diagnose and monitor of emission of gas from human body because of restrictive diagnosis method. As a result, we cannot measure consecutively of expiration collection. In addition, in the bad breath diagnosis, as for the method with gas chromatography and a gas sensor, there were some problems with handiness and selectivity (Natale et al., 2014). For example, a medical doctor and a third party may directly smell and diagnose the bad breath of the patient. However, this diagnosis is subjective and inaccurate, because the sensing of bad breath depends on the olfactory senses of an individual. Since modern breath analysis started in the 1970s, gas chromatography has been used to identify more than 200 components in human exhaled breath (Lindinger et al., 1998). However, a large volume of exhaled breath was required for diagnosis and the liquid nitrogen or liquid argon was used for cryogenic pre-concentration, which was necessary for sample analysis (Philipp et al., 2004). Isotopes of ^{13}C used in breath tests in the diagnosis of *Helicobacter pylori* infections, have been successfully used as diagnostic method (Romagnuolo et al., 2002). Furthermore, a gas emission from skin surface is lower

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concentration than that of breath gas expiration. Thus, highly sensitive techniques for continuously and selectively measuring volatile chemicals generated by the body are essential for non-invasive diagnosis.

It has been reported that the volatile chemical ingredient associated with disease and the physical physiological state including in skin gas like expiration. For example, acetone, hydrogen, alcohol was included in volatile chemical components, which was well known to researchers (Naitoh et al., 2000; Natale et al., 2000; Rock et al., 2008; Costello et al., 2014). However, sensors and measurement systems for volatiles are expensive and cumbersome to use in a medical facility. Furthermore, gas chromatographs and mass spectrometers, which are used to analyze these compounds, have demanding requirements for installation, and monitoring temporal changes in emissions of gaseous components is difficult. A bracelet-type sensor (SCRAM^{TM}) was used to monitor ethanol concentrations in transdermal gas to measure the quantity of alcohol consumed. This sensor, which could be used in clinical and practical applications (Sakai et al., 2006; Barnett et al., 2011) was used to monitor transdermal alcohol emissions every 30 minutes for 2 weeks. Criteria for detecting alcohol from human skin for this sensor provided an accurate and unconventional approach for use with non-mandated clients.

A number of biosensors have been developed for the measurement of methanol, ethanol and lower alcohols. The measurement of ethanol, in particular, is important essential for forensic science, clinical chemistry and analytical chemistry. In addition, food products, beverages and the wine industry are interested in analytical methods to control the quality and processing of the food products (Wen et al., 2007). A standard alcohol biosensor is composed of an alcohol oxidase immobilized membrane and a commercial oxygen sensor. The quantity of ethanol is determination by the change in the level of dissolved oxygen in the solution. Many sensor technologies employing enzymatic reactions have been developed, such as biochemical gas sensors (bio-sniffers) for gaseous formaldehyde and ethanol, as well as a NADH-dependent fiber-optic biosensor for the determination of gaseous components determination (Kudo et al., 2010, 2012). Also the enzyme-based chemical gas sensors are highly selective and sensitive for target chemicals.

We recently obtained human body information by various kinds of methods for the treatment of a variety of diseases. Mainly, blood and urine have been used for the screening of diseases because of high reliability sample of human body. Recently, sweat, exhaled breath and saliva also used for sample for it. In this paper, we focused on the imaging of transdermal emissions of gaseous ethanol from human palms at hands with alcohol metabolism in human body after oral alcohol administration. This method enables non-invasive, pain-less and blood-less monitoring of information of human body. We can obtain various information of human body by analyzing the ingredient included in transpired emissions. In our previous study, we developed an imaging system of gaseous ethanol based on chemiluminescence measurement employing an EM-CCD camera for breath gaseous ethanol imaging (Wang et al., 2010; Arakawa et al., 2013a, 2013b). However, measurements of transdermal human gas through the system have not been sufficiently evaluated. Therefore, in this study, we developed a novel non-invasive temporal imaging system of transdermal gaseous ethanol to analyze alcohol metabolism in real-time. We measured the changes in chemiluminescence intensity due to emission of transdermal gaseous ethanol after alcohol administration.

2. Experimental

2.1. Chemicals and apparatus

A system for imaging the movement of ethanol was developed as shown in Fig. 1. This imaging system consisted of an electro-multiplying CCD camera (L3C95-05, pixel size: $15 \times 35.5\ \mu m^2$, image format: 768×244 pixels, spectral range: 400–1060 nm, e2v technologies limited, United Kingdom) and a video encoder (GV-MDVD3, I-O DATA, Japan). Data analysis of the recorded chemiluminescence was analyzed by Cosmos 32 software (Library Inc., Japan). All solutions were prepared in deionized distilled water obtained from a Milli-Q purification system (Millipore Co., USA). Cotton mesh substrates used for imaging (cotton: 100%, thickness: 1 mm, and interval size: 1 mm, Pip-Fujimoto Co., Japan) was evaluated for enzyme stabilization. Alcohol oxidase (AOD, E. C.1.1.3.13, A2404-1kU, 10–40 units mg^{-1} protein, from *Pichia pastoris*, Sigma-Aldrich Co., USA) and horseradish peroxidase (HRP, E. C.1.11.1.7, 169-10791, 100 units mg^{-1}, Wako Pure Chemical Industries, Ltd., Japan), photo-crosslinkable poly(vinyl alcohol) containing stilbazolium groups (PVA-SbQ, type: SPH, 9C-10L, 10.4 wt%, Toyo Gosei Co., Ltd., Japan) were used for enzyme stabilization on the mesh substrate. A 5.0 mmol/l luminol (01253-60, Kanto Chemical Co., Inc., Japan) solution was prepared in Tris–HCl buffer solution (100 mmol/l) for measurement of chemiluminescence generated by ethanol (Wang et al., 2011). In addition, to improve the intensity of gaseous ethanol, we selected luminol sodium salt HG (5-amino-2,3-dihydro-1.4-phathalazinedione sodium salt, Lot. CDR7140, Wako Pure Chemical Industries, Ltd.) and eosin Y (Sodium Tetrabromofluorescein, Lot. CDM1099, Wako Pure

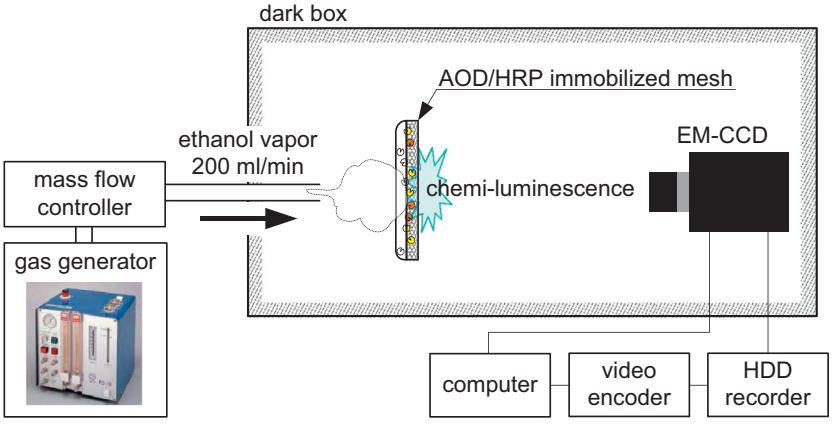

Fig. 1. Schematic of the gaseous ethanol imaging system for standard gaseous ethanol. This system consisted of AOD and HRP immobilized on mesh substrate, an electro-multiplying CCD camera and a standard gas generator machine.