Plasma surface modifications of nano-structured materials and their applications to virus detection system

M. Nagatsu[1,2], A. Sakudo[3], A. Viswan[1], T. Abuzairi[1,4], H. Chou[2], M. Okada[2], E. Yang[1], I. Motrescu[1,5], M. A. Ciolan[1,6], R. W. Purnamaningsih[4], N. R. Poespawati[4] and D. Luca[6]

[1]*Shizuoka University, Graduate School of Science and Technology, Hamamatsu 432-8561, Japan*
[2]*Shizuoka University, Graduate School of Engineering, Hamamatsu 432-8561, Japan*
[3]*University of the Ryukyus, Faculty of Medicine, Nishihara, Okinawa 903-0215, Japan*
[4]*University of Indonesia, Department of Electrical Engineering, Depok 16424, Indonesia*
[5]*University of Agricultural Science and Veterinary Medicine, Department of Science, Iasi 700490, Romania*
[6]*Alexandru Ioan Cuza University, Faculty of Physics, Blvd. Carol I nr. 11, Iasi 700506, Romania*

In this study, we present the recent research progress on biomedical applications of plasma processing. The main topics are; (1) fabrication of the nano-structured materials by plasma processing and development of the high-sensitive virus detection system using surface functionalized carbon-encapsulated magnetic nanoparticles, (2) functionalization of the surfaces locally by an ultrafine atmospheric pressure plasma jet for developing biochip device, and (3) development of the plasma surface functionalization of ZnO nanophosphors for bioimaging.

1. Introduction

Plasma processing has been proven its numerous advantages in the surface functionalization of the polymer, metallic materials, nano-structured materials such as carbon nanotubes and various kinds of nanoparticles(NPs). An important subject of plasma processing is to understand the functionalization mechanism so that a better control of the functional group could be achieved. In this study, we will present our recent progress on the fabrication of nano-structured materials and their surface functionalization by plasma chemical modification, and immobilization of the biomolecules onto the surface of nano-structured materials for bio-medical application.

Recently, carbon-encapsulated metal nanoparticles have attracted considerable interest in various industrial applications. The incorporation of both metal nanoparticles and carbon in stable core-shell system provide the improvement of their great advantageous properties which make them potentially applicable in various areas such as magnetic data storage, magnetic fluid, magnetic inks[1], catalyst support[2], magnetic separation, electrode, additives in many uses, conductive pasta, conductive coating, biotechnology and biomedicine applications[3-8] .

In this study we will present our recent results on the fabrication of nano-structured materials by dc arc discharge and laser ablation method, functionalization of their surfaces by plasma chemical modification at low-pressure and atmospheric pressure, and finally to immobilization of the relevant biomolecules onto the surface of nano-structured materials for biomedical application.

2. Carbon-encapsulated magnetic nanoparticles for virus detection

Carbon-encapsulated metal nanoparticles were prepared by arc discharge [9,10], which has been described in a previous study [11]. An arc discharge was generated by applying a dc current of 150–200 A at a potential of about 25 V between the anode and the cathode. A graphite electrode molded with metal powder was used as the anode, while a graphite rod as the cathode. The chamber was evacuated to about 1 Pa by a rotary pump. A He:CH_4 gas mixture with a ratio of 8:2 was beforehand introduced into the chamber until the pressure reached 1.3×10^4 Pa. Figure 1 shows a size distribution of carbon-encapsulated iron nanoparticles and a typical high-resolution TEM image. The particles mainly have an average diameter of 20 nm in the range 10–50 nm.[12]

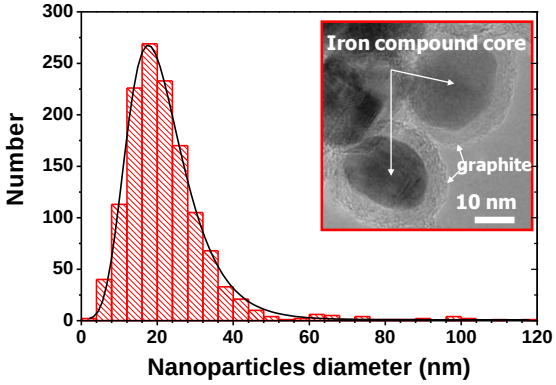

Fig. 1 HR-TEM image and size distribution of carbon-encapsulated NPs.[12]

