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Formation of Photosensitizing Crystalline C_{60} Particles by Ink-Jet Method

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ABSTRACT

The crystalline fullerene C_{60} particles were formed and immobilized on poly(dimethylsiloxane) (PDMS) substrates under the various discharge conditions by an ink-jet method, and investigated for the reactive oxygen species (ROS) generation property under visible light irradiation. The particles were synthesized by discharging a toluene solution dissolved C_{60} and poly(methyl methacrylate) (PMMA) with the ink-jet spotting system. The ROS generation was evaluated by comparisons of the fluorescence intensities measured for the formed particles under green laser irradiation and in a dark room using fluorescent dyes, 2',7'-dichlorofluorescein diacetate and dihydroethidium. The results of transmission electron microscope (TEM) observation showed that the formed particles consisted of crystalline C_{60}. The optimal ink-jet discharge conditions for synthesizing the particles to generate more ROS were found. In the case of the optimal conditions, the structure in which the needle-like particles were three-dimensionally formed was confirmed. The surface area of the crystalline C_{60} particles was calculated using the SEM observation results, and it was suggested that when the needle-like finer particles were three-dimensionally formed under the optimal conditions, increasing the surface area lead to an increase in the ROS generation amount.

Keywords: Fullerene; Particle; Ink-Jet; Reactive Oxygen Species

1. Introduction

Fullerenes, carbon allotropes, represent the unique physical and chemical properties, and have incited a considerable hope of their potential for uses in biomedical application. One of the biologically most relevant features of fullerenes is mediating generation and quenching of reactive oxygen species (ROS) under visible light irradiation [1]. As recent studies on ROS generation by fullerenes, Sayes and co-workers reported that pure C_{60} brought into water by means of solvent extraction formed water-stable crystalline aggregates (called nano-C_{60} or nC_{60}) in the size of about 100 nm to generate high amount of ROS, and kill both normal and tumor cells at extremely low concentrations [2]. Also, by Markovic and co-workers, the cytotoxicity/ROS production of different nC_{60} suspensions prepared using tetrahydrofuran, ethanol and water were examined for investigating the mechanisms of the cytotoxicity [3]. In this way, many studies on ROS generation by C_{60} have been performed using solutions with colloidal dispersions of C_{60}. While, we have up to date studied an method to immobilize C_{60} at the specific locations on the surface of a polymer substrate, for the purpose of applying the photosensitizing properties of C_{60} as the function of microfluidic and microarray chips, called μ-TAS (micro total analysis systems). The μ-TAS is a technology having the ability to perform the chemical operations such as mixing, reaction and separation by inserting a small amount of solution into various microstructures formed on the chip, and has recently become a candidate for the biological applications [4].

On the other hand, an ink-jet technology has been widely used for not only the direct-writing in the printing field, but also the manufacturing of micro-optical parts and polymer electronics [5]. One of important features of the versatile ink-jet method is modifying a surface locally by means of discharging extremely small amount of solution on the local area. As for the applications of the ink-jet method to μ-TAS, the microarrays with covalent attachment of DNA were fabricated by discharging 5'-terminal-thiolated oligonucleotides to a glass surface using a Bubble Jet method [6], and very recently Abe and co-workers demonstrated "all-inkjet-printed" microfluidic multianalyte chemical sensing paper for the simultaneous determination of pH, total protein and glucose in clinically relevant concentration ranges for urine analysis [7].

In this study, by discharging a fullerene solution using the ink-jet method, crystalline C_{60} particles were synthesized and immobilized at the specific locations on poly (dimethylsiloxane) (PDMS) substrates, and evaluated for the ROS generation under visible light irradiation [8]. In addition, the effects of the discharge conditions and the structure of formed particles on the ROS generation were investigated [9].

2. Experimental

2.1. Ink-Jet Discharging

A picojet 2000-CW ink-jet spotting system (Microjet Co. Ltd., Japan) having the ability to discharge extremely small amount of a solution was used in this study. This system is equipped with a three-axis and microposition-ing system of 1 μm accuracy, and a piezo-driven nozzle with 30 μm in diameter. A stroboscopic camera system allows visual monitoring to adjust piezo voltages and pulse durations for reliable droplet ejection. The vertical separation between the nozzle and the substrate was typically 0.5 mm. As the solution, a fullerene solution was prepared by dissolving C_{60} (Frontier carbon corporation: nanom purple ST) of 35 mg and poly(methyl methacrylate) (PMMA) of about 10 mg in toluene of 40 ml. PDMS flat substrates were fabricated with SILPOT 184 W/C (Dow Corning Toray Co. Ltd.). As the discharge conditions, the number of droplets per one discharge, “droplets per discharge”, was varied from 10 to 20, and the droplets were discharged 10 to 40 times onto the same spots on the PDMS substrates. The product of droplets per discharge and the number of discharges is expressed as “total droplets”. The 11 × 11 spots were arranged with the distance of 250 μm between the centers to form the shape of a square.

