GENERATION OF REACTIVE OXYGEN SPECIES BY CRYSTALLINE C_{60} PARTICLES SYNTHESIZED USING THE INK-JET METHOD

Fusako Sasaki[1] *and Masahito Ban*[2]

[1] Systems Engineering Major, Graduate School, Nippon Institute of Technology, Saitama 345-8501, Japan
[2] Dept. of Innovative Systems Engineering, Nippon Institute of Technology, Saitama 345-8501, Japan

Introduction

The microfluidic chip whose system is often called μ-TAS (micro total analysis systems) is a technology having the ability to perform the various chemical operations by inserting a small amount of solution into the chip, and has recently become a candidate for the biological applications [1]. One of the key technologies necessary to carry out the operation is forming a functional surface in a localized region, and an ink-jet printing technology is capable of locally modifying a surface by means of discharging extremely small amount of solution on a target area. Very recently "all-inkjet-printed" microfluidic multianalyte chemical sensing paper was demenstrated for the simultaneous determination of urine analysis [2]. On the other hand, one of the biologically most relevant features of fullerenes is mediating generation and quenching of reactive oxygen species (ROS) under visible light irradiation [3]. Sayes and co-workers reported that pure C_{60} brought into water by means of solvent extraction formed water-stable crystalline aggregates to generate high amount of ROS and kill both normal and tumor cells [4]. We recently showed that the ink-jet method using a fullerene solution was able to synthesize crystalline C_{60} particles in the size range of a few μm fixed on a substrate, and the particles had the ability to generate ROS under visible light irradiation [5]. In addition, for the purpose of applying the photosensitizing properties of fullerenes as the function of μ-TAS, the crystalline C_{60} particles were formed in poly(dimethylsiloxane) (PDMS)-based microchambers using the ink-jet method [6].

In this study, the ROS generation under visible light irradiation was investigated for the particles synthesized under various ink-jet discharge conditions, and the relationship between the shape and the structure of the particles, and the ROS generation amount was evaluated.

Experimental

A picojet 2000-CW inkjet spotting system (Microjet Co. Ltd., Nagano, Japan) was used in this study. This system is equipped with a three-axis and micropositioning system of 1 μm accuracy, and a piezo-driven nozzle with 30 μm in diameter. A fullerene solution, namely, solution of C_{60} (Frontier carbon corporation: nanom purple ST, 35mg) and poly(methyl methacrylate) (PMMA) (about 10mg) dissolved in toluene of 40ml was prepared. The PDMS substrates were fabricated using a soft lithographic method with SILPOT 184 W/C (Dow Corning Toray Co. Ltd.). The droplets per discharge and the total droplets were varied as the discharge conditions. The droplets per discharge of 10 to 40 were spotted 10 to 40 times on the PDMS substrates. The spotted surfaces of the PDMS substrates were observed by a scanning electron microscope (SEM).

Using a fluorescence spectrometer (Hitachi: F-7000), the production of ROS was determined by measuring the intensity of green fluorescence (530nm) emitted by a fluorescent dye, 2',7'-dichlorofluorescein diacetate (DCF-DA) with 488nm excitation [5]. Here are details of the procedure. Initially, DCF-DA was soluted in pure water to obtain a DCF-DA solution. Second, the DCF-DA solution was degased by flowing argon gas in it at a flow rate of 200 sccm for 1h. The PDMS substrate with the area spotted by the ink-jet was immersed in the DCF-DA solution in a Quartz cell. And a green laser (532nm, 1mW) was irradiated to the spotted area on the substrate (P-PDMS irradiation) up to 3h, and fluorescence intensities were measured by using the fluorescence spectrometer at a given time. As the comparison, the spotted PDMS substrate in dark room (P-PDMS dark) was evaluated up to 3.5 h in the same manner, and pure water was measured as a control (pure water).

