Design of Biointerface by Nonequilibrium Atmospheric Plasma Jets -Approach from Plasma Susceptible Polymers-

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1. Introduction

According to a progress in bio-related science and technology such as a high-technique medical therapy, medical treatments and diagnostics, numerous types of medical devices and tools have been developed. In order to give best performances on the bio-devices, surface of the devices must be precisely controlled. For example, surface of catheter must be not only lubricant but also non-biofouling to fluently control in blood vessel. Strong demands on non-biofouling surface are also available to bio-related labwares such as tubes, cuvettes, vials and dishes¹. Since versatile types of plastics are employed for bio-devices, it is not easy to modify consistently to improve biocompatibility of the surfaces. In order to increase biocompatibility of the versatile plastic surfaces, new simple methods must be developed.

Plasma surface modification has been employed to reform characteristics of plastic surface. For example, plasma treatments are applied to contact lens surface to improve both the lubricity and the biocompatibility². Plasma treatments are also applied to laminate films to

improve their adhesive tendency³. However, conventional plasma has several issues. One of the most important issues is that it can hardly control surface characteristics due to the extremely strong power and temperature of the plasma source. Most of plastic surfaces are damaged and certain numbers of surface polymer linkages were cleaved in addition to oxidization and other miscellaneous reactions. In addition, the conventional plasma must be utilized under high vacuum conditions^{4,5}.

We have recently been applying non-equilibrium atmospheric pressure plasmas (LF-plasma jet), which can be generated by low frequency (LF) pulsed high voltage (10 kV, 10KHz) to He flow⁶. In the system, plasma jets are generated with a single powered electrode and with or without a dielectric tube for He gas. Especially in the case with a dielectric tube, metastable helium plasma species can be generated without increase in surrounding gas temperature. Such dielectric barrier discharge plasma jets can induce chemical reactions of the solute or solution without any increase in the liquid temperature. We have recently published on plasma jet-supported preparation of

gold nanoparticles having extremely narrow size distribution along with highly dispersion characteristics⁷.

Since the LF-plasma jet gives mild and stable plasma sources mentioned above, it is applicable for surface modifications for new biointerface. In order to create consistent surface from versatile kinds of materials, especially plastic devices, new surface modification agent, which is susceptible for the LF-plasma jet must be designed. In this study, poly(chloromethylstyrene)-*r*-poly(2-methoxyethyl acrylate) random copolymers were prepared as an LF-plasma jet susceptible surface coating materials. The surface characterizations of the LF-plasma jet treated surface are also described.

2. Experiments

2.1 Chemicals: Chloromethylstyrene (CMS), which was kindly provided by Seimi Chemical Inc., and commercial 2-methoxyethyl acrylate (MEA) were distilled under reduced pressure. Dioxane was purified by conventional distillation.⁸ Azobisisobutyronitrile (AIBN) was crystallized from methanol. Other reagents were used as received.

2.2.Synthesis of poly(chloromethylstyrene)-*r*-poly(2-methoxyethyl methacrylate) random copolymer (PCM): To a dioxane (50mL) in 100 mL round bottomed flask equipped with three way stopcock, predetermined ratio of CMS and MEA (total 100 mmol) and AIBN (1mmol) were added under nitrogen atmosphere. The mixture was allowed to react at 65 °C. After the reaction, the solution was poured to large amount of methanol or hexane. The precipitate was obtained by decantation and dissolved in dioxane again. This purification was repeated three times to obtain pure polymers. The obtained polymers were finally freeze-dried with benzene to obtain white powder. Polymerization conditions and yields are summarized in Table 1.

Figure 1. Illustration of low frequency dielectric barrier discharged plasma jet apparatus

2.3 Low frequency dielectric barrier discharged plasma jet (LF-plasma jet): A schematic diagram of the plasma jet apparatus is shown in Figure 1. A glass tube was used to flow He gas. A Cu film with an electrically conductive adhesive tape was attached to the outside of

the tube as the high voltage electrode. Plasmas used in this study were generated by 10 kV with 10 kHz pulsed high voltage applied to the electrode with He gas flow of 40 L/min.

Table 1. Results of copolymerization of CMS with MEA^a

Run	Time H	CMS (%) ^b in		10 ⁻⁴ x Mn ^c	Mw/ Mn	Yield ^d %
		feed	polym			
1	2	0	0	3.0	2.1	78.4
2	2	5	9	3.0	1.7	45.8
3	2	10	27	3.1	1.6	17.5
4	5	20	34	2.5	1.4	43.7
5	5	30	45	1.8	1.7	42.4
6	5	70	80	2.4	1.6	28.4
7	12	100	100	1.9	1.6	57.9

^aPolymerization Temp.: 65°C; Solv. Dioxane.

^bNMR data, ^cSEC data, ^dGC data

2.4. Plasma treated surface modification: Toluene solution of PCM (5 wt%) was spin-coated on polypropylene (PP) surface (500 rpm for 5 s then 2,000 rpm for 20 s). LF-Plasma was irradiated to the obtained surface for certain period. The obtained surface was analyzed by contact angle and ζ -potential measurements.