2.2. Structural Evaluation

The surfaces of the PDMS substrates discharged the fullerene solution by the ink-jet spotting system were observed by a scanning electron microscope (SEM). A transmission electron microscope (TEM) was used for observations of the fullerene solution discharged on a TEM grid using the ink-jet spotting system.

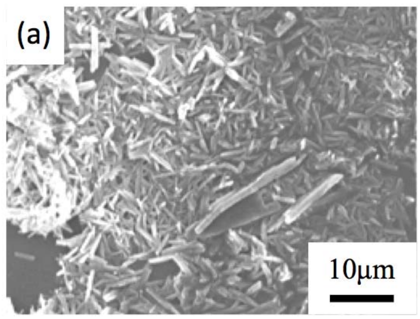
2.3. ROS Generation Evaluation

The production of ROS was determined by measuring the fluorescence intensities emitted by fluorescent dyes; 2’,7’-dichlorofluorescein diacetate (DCF-DA) with 488 nm excitation and 530 nm emission, and dihydroethidium (DHE) with 488 nm excitation and 600 nm emission. The evaluation was performed by the following procedure. First, DCF-DA and DHE were soluted in pure water to obtain a DCF-DA solution (6.0 μM) and a DHE solution (2.3 μM), respectively. Second, each solution was degassed by flowing argon gas in it at a flow rate of 200 sccm for 1h. The PDMS substrates ($5 \times 5 \times 1^t$ mm) with the areas spotted by the ink-jet system were immersed in the DCF-DA and DHE solutions in Quartz cells. And then green lasers (532 nm, 1 mW) were irradiated to the spotted areas on the substrates up to 4 h (“irradiation”), and fluorescence intensities were measured by using the fluorescence spectrometer (Hitachi: F-7000) at given times. As the comparison, the spotted PDMS substrates immersed in the DCF-DA and DHE solutions in a dark room were evaluated up to 4 h in the same manner (“dark”), and pure water was measured as a control (“pure water”).

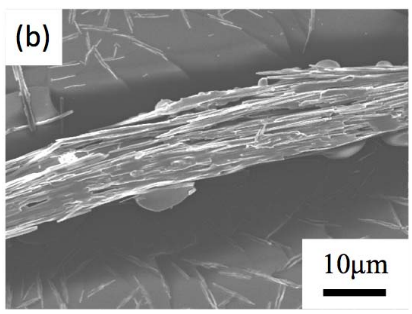
3. Results and Discussion

3.1. Structural Evaluation

Figure 1(a) shows a typical image obtained from the SEM observation of the area spotted on a PDMS substrate by the ink-jet spotting system. In this case, ten droplets were discharged 40 times onto the same area. As the comparison, the fullerene solution of 10 μl was pipetted off onto a PDMS substrate, shown in a typical SEM image of **Figure 1(b)**. It is obvious from **Figure 1(a)** that fine needle-like particles with a few μm size were numerously precipitated on the substrate. As can be seen in **Figure 1(b)**, the particles formed by the pipetting had much larger size, and were likely the aggregate consisted of a number of particles. The results indicated that



(a)

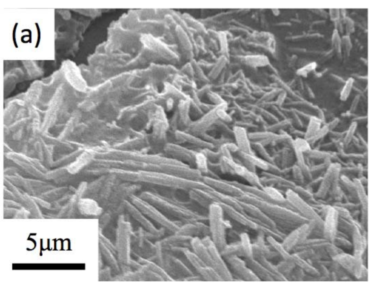


(b)