After fabricating the nanoparticles, they were treated by using an inductively coupled

radio-frequency (RF) plasma device with 200 mm in both diameter and height, as shown in Fig. 2. A helical water-cooling copper pipe was coupled to an RF power generator at 13.56 MHz via a matching network. The input RF power was typically about 80 W. Samples were set in a glass dish that was placed on the stage inside the chamber. Two-step plasma treatment was performed here. As the first step, pretreatment was performed with Ar plasma at 50 Pa. Subsequently, Ar/NH_3 or Ar/H_2O gas plasma was used as the post-treatment to introduce amino or carboxyl groups, respectively. During the plasma processing, the gate chamber was closed to prevent the nanoparticles from flowing to the turbo pump system.

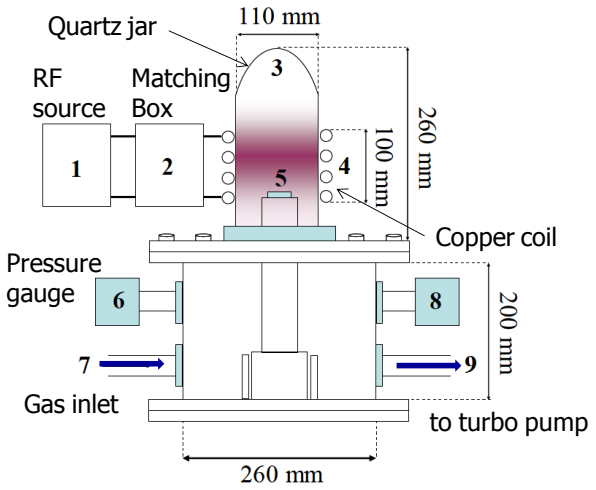

Fig. 2 Experimental setup of an inductively coupled radio-frequency plasma device.[11,12]

The amino group population of the plasma-treated nanoparticles was analyzed by the chemical derivatization method using sulfosuccinimidyl 6-[3´(2-pyridyldithio)-propionamido] hexanoate (sulfo-LC-SPDP) according to the specific chemical procedure, as shown in Fig. 3. The plasma modified nanoparticles (250 μg) were suspended by bath sonication

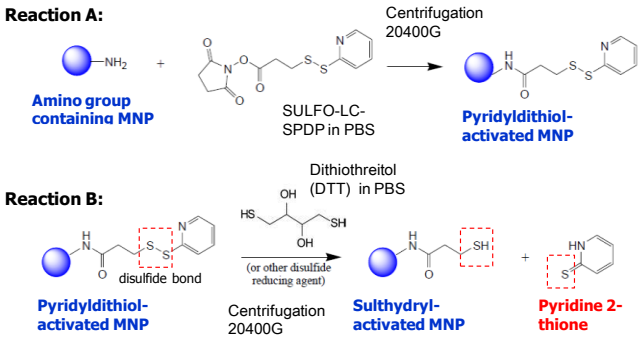

Fig. 3 Illustration of chemical derivatization method to analyze amino group population of carbon-encapsulated magnetic nanoparticles(MNPs).

in 200 μl of 10 mM sulfo-LC-SPDP in phosphate buffer saline (PBS) and reacted for 30 min under light shielding conditions, repeating the ultrasonication every 5 min. The treated nanoparticles were washed three times with PBS solution and collected magnetically. The centrifugation was performed for 5 min with a gravitational force of 20,400 *G*. The nanoparticles with sulfo-LC-SPDP complexes were then reacted with 20 mM dithiothreitol (DTT) in PBS and reacted under light shielding conditions. After a 15 min reaction, 5 min centrifugation at 20,400 *G* was performed and the cleavage product pyridine-2-thione(P2T) liberated from the sulfo-LC-SPDP was determined by spectrophotometry at 343 nm.

The number of amino groups in 250 μg of the modified nanoparticles was quantitatively determined from the calibration curve or by theoretical evaluation using the extinction coefficient of pyridine-2-thione at 343 nm: 8.08 x 10^3 $M^{-1}cm^{-1}$. The number of amino groups per nanoparticle was then calculated when the number of nanoparticles per gram was 1.14 x 10^{14}, which was estimated by measuring the ratio of the mass of the nanoparticles to their volume under the assumption that the nanoparticles have a regular spherical shape mainly of 20 nm diameter determined from the nanoparticle size distribution taken by HR-TEM. The amino group population of the plasma-treated nanoparticles was analyzed by UV absorption technique using the chemical procedure with SPDP and DTT reagents. Figure 4 shows the numbers of amino groups introduced onto the nanoparticle surfaces as a function of Ar/NH_3 gas mixture ratio in RF plasmas, together with the NH emission line intensity measured by optical emission spectroscopy.

