# APPLICATION OF INKJET-FABRICATED CRYSTALLINE $C_{60}$ PARTICLES GENERATING REACTIVE OXYGEN SPECIES UNDER VISIBLE LIGHT IRRADIATION TO MICROARRAY CHIPS

**F. Sasaki*** **and M. Ban**
*Nippon Institute of Technology, JAPAN*

## ABSTRACT

This paper reports microarray chips equipped with wells encapsulating three-dimensionally formed μm-size crystalline $C_{60}$ particles to generate reactive oxygen species (ROS) by irradiation of visible light, which does not affect biological materials. The crystalline $C_{60}$ particles are synthesized and immobilized on the bottoms of wells by an inkjet printing technology. The optimal inkjet conditions for synthesizing the particles to generate more ROS were found, and in the conditions the structure in which the needle-like particles were three-dimensionally formed was confirmed. The particles are capable of being used as a ROS generation source for evaluating cellular response to oxidative stress.

**KEYWORDS:** Fullerene, Inkjet, Reactive oxygen species, Oxidative stress

## INTRODUCTION

One of the key technologies necessary to fabricate microfluidic chips is forming a functional surface in a localized region on polymer materials used as the chip substrate. An inkjet printing technology is capable of locally modifying a material surface by means of discharging extremely small amount of solution on the target area. On the other hand, fullerenes, a third carbon allotrope, represent the unique physical and chemical properties, and have incited a considerable hope of their potential for uses in biomedical application. It is well known that fullerenes have ROS generation property, and as recent relevant studies water-stable colloidal $C_{60}$ aggregates were reported to generate high amount of ROS and mediate cytotoxicity. 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 range of about 60 nm, to generate high amount of ROS and kill both normal and tumor cells [1]. Also, by Z. Markovic and co-workers, the cytotoxicity / ROS production of $nC_{60}$ colloids prepared using tetrahydrofuran, ethanol and water was examined [2].

Meanwhile, our group presented that the μm-size particles consisting of crystalline $C_{60}$ were synthesized by the inkjet method and generated ROS under visible light irradiation [3-5]. The crystalline $C_{60}$ particles differ from the colloidal $C_{60}$ in being immobilized in the specific area on a substrate by the inkjet method, and therefore that permits applications of $C_{60}$ to μ-TAS. In this paper, it is reported that the crystalline $C_{60}$ particles with the optimal three-dimensional structure generating more ROS were encapsulated into wells of a microarray chip.

## EXPERIMENTAL

Figure 1 shows a conceptual diagram of a picojet 2000-CW inkjet spotting system (Microjet Co. Ltd., Japan) 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, and has the ability to eject a single droplet with diameter in 20 to 30 μm. A fullerene solution, namely, solution of $C_{60}$ (Frontier carbon corporation: nanom purple ST, 35 mg) and poly(methyl methacrylate) (PMMA) (about 10 mg) dissolved in toluene of 40 ml was prepared. The poly(dimethylsiloxane) (PDMS) substrates equipped with microchambers were fabricated using a soft lithographic method with SILPOT 184 W/C (Dow Corning Toray Co. Ltd.). The diameters and the depth of the microchambers were 500 and 1000 μm, and about 40 μm, respectively. The droplets per discharge, the total droplets and the interval time between the discharges were varied as the discharge conditions. The droplets per discharge of 10 to 40 were spotted 10 to 40 times with no interval time on the flat PDMS substrates. Also, the droplets per discharge of 10 to 100 were spotted 5 to 100 times with up to 2 min of the interval time into the microchambers. The vertical separation between the nozzle and the substrate was typically 0.5 mm.

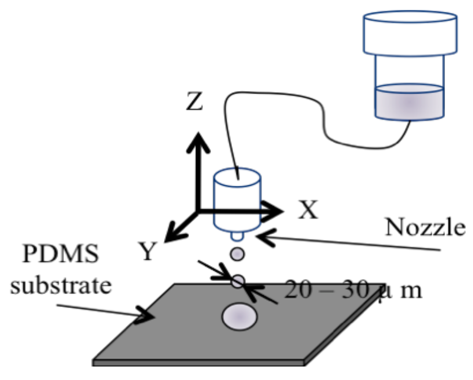


*Figure 1: Schematic diagram of the used inkjet system.*

The spotted flat surfaces and microchambers 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 (530 nm) emitted by a fluorescent dye, 2',7'-dichlorofluorescein diacetate (DCF-DA) with 488 nm excitation. 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 inkjet was immersed in the DCF-DA solution in a Quartz cell. And a green laser (532 nm, 1 mW) was irradiated to the spotted area on the substrate ('under visible light 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 ('in a dark room') was evaluated up to 3.5 h in the same manner, and pure water was measured as a control (pure water).