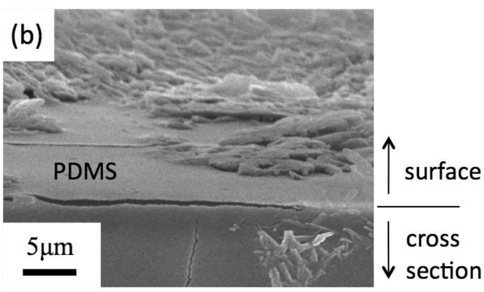
Figure 1. Typical SEM images of the area (a) Spotted on a PDMS substrate with the discharge conditions, the droplets per discharge of 10 and the total droplets of 400, by the ink-jet spotting system; and (b) Pipetted off a fullerene solution of 10 μl onto a PDMS substrate.

the ink-jet process was responsible for the formation of finer particles. For the ink-jet spotting the volume of the droplet discharged was extremely small, several pl, while, in the case of conventional pipetting, approximately 0.1 μl at minimum, and therefore it is suggested that the rapid evaporation of a solvent occurred to inhibit the growth of particles. A typical SEM image of the ink-jet spotted surface is shown in **Figure 2(a)**, being magnified than the image of **Figure 1(a)**. It was demonstrated that numerous needle-like particles with about 1 μm in diameter seemed to be partially embedded in a film-like residue, which might be remaining PMMA. **Figure 2(b)** shows a perspective SEM image of same spotted surface as **Figure 2(a)**, indicating that the film-like PMMA residue including the particles adhered properly to the PDMS substrate. It was implied that the PMMA played an important roll in immobilizing the particles on the PDMS surface.

Figure 3 indicates the TEM observation results for (a) particles on the TEM grid and (b) the magnification of the edge (circle in (a)) of a particle. The particle shown in **Figure 3(a)** had a needle or brush-like shape, which was in good agreement with a typical shape of particles formed on the PDMS substrates by various discharge conditions in this study. **Figure 3(b)** suggests that the lattice spacings between adjacent lattice plains are about 0.50 and 0.81 nm, corresponding to the (110) and (111) plane spacings of face-center-cubic (fcc) C_{60} crystal, respectively [10]. Therefore, the observation results implied that the particles formed on the PDMS substrate (see **Figure 1(a)** and **Figure 2**) consisted of crystalline C_{60}.

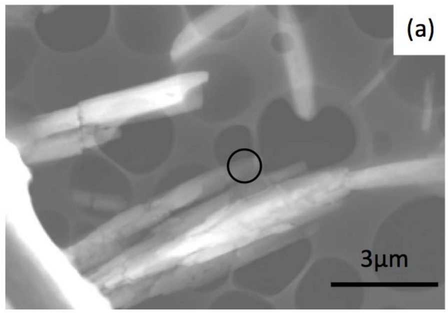


(a)

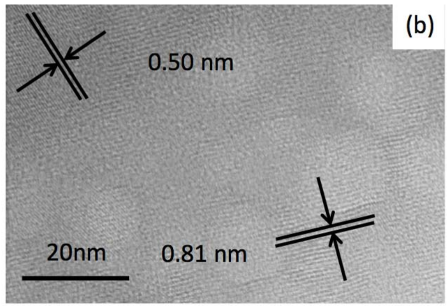


(b)

Figure 2. Typical SEM images of the ink-jet spotted PDMS substrate, (a) The surface and (b) The perspective view.



(a)

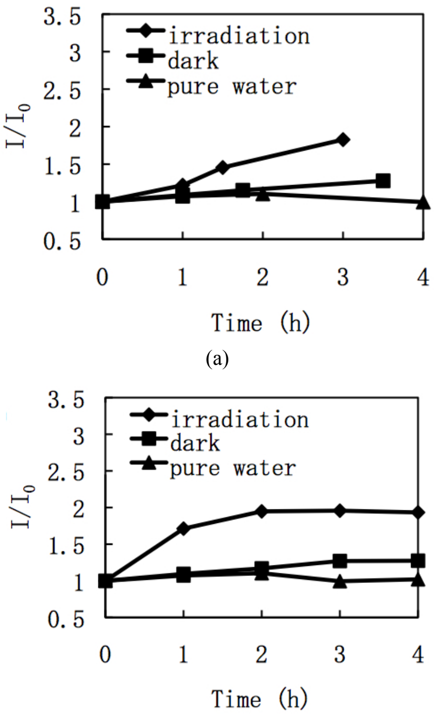


(b)

Figure 3. TEM observation images of (a) Particles on a TEM grid and (b) The magnification of the edge of a particle seen in (a).