Results and Discussion

Fig. 1 shows typical images obtained from the SEM observation results of the films formed on the PDMS substrates by discharging droplets using the ink-jet spotting system. Figs. 1 (a) and (b) show that needle-shaped particles with 0.5-1.0 μm in diameter and 3 to 10 μm length were formed on the substrate. The particles seen in Fig. 1 (b) were formed much numerously and three-dimensionally than those seen in Fig. 1 (a), and, existed in a state held in the film. With regard to Fig. 1 (c), the particles having a shape of relatively grain were synthesized mixed in the needle-shaped particles.

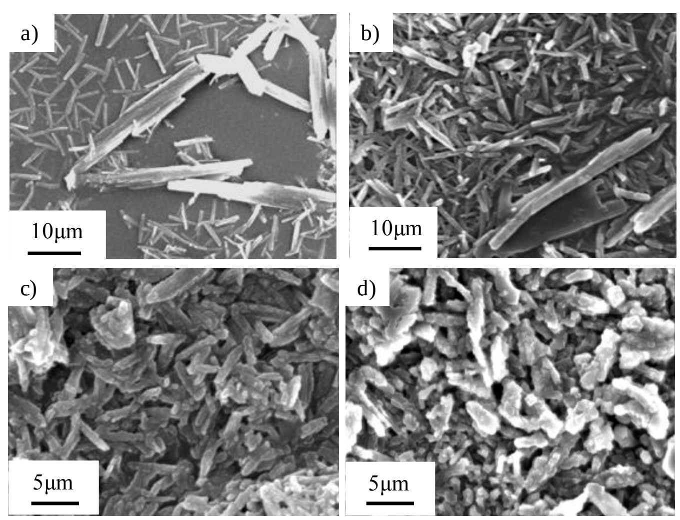

Fig. 1 The SEM observation results of the crystalline C_{60} particles with the droplets per discharge and the total droplets of (a) 10 and 200, (b) 10 and 400, (c) 15 and 300, and (d) 40 and 800, respectively.

Moreover, Fig. 1 (d) shows that the grain-shaped particles were formed sticking to each other. Additionally, the followings were indicated from the SEM observation results of all PDMS substrates spotted by the ink-jet system. With an increase in the droplets per discharge and the total droplets, the particle formation state was transformed from planar into three-dimensional structure and the ratio of the grain-shaped to the needle-shaped particles was increased.

Fig. 2 shows the measurement results by a fluorescence spectrometer for the PDMS substrates spotted under the conditions; 10 droplets per discharge and 400 total droplets. In this figure, the variations in an intensity (I) of DCF-DA emitted green fluorescence normalized to the intensity value (I_0) obtained at 0 h (before the measurement) are plotted as a function of the measurement time. Concerning the crystalline C_{60} particle formed PDMS substrate irradiated a green laser (P-PDMS irradiation) and the same PDMS substrate in the dark room (P-PDMS dark), the values of I/I_0 monotonously increased with an increase in the measurement time, and I/I_0 for pure water maintained a virtually constant value. In the case of P-PDMS irradiation, the increasing rate of I/I_0 against the measurement time was larger than that of P-PDMS dark, so that it was suggested that the crystalline C_{60} particles had the ability to generate ROS by green laser irradiation. I/I_0 was rising with respect to not only P-PDMS irradiation but also P-PDMS dark, and that is thought to be an influence of oxidation of DCF-DA by dissolved oxygen in the solution.

Fig. 3 shows the ratio of $(I-I_0) / I_0$, the increasing rate of P-PDMS irradiation to that of P-PDMS dark at the measurement time of 3h as a function of the droplets per discharge. The figure indicated that the larger ratio was obtained in the case of the discharges under the conditions in the droplets per discharge of 10 to 20 and the total droplets of 400 compared to the other discharge conditions. The result revealed that the optimal discharge conditions to form the particles for generating more ROS were in the range of both 400 total droplets and the smaller droplets per discharge. Since the finer needle-shape particles were three-dimensionally formed under the optimal discharge conditions, as observed in the image of Fig. 1 (b), the particles are suggested to have larger total surface area. Meanwhile, the particles formed under the condition of the total droplets of 800 showed smaller total surface area compared to the case of 400 total droplets, because the particles were stuck together to be larger clumps, as seen in Fig. 1 (d).