## RESULTS AND DISCUSSION

Figure 2 shows the SEM images of a) 11 × 11 inkjet-spotted areas on the flat PDMS substrate and b) the magnification of one spotted area shown in a). Figure 2 b) demonstrates that numerous crystalline $C_{60}$ particles were three-dimensionally formed in a state fixed in a PMMA film as shown in the illustrated cross-sectional diagram of Figure 2 c). The Raman analysis and the TEM observation results suggested that the synthesized particles comprised of crystalline $C_{60}$ [3].

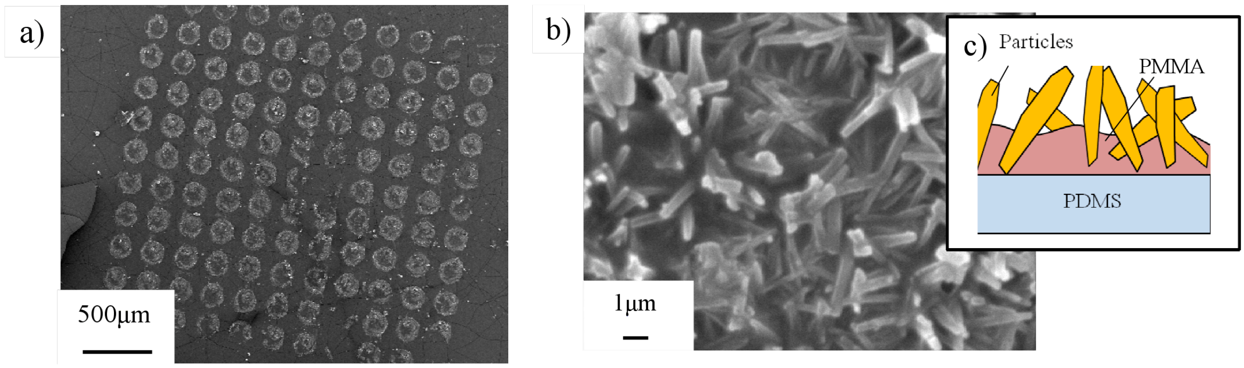


*Figure 2: SEM observation images of crystalline $C_{60}$ particles synthesized using the inkjet system.*

In Figure 3, 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 for the PDMS substrate spotted under the conditions; 10 droplets per discharge and 400 total droplets. The measured intensities of the fluorescence implied that ROS generation was clearly confirmed under visible light irradiation from the difference in increasing rates of the intensities. As the results of fluorescence measurements for the particles formed at various discharge conditions, the ratios of 'under visible light irradiation' to 'in a dark room' for the increasing rates of intensities in 3 h are shown in Figure 4. It is noted that the optimal discharge conditions, the droplets per discharge of 10 to 20 and the total droplets of 400, for generating more ROS exists, and it was found that under the conditions the finer needle-like particles have the three-demensionally formed structure seen in Figure 2 b).

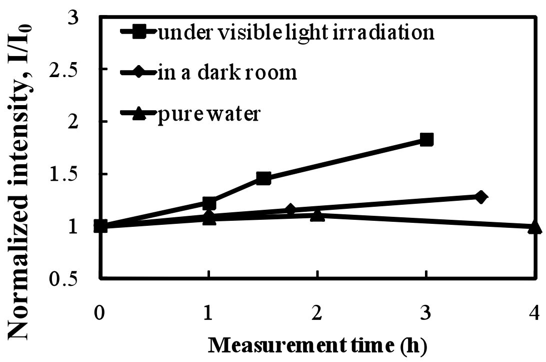


*Figure 3: Variation in normalized intensities of DCF-DA emitted fluorescence (530 nm) for the crystalline $C_{60}$ particles 'under visible light irradiation' and 'in a dark room'.*

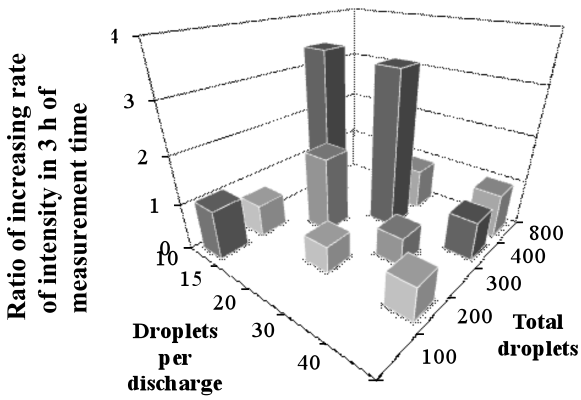


*Figure 4: Ratio of increasing rate of intensity of 'under visible light irradiation' to that of 'in a dark room' in 3 h. An increment in the ratio implies more ROS generation.*