3.2. ROS Generation Evaluation

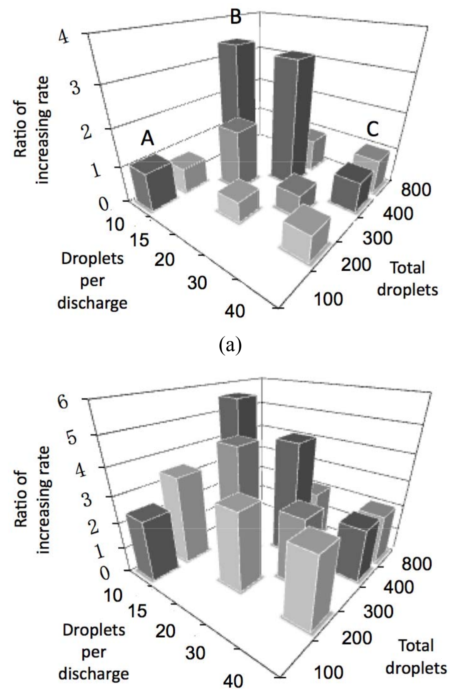
Figure 4 demonstrates the measurement results by a fluorescence spectrometer, (a) and (b) in the cases of using DCF-DA and DHE fluorescent dyes, respectively, for the PDMS substrates formed crystalline C_{60} particles under the conditions; the droplets per discharge of 10 and the total droplets of 400. In **Figure 4(a)**, the variations in an intensity (I) of DCF-DA emitted green fluorescence (488 nm) normalized to the initial 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 (irradiation) and the same PDMS substrate in the dark room (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 "irradiation", the increasing rate of I/I_0 against the measurement time was larger than that of "dark", so that it was suggested that the crystalline C_{60} particles had the ability to generate ROS, hydrogen peroxide, by green laser irradiation. As is the case in **Figure 4(a)**, **Figure 4(b)** indicates the variations of I/I_0 obtained from red fluorescence emission (600 nm) by DHE as a function of the measurement time. I/I_0 of "irradiation" was higher than that of "dark", implying that the crystalline C_{60} particles mediated the generation of ROS, superoxide anion, under green laser irradiation. The reason why I/I_0 of not only "irradiation" but also "dark" were



(a)

(b)

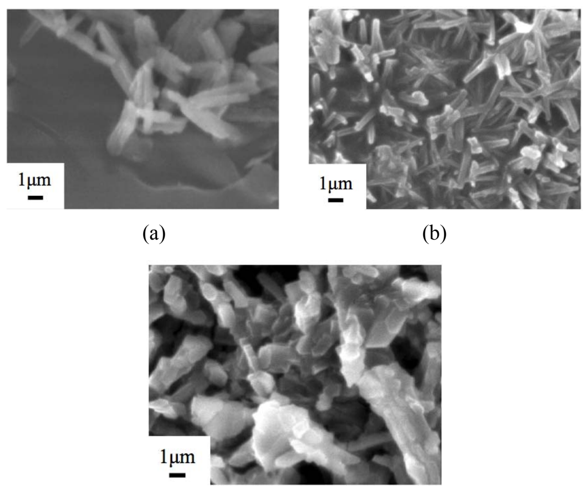
Figure 4. Variations in intensities (I) of (a) DCF-DA and (b) DHE emitted fluorescences normalized to the initial intensity values (I_0) obtained at 0 h as a function of the measurement time for the PDMS substrate spotted under the conditions of 10 droplets per discharge and 400 total droplets.



(a)

(b)

Figure 5. Ratios of "irradiation" to "dark" for the increasing rates of intensities in 3 h as functions of both the droplets per discharge and the total droplets; (a) and (b) in the cases of using DCF-DA and DHE fluorescent dyes, respectively.



(a) (b)

(c)

Figure 6. Typical SEM observation images of the crystalline C_{60} particles formed on the PDMS substrates represented as (a), (b) and (c) in Figure 5(a). The droplets per discharge and the total droplets are (a) 10 and 100; (b) 10 and 400; and (c) 40 and 800, respectively.

increased is thought to be an influence of oxidation of DCF-DA and DHE by dissolved oxygen in a solution, since the amount of the dissolved oxygen after the measurement of 4 h indicated the value near that before the degassing.