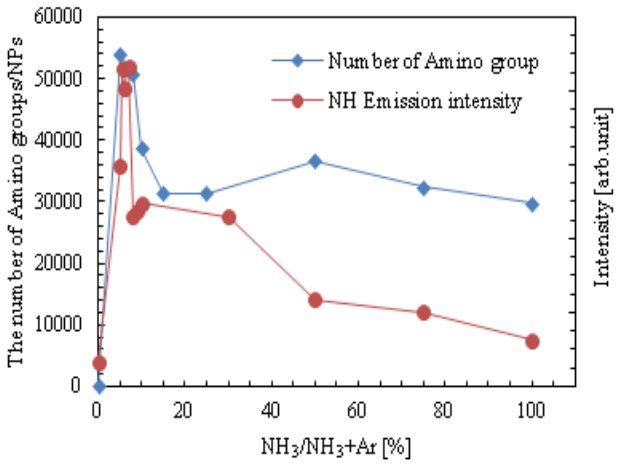

Fig. 4 Numbers of amino groups introduced onto the carbon-encapsulated iron nanoparticles surfaces versus Ar/NH_3 gas mixture ratio in RF plasmas.

Based upon the present results of surface modification of carbon-encapsulated metal nanoparticles, we have carried out the experiments on virus detection using these surface-functionalized nanoparticles. The preliminary experiments indicated the promising results for the future application to biomedical research fields. Figure 5 shows the procedure of the viral collection test using amino-modified carbon-encapsulated magnetic nanoparticles, where the antibody immobilized iron nanoparicles were incubating with a dilute suspension of influenza A virus H1N1 in phosphate-buffered saline. With anti-influenza A virus hemagglutinin (HA) antibody C179 (Takara bio, Japan), which recognizes amino acid residues of HA, we confirmed the virus concentration of 17.3-fold in the present technique, as shown in Fig. 6, where sample before incubation was denoted as BF, supernatant after the incubation as SP, and the magnetic collected fraction as BD, respectively.[13]

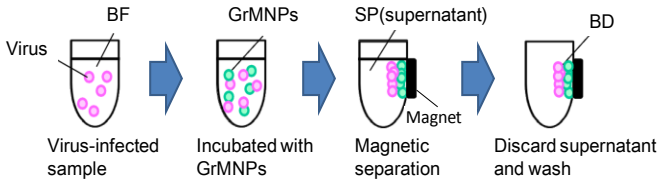

Fig. 5 Illustration of capturing procedure of influenza A virus by antibody-immobilized ion nanoparticles by magnet[13].

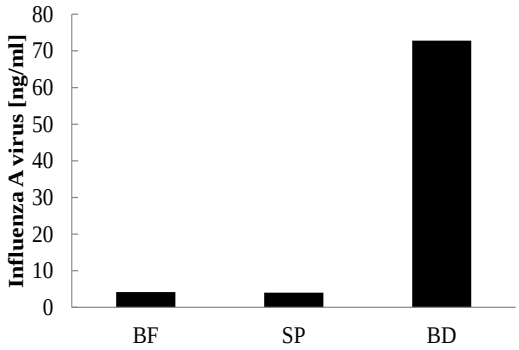

Fig. 6 Result of virus concentration of influenza A virus by antibody-immobilized iron nanoparticles[13].

3. Surface functionalizion of CNT dot-array by ultrafine APPJ for developing biochip device

To realize maskless functionalization of a carbon nanotube (CNT) dot array, a localized surface functionalization technology using an ultrafine atmospheric pressure plasma jet (APPJ) was developed.[14] The surface functionalization onto individual vertically aligned CNT was carried out by two stages of plasma treatments: pre-treatment for activation of the CNT surface and post-treatment for surface functionalization. Figure 7 shows photos of CNT dot-array treated by an ultrafine APPJ and FE-SEM image of the tip of ultrafine APPJ with aperture size of 100 nm. Figure 8 shows the illustration of maskless surface functionalization of CNT dot-array substrate by using the ultrafine APPJ.