2.5 Instruments: Size exclusion chromatography (SEC) measurements were carried out using a TOSOH HLC-8120 equipped with TSK gel columns (Super HZ4000 and Super HZ3000) and an internal refractive index (RI) and an ultraviolet (UV) detectors. THF with 5 % (v/v) triethylamine was used as eluent at a flow rate of 0.35 mL min⁻¹ at 40 °C. The ¹H NMR spectra were obtained using chloroform-d with a JEOL EX270 spectrometer at 270 MHz. Glass transition temperatures were obtained by differential scanning calorimeter (DSC; Seiko Instruments Inc. EXSTRAR6000 DSC/TG/ DTA). Contact angles of water on the prepared surfaces were measured using a contact angle analyzer (CA-X, Kyowa Interface Science Co., Ltd., Asaka, Japan) as follows: a liquid droplet was gently placed onto the surface, and contact angles were measured 10 s after placement by the $\theta/2$ method. Net surface charge of the surface was

Scheme 1. Poly(chloromethylstyrene)-*r*-poly(2-methoxyethyl acrylate) PCM

measured using a zeta potential analyzer (He-Ne laser, Zetasizer Nano ZS, Malvern, UK) at 25 °C.

3. Results and Discussion

3.1 Synthesis of poly(chloromethylstyrene)-*r*-poly(2-methoxyethyl acrylate) (PCM): Copolymerization reaction of CMS with MEA proceeded smoothly and no gel formation was observed. Figure 2 shows typical SEC and ^1H NMR spectrum of the obtained random copolymer (PCM(45)), where number in parenthesis denotes weight fraction of CMS in (%). The average molecular weights and the distributions of the obtained copolymers were $1.8 - 3.0 \times 10^4$ and $1.5 - 2.1$, respectively. From the NMR spectrum, signals based on both monomers are shown, confirming the random copolymerization. A glass transition temperature (T_g) of the obtained copolymers were determined by DSC. As can be seen in Figure 3, the T_g s increased proportionally with the increasing CMS contents in the copolymer. On the basis of these results, it is confirmed that PCM random copolymers shown in Scheme 1 were obtained.

3.2 Evaluation of plasma treated surface: Since versatile materials are utilized for labware, simple and consistent surface modification must be required. As shown in Figure 1, since the LF-plasma jet used in this study is not only simple structure but also easy-handling. If it works efficiently, it becomes unique surface modification technique. One of the most important points of this LF-plasma jet modification is to prepare surface modification agents, which possesses suitable chemical structure. Our idea was to incorporate chloromethyl groups as a side chain of the biocompatible copolymers. If the chloromethyl group reacts with LF-plasma

effectively, simple surface modification will be achieved. Polypropylene (PP) was used as a model plastic surface because it is one of the most stable hydrocarbon polymers. After PCM solution was spin-coated on the PP surface, the surface was irradiated by LF-plasma jet. Contact angles (CA) were measured to assess surface of the samples. Contrary to the CA of PP surface (ca. 100°), PCM(45) coated surface decreased its CA to ca. 80° as

Figure 3. Plots of glass transition temperature (T_g) vs. CMS contents in PCM copolymer

shown in Figure 4. When the PCM coated surface was soaked in solvent, however, the CA increased to 100°, indicating that the coated PCM was completely washed away. When the PCM surface was treated with the LF-plasma jet, the CA decreased significantly to 60°. The CA of the LF-plasma treated surface did not change after solvent washing. The fact means that the effective plasma-induced reaction took place not only in the coating but also between coating and substrate. Effect of LF-plasma treatments on the copolymer compositions was investigated using CA measurements as shown in Figure 5. The CA of the PCM coating surface gradually increased from 60° to 80° with the increasing CMS

contents due to the hydrophobicity of CMS moiety. On the contrary, the CA of the LF-plasma treated surface decreased with the increasing CMS contents, which means that chloromethyl group in CMS unit plays an important role for LF-plasma reaction. On the basis of these results, it is confirmed that PCM coated surface easily react with

Figure 2. Size exclusion chromatography (left) and ^1H NMR spectrum (right) of poly(chloromethylstyrene)-*r*-poly(2-methoxyethyl acrylate) (PCM(45))

LF-plasma to firmly fix to plastic substrate.

ζ -Potential measurements of the LF-plasma treated surface were also carried out. The ζ -potential of the PCM polymer showed ca. -20 to -30 mV regardless of the composition. The negative ζ -potential is probably due to the initiator fragment at the end of polymer chain. After the LF-plasma treatments, the ζ -potential decreased to become -35 to -55 mV. Increasing in negative charge is probably due to the carboxylic acids, which is generated by the plasma oxidation reaction.

Figure 4. Contact angles of substrate surfaces before (left) and after (right) solvent washing

The LF-plasma treated PCM surface showed extremely low protein adsorption character (data not shown). One of the other important characters of the LF-plasma treated PCM surface is to install other substance using remaining chloromethyl groups. Chloromethyl group is known to easily react with primary- or secondary-amino groups under suitable conditions. For example, when LF-plasma treated

Figure 5. Variation of contact angles of substrate surfaces with (gray) and without (dark) the LF-plasma treatment as a function of CMS content in PCM copolymers

surface was soaked in triethylenetetramine solution for 1d at room temperature, the ζ -potential increased to -10 mV. The cationic charge in triethylenetetramine increased the surface charge of the material. The obtained LF-plasma

treated surface is thus anticipated as high performance biointerface, possessing installation ability of functional substances.

4. Conclusions

For high performance biointerface, poly(chloromethylstyrene)-*r*-poly(2-methoxyethyl acrylate) (PCM) was synthesized by a radical copolymerization. The obtained PCM showed LF-plasma susceptibility, which was confirmed by both contact angle and ζ -potential measurements. Remaining chloromethyl groups in the LF-plasma treated PCM surface showed enough reactivity for installing amino containing substances, which can be anticipated as immobilization of biomolecules such as proteins and DNA because that they have amino groups. In addition, the LF-plasma treated PCM surface showed protein non-fouling character. The obtained LF-plasma treated surface is thus anticipated as high performance biointerface, possessing installation ability of functional substances.

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6. References

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