The SEM image of Figure 5 a) demonstrates that the crystalline $C_{60}$ particles having the optimal structure (see Figure 5 b)) to generate more ROS were encapsulated in a circular pattern inside a PDMS-based 1000 μm-diameter microwell. Figure 6 is the optical microscope photograph, indicating that a 500 μm-diameter microwell encapsulating the particles is equipped with the inlet and outlet microchannels located in the upper part as the key components of PDMS-based microarray chip.

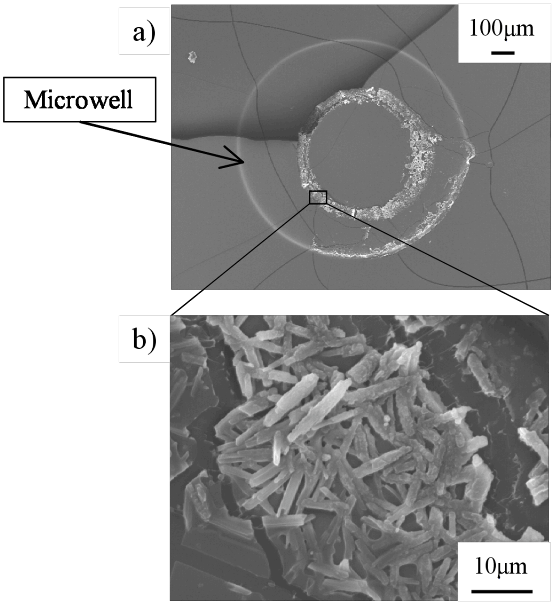


*Figure 5: SEM observation images of crystalline $C_{60}$ particles encapsulated inside a microwell of PDMS-based microarray chip.*

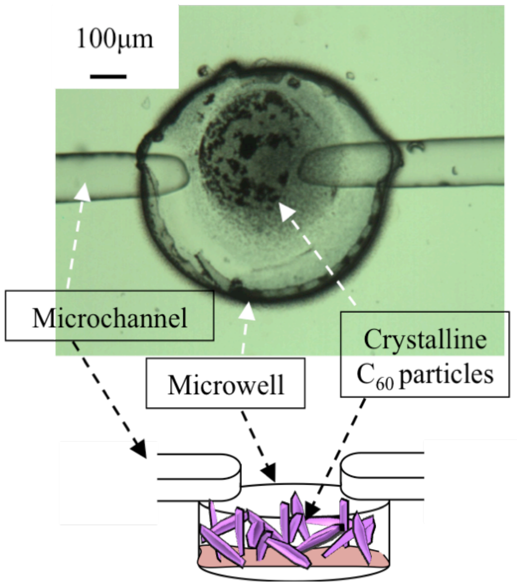


*Figure 6: Optical microscope observation image of a crystalline $C_{60}$ particle encapsulated PDMS-based microwell having the inlet and outlet microchannels located in the upper part.*

## CONCLUSION

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 the finer needle-like particles were three-dimensionally formed under the optimal conditions. It is suggested that there is a possibility that increasing the surface area of crystalline $C_{60}$ particles lead to an increase in the ROS generation amount. The numerous needle-shaped particles, which were similar to those formed with the optimal conditions to generate more amount of ROS, were successfully encapsulated in a circular pattern inside the microwells of 500 and 1000 μm diameter.

## REFERENCES

[1] C. M. Sayes, J. D. Fortner, W. Guo, et. al., The differential cytotoxicity of water-soluble fullerenes, Nano Lett., 4, pp. 1881-1887, (2004).
[2] Z. Markovic, B. T-Markovic, D. Kleut, et. al., The mechanism of cell-damaging reactive oxygen generation by colloidal fullerene, Biomaterials, 28, pp. 5437-5448, (2007).
[3] 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.
[4] M. Ban and F. Sasaki, Crystalline $C_{60}$ particle formation in PDMS-based micro chamber by the ink-jet method, APCE 2009 & APLOC 2009 Program & Abstract, 2009, Shanghai, 95.
[5] F. Sasaki and M. Ban, Formation of photosensitizing crystalline $C_{60}$ particles in PDMS-based micro chamber by the ink-jet method, 11th International Conference on Microreaction Technology (IMRET 11) Book of Abstracts, 2010, Kyoto, 382.

## CONTACT

*F. Sasaki, futa1104@hotmail.co.jp