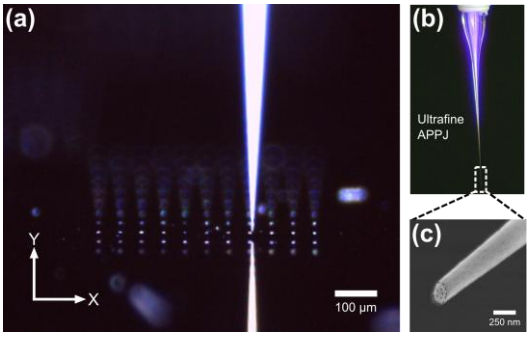

Fig. 7 (a) Photo of CNT dot-array treated by an ultrafine APPJ. (b) An image of ultrafine APPJ. (c) FE-SEM image of the tip of ultrafine APPJ with aperture size of 100 nm.[14]

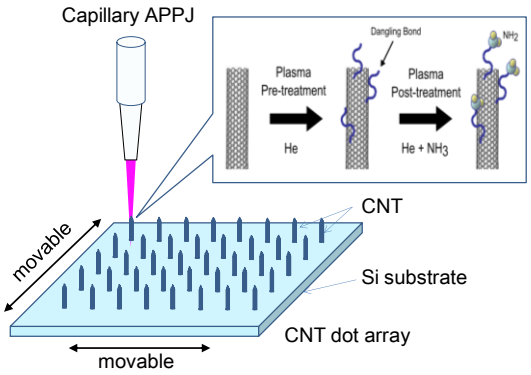

Fig. 8. Illustration of surface processing with ultrafine APPJ.

To realize maskless functionalization, plasma surface functionalization was conducted on individual vertically aligned CNT dot-array to create a line pattern by computer-controlled x-y stage. Figure 9(a) depicts the bright field image of CNT dot-array to show the area treated in a line pattern by ultrafine APPJ. Figures 9(b) and 9(c) show the successful line pattern of amino and carboxyl group functionalization respectively, using 1 μm aperture size of the APPJ.

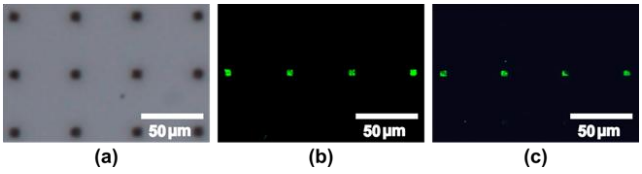

Fig. 9 (a)Bright field image of CNT dot-array. Fluorescence microscope images of line-patterning with (b)amino group and (c) carboxyl group functionalization by the ultrafine APPJs.[14]

4. Surface functionalization of ZnO nanoparticles for bioimaging

ZnO nanoparticles were fabricated by a laser ablation method.[15,16] Our interest focuses on amine group modification onto the surface of ZnO nanoparticles by plasma processing. The amine groups introduced on the surface of the NPs can be used to link with ligand molecules, as it is illustrated in Fig. 10. Here, we used NH_3 surface wave plasma for surface modification of the ZnO nanoparticles. To examine the amino groups modification, we used a conventional chemical derivatization method using Dextran with fluorescent isothiocyanate (FTIC) which selectively connected with amino groups, or a combination of Dextran and fluorescent dyes of 6-DTAF which selectively connected with OH groups. Figure 11 shows the results of fluorescent spectroscopy measurement. The present results shows that amino group functionalization of ZnO nanoparticles are successfully performed by NH_3 plasma modification. The present results suggest the surface-functionalized ZnO nanophosphors can be utilized for detection of virus detection system.

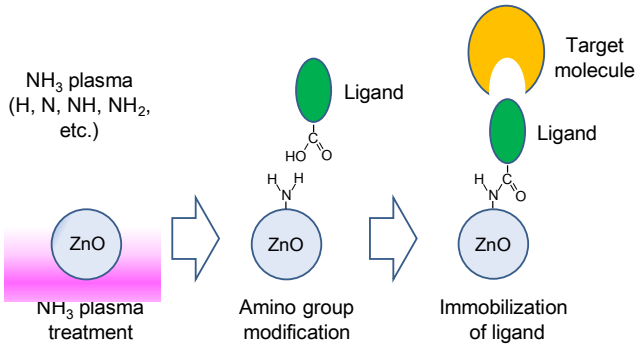

Fig. 10 Illustration of biofunctionalization process of ZnO NPs for application to bioimaging.[16]