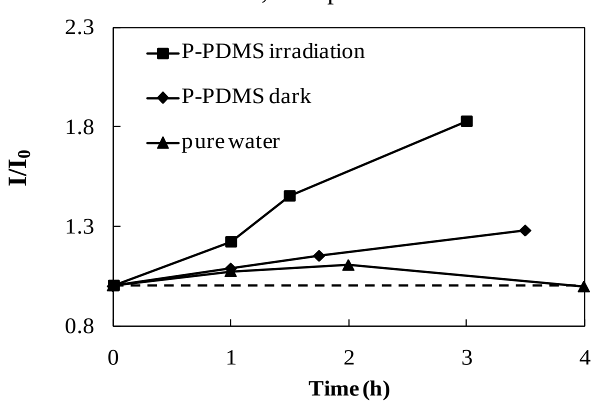

Fig. 2 The measurement results of fluorescence intensity (10 droplets per discharge and 400 total droplets).

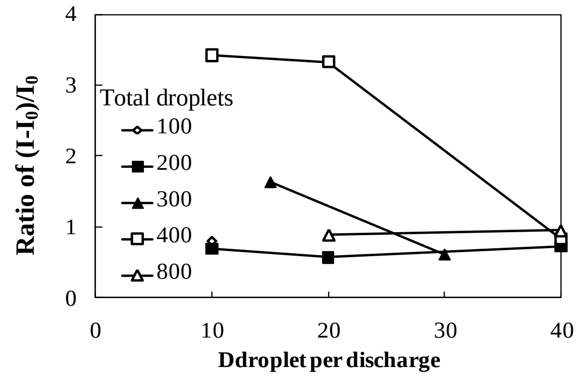

Fig. 3 The variations of ratio of $(I-I_0)/I_0$ as a function of the droplet per discharge.

Conclusions

The crystalline C_{60} particles were formed on the PDMS substrates under the various discharge conditions by the ink-jet system, and investigated for the relationships among the conditions, the shape and the structure of the particles, and the ROS generation.

The particle precipitation state was transformed from planar into three-dimensional structure and the particle shape was changed from needle-like to grain-like, with an increase in the droplets per discharge and the total droplets. The optimal ink-jet discharge conditions, the droplets per discharge of 10 to 20 and the total droplets of 400, existed for synthesizing the crystalline C_{60} particles to generate more ROS. It was indicated that the finer needle-like particles were three-dimensionally formed under the optimal conditions. It was found that there was a possibility that increasing the surface area of the crystalline C_{60} particles lead to an increase in the ROS generation amount considering that the surface area of the particles with the optimal conditions was larger.

References

[1] P. S. Dittrich, K. Tachikawa, A. Manz. Micro total analysis systems. latest advancements and trends. Anal. Chem. 2006;78: 3887-3907.
[2] K. Abe, K. Suzuki, D. Citterio. Inkjet-printed microfluidic multianalyte chemical sensing paper. Anal. Chem. 2008;80: 6928.
[3] E. Nakamura, H. Isobe. Functionalized fullerenes in water. H. Am. Chem. Soc. 2003;36:807-815.
[4] C. M. Sayes, J. D. Fortner, et al. The differential cytotoxicity of water-soluble fullerenes. Nano Lett. 2004;4:1881-1887.
[5] F. Sasaki, M. Suzuki, M. Ban. Evaluation of photosensitizing properties of crystalline C_{60} particle synthesized by Ink-jet method. Carbon 2009 Presentation extended abstracts. 2009;Biarritz:ID426.
[6] M. Ban, F. Sasaki. Crystalline C_{60} particle formation in PDMS-based micro chamber by the ink-jet method. APCE 2009 & APLOC 2009 Program & Abstracts. 2009; Shanghai,:95.