As the results of fluorescence measurements for the particles formed at various discharge conditions, the ratios of "irradiation" to "dark" for the increasing rates of intensities in 3 h are shown in **Figure 5** as functions of both the droplets per discharge and the total droplets; (a) and (b) in the cases of using DCF-DA and DHE fluorescent dyes, respectively. Note that an increase in the ratio means an increase in the amount of generated ROS; hydrogen peroxide and superoxide anion in the cases of (a) and (b), respectively. The figure indicated that the larger ratios were 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 results revealed that the optimal discharge conditions to form the particles for generating more ROS existed. **Figure 6** shows typical images obtained from the SEM observations of the crystalline C_{60} particles formed on the PDMS substrates represented as A, B and C in **Figure 5(a)**. Namely, the droplets per discharge and the total droplets are A 10 and 100, B 10 and 400, and C 40 and 800, respectively. **Figure 6(a)** shows that needle-shaped particles with 0.5 to 1.0 μm in diameter and several μm length were formed on the substrate. The particles seen in **Figure 6(b)** had finer needle-like shape and were much numerously formed than those in

Figure 6(a). For **Figure 6(c)**, the particles having a shape of relatively grain were synthesized, and formed sticking to each other. Therefore, it is noted that at the optimal discharge conditions for generating more ROS, that is to say, the droplets per discharge of 10 to 20 and the total droplets of 400, the finer needle-like particles were three-dimensionally formed as seen in **Figure 6(b)**. Additionally, the SEM observation results of all ink-jet spotted PDMS substrates suggested that the particles formation state was transformed from planar into three-dimensional structure, and from needle-shaped into grain-shaped particles with an increase in both the droplets per discharge and the total droplets.

To investigate the relation between the formation state of particles and the ROS production, the total surface area of the crystalline C_{60} particles formed on the PDMS substrate was estimated by the following calculation procedure. The total amount of the fullerene solution discharged was calculated by the product of an amount of one droplet (4.2 pl, supposing that the diameter was 20 μm) and the number of total droplets. The weight of all crystalline C_{60} particles formed on the PDMS substrate was calculated with the total discharged amount and the concentration of the C_{60} dissolved in the solution, 35 mg/ml. Here, the weight of one crystalline C_{60} particle was obtained using the density of C_{60}, 1.73 g/cm^3 [11], and the sizes of the crystalline C_{60} particles measured from the SEM images. Assuming that all dissolved fullerene in the fullerene solution was precipitated as the crystalline C_{60} particles, the number of the crystalline C_{60} particles formed on the PDMS substrate was obtained from the weight of one crystalline C_{60} particle and the calculated weight of all crystalline C_{60} particles formed on the PDMS substrate. Multiplying the surface area of one crystalline C_{60} particle, which was calculated from the size measured using the SEM observation results, by the number of the crystalline C_{60} particles, the total surface area of the crystalline C_{60} particles formed on the PDMS substrate was obtained.

For the crystalline C_{60} particles formed in the various discharge conditions, the variation of the ratio of increasing rate in the case of using DCF-DA (see **Figure 5 (a)**) as a function of the calculated total surface area of the particles is shown in **Figure 7**. The figure revealed that there appeared to be two tendencies (plots of group I and II) of the ratio of increasing rate against the total surface area of the particles. That is to say, for group I and II, the ratios of increasing rate were increased and almost unchanged with an increase in the surface area of the particles, respectively. The crystalline C_{60} particles belonging to group I had a three-dimensionally formed finer structure as observed in the image of **Figure 6(b)**, and it was suggested that larger exposed particle surface led to more production of ROS. Meanwhile, for the crystalline C_{60} particles in group II the increment of the surface area made little contribution to ROS generation because it was highly probable that the particles were stuck together to form larger clumps, as seen in **Figure 6(c)**.

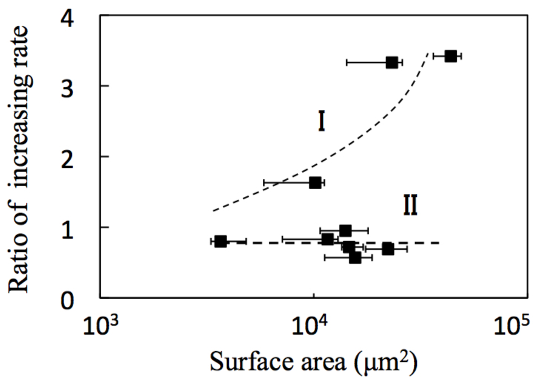


Figure 7. Variation in the ratio of increasing rate as a function of the total surface area calculated for the crystalline C_{60} particles formed under the various discharge conditions. The dotted lines are drawn to guide the eye.