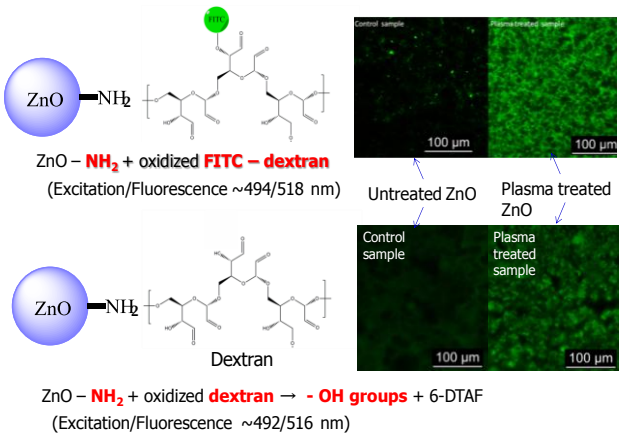

Fig. 11 Fluorescent images of NH_3 plasma amino-modified ZnO nanoparticles for two different methods, FITC-Dextran (upper) or a combination of Dextran and fluorescent dyes(lower).

5. Conclusion

In this study, we presented the recent experimental results on various plasma surface modification techniques of carbon-encapsulated metal nanoparticles fabricated by dc arc discharge, CNT dot array for biochip devise and ZnO nanoparticles for bioimaging. We have also shown the amino and carboxyl modification of carbon-encapsulated nanoparticles by low-pressure RF plasmas and CNT dot array by ultrafine APPJs. With amino group modified carbon-encapsulated iron nanoparticles, we successfully demonstrated the influenza A virus concentration by immobilizing antibody onto the surface-functionalized magnetic nanoparticles.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (No. 21110010), and Challenging Exploratory Research (No. 25600120) from the Japan Society for the Promotion of Science (JSPS).

References

[1] M. E. McHenry, S. A. Majetich, E. M. Kirkpatrick, Mater. Sci. Eng.: A 1995, 204, 19.
[2] L. Kong, X. Lu, X. Bian, W. Zhang, C. Wang, ACS Appl. Mater. Interfaces, 2010, 3, 35.
[3] G. Pastorin, Pharm. Res. 2009, 26, 746.
[4] Q. A. Pankhurst, N. K. T. Thanh, S. K. Jones, J. Dobson, J. Phys. D: Appl. Phys. 2009, 42, 224001.
[5] A.-H. Lu, E. L. Salabas, F. Schuth, Angew. Chem. Int. Ed. 2007, 46, 1222.
[6] S. Kim, E. Shibata, R. Sergiienko, T. Nakamura, Carbon, 2008, 46, 1523.
[7] A. Ito, M. Shinkai, H. Honda, T. Kobayashi, J. Biosci. Bioeng. 2005, 100, 1.
[8] C. C. Berry, J. Phys. D: Appl. Phys. 2009, 42, 224003.
[9] M. Nagatsu, T. Yoshida, M. Mesko, A. Ogino, T. Matsuda, T. Tanaka, et al., Carbon, 2006, 44, 3336.
[10] Y. Saito, T. Yoshikawa, M. Okuda, N. Fujimoto, et al., Chem. Phys. Lett. 1993, 212, 379.
[11] T. E. Saraswati, T. Matsuda, A. Ogino, M. Nagatsu, Diam. Relat. Mater. 2011, 20, 359.
[12] T. E. Saraswati, A. Ogino, M. Nagatsu, Carbon, 2012, 50, 1253.
[13] A. Sakudo, H. Chou, K. Ikuta, M. Nagatsu, Bioorg. Med. Chem. Lett. 2015, in press.
[14] T. Abuzairi, M. Okada, N. R. Poespawati, R. Wigajatri, M. Nagatsu, et.al., Carbon, 2015, in press.
[15] M. A. Ciolan, I. Motrescu, D. Luca, M. Nagatsu, Jpn. J. Appl. Phys., 2014, 53 010207.
[16] M. A. Ciolan, I. Motrescu, D. Luca, M. Nagatsu, Dig. J. Nanomater. Bios., 2014, 9, 483.