4. Conclusion

The crystalline C_{60} particles were formed on the PDMS substrates under the various discharge conditions by the ink-jet spotting system, and investigated for the relationships among the conditions, the shape and the structure of particles, and the ROS generation. The fluorescence measurement results implied that the formed crystalline C_{60} particles mediated the generation of ROS, hydrogen peroxide and superoxide anion. 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 from the SEM observation results that under the optimal conditions the finer needle-like particles were three-dimensionally formed. It was found that there was a possibility that increasing the surface area of crystalline C_{60} particles led to an increase in the ROS generation amount, considering that the particles formed under the optimal conditions had larger surface area.

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REFERENCES

[1] E. Nakamura and H. Isobe, "Functionalized Fullerenes in Water," *Accounts of Chemical Research*, Vol. 36, No. 11,

2003, pp. 807-815.

[2] C. M. Sayes, J. D. Fortner, W. Gyo, D. Lyon, A. M. Boyd, K. D. Ausman, Y. J. Tao, B. Sitharaman, L. J. Wilson, J. B. Hughes, J. L. West and V. L. Colvin, "The Differential Cytotoxicity of Water-Soluble Fullerenes," *Nano Letters*, Vol. 4, No. 10, 2004, pp. 1881-1887. doi:10.1021/nl0489586

[3] Z. Markovic, B. Todorovic-Markovic, D. Kleut, N. Nikolic, S. Vranjes-Djuric, M. Misirkic, L. Vucicevic, K. Janjetovic, A. Isakovic, L. Harhaji, B. Babic-Stojic, M. Dramicanin and V. Trajkovic, "The Mechanism of Cell-Damaging Reactive Oxygen Generation by Colloidal Fullerene," *Biomaterials*, Vol. 28, No. 36, 2007, pp. 5437-5448. doi:10.1016/j.biomaterials.2007.09.002

[4] P. S. Dittrich, K. Tachikawa and A. Manz, "Micro Total Analysis Systems. Latest Advancements and Trends," *Analytical Chemistry*, Vol. 78, No. 12, 2006, pp. 3887-3907. doi:10.1021/ac0605602

[5] B.-J. de Gans, P. C. Duineveld and U. S. Schubert, "Ink-jet Printing of Polymers: State of the Art and Future Developments," *Advanced Materials*, Vol. 16, No. 3, 2004, pp. 203-213. doi:10.1002/adma.200300385

[6] T. Okamoto, S. Tomohiro and Y. Nobuko, "Microarray Fabrication with Covalent Attachment of DNA Using Bubble Jet Technology," *Nature Biotechnology*, Vol. 18, No. 4, 2000, pp. 438-441.

[7] K. Abe, K. Suzuki and D. Citterio, "Inkjet-Printed Microfluidic Multianalyte Chemical Sensing Paper," *Analytical Chemistry*, Vol. 80, No. 18, 2008, pp. 6928-6934. doi:10.1021/ac800604v

[8] F. Sasaki, M. Suzuki and M. Ban, "Evaluation of Photosensitizing Properties of Crystalline C_{60} Particle Synthesized by Ink-Jet Method," *Carbon* 2009 *Presentation Extended Abstracts*, Biarritz, 14-19 June 2009, Article ID: 426.

[9] F. Sasaki and M. Ban, "Application of Inkjet-Fabricated Crystalline C_{60} Particles Generating Reactive Oxygen Species under Visible Light Irradiation to Microarray Chips," *The* 14*th International Conference on Miniaturized Systems for Chemistry and Life Sciences* (*µTAS* 2010), Groningen, 3-7 October 2010, Article ID: 0268.

[10] W. I. F. David, R. M. Ibberson and T. Matsuo, "High Resolution Neutron Powder Diffraction: A Case Study of the Structure of C_{60}," *Proceedings of the Royal Society A*, Vol. 442, No. 1914, 1993, pp. 129-146. doi:10.1098/rspa.1993.0095

[11] P. A. Heiney, J. E. Fisher, A. R. McGhie, W. J. Romanow, A. M. Denenstein, J. P. McCauley Jr., A. B. Smith and D. E. Cox, "Orientational Ordering Transition in Solid C_{60}," *Physical Review Letters*, Vol. 66, No. 22, 1991, pp. 2911-2914. doi:10.1103/PhysRevLett.66.2911

