Plasma-Assisted Immobilization of Bio-Molecules on LDPE Surface

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Considerable interest has focused on the immobilization of functional molecules, such as DNA, enzyme and protein, onto the water-insoluble supports, which has been used as a bio-chip for μ-TAS (micro total analysis system). Especially the development of “DNA chips”, on which many kinds of oligo-DNA are fixed, has revolutionized the fields of genomics and bioinformatics.1,2 This technology promises to monitor the whole genome on a single chip so that researchers can have a better understanding of the interactions among thousands of genes simultaneously. However, current biochips leave several points to be refined. One is the question of chip reusability.3 All current biochips are disposable and lack of reusability, in part because the current devices are not physically robust. For example, nucleic acid probes tend to break away from a supporting glass plate.

We have recently reported a novel method to introduce a durable surface wettability and minimize its decay with time on several hydrophobic polymers (polyethylene-naphthalate, polyethylene, nylon-12).4,5 This method involves

Fig. 1 Immobilization of bio-molecules onto hydrophobic polymer surface.

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Scheme 1  Immobilization of DNA on polymer surface by using DCC and DMTMM

a sorption of vinylmethylether-maleic anhydride copolymer (VEMA), which is commercially known as a GANTREZ, into the surface layer and the immobilization by plasma-assisted cross-link reaction, followed by hydrolysis of VEMA to generate hydrophilic carboxylic groups (VEMAC) on the surface. The durable surface wettability thus introduced has been confirmed by not only the measurement of water contact angle but also demonstration of long term stability of the surface lubricity on the urethane-made catheter. It is expected, therefore, that the reusable bio-chip can be fabricated by the bond formation of biomolecules with the carboxyl group on the surface prepared by this method. (Fig. 1)

In this communication, we report an extended work on the plasma-assisted immobilization of oligo-DNA on low-density polyethylene (LDPE) surface. LDPE was chosen for an initial study as one of the attractive polymers for DNA and enzyme immobilization because of its low cost, chemical and mechanical properties, and ready availability in a various forms (e.g. film, beads, nets, tubes, sheets, etc.).

A LDPE-VEMAC sheet, which is of a durable wettability, was prepared according to the previous method.6,7 LDPE sheet (10mm × 30mm) was soaked in cyclohexanone solution containing 2% VEMA for 24h at 75°C to deposit VEMA into LDPE surface layer, and dried. Then, the LDPE sheet was soaked in THF for 10s to remove the excess VEMA. After drying, the LDPE thus treated was submitted to Ar plasma-irradiation to immobilize the VEMA onto LDPE surface layer. After plasma-irradiation, the hydrolysis of maleic anhydride linkage in VEMA was conducted by immersing the LDPE sheet in 1N NaOH aqueous solution. The hydrolyzed LDPE-VEMAC sheet was soaked in dil. HCl at room temperature, washed with water fully and dried.

For a procedure to immobilize an oligo-DNA on the surface of LDPE-VEMAC sheet, it is considered that 5'-aminolinker oligo-DNA, which possesses an aminohexyl group as a 5'-terminal group of DNA and is commercially available, is allowed to react with the carboxylic group on the surface of LDPE-VEMAC sheet. The n-butylamine as a model compound for a preliminary experiment was immobilized onto the surface of LDPE-VEMAC sheet using two kinds of condensation reagents, dicyclohexylcarbodiimide (DCC)8,9 and 4-(4,6-di-methoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride(DMTMM).10,11 (Scheme 1)

Figure 2 shows the change of contact angle of LDPE-VEMAC sheet surface treated with n-butylamine. It is seen that the contact angle values of LDPE-VEMAC sheet were about 30°, and those treated with n-butylamine increased to a considerable extent. In the reaction using DMTMM, the contact angle value of the LDPE-VEMAC sheet
Fig. 3 FTIR-ATR spectra of (A) LDPE sheet, (B) LDPE-VEMAC sheet and (C) LDPE-VEMAC sheet reacted with n-butylamine.

Fig. 4 The scan image of the fluorescence intensity for a set of probes designed on LDPE-VEMAC-DNA sheet.
(A): LDPE-VEMAC-DNA, (B): hybridization with PM, (C): hybridization with MM.

increased up to about 60°, more effectively than that in the reaction using DCC. The more increase of the contact angle value using DMTMM as a condensation reagent suggests that n-butylamine was immobilized more effectively onto LDPE-VEMAC sheet.

In order to confirm the formation of amide bond, FTIR-ATR spectrum of LDPE-VEMAC sheet treated with n-butylamine using DMTMM was measured. Figure 3 shows representative FTIR-ATR spectra of LDPE sheet (A), LDPE-VEMAC sheet (B) and LDPE-VEMAC sheet treated with n-butylamine (C). It is clearly seen that the characteristic peaks of carboxyl group were observed at 1719 and 1626 cm\(^{-1}\) in the spectrum (B), and that the characteristic peaks of amide group of 1649 and 1552 cm\(^{-1}\) were observed in the spectrum (C). The result also suggests that n-butylamine was immobilized onto the surface of LDPE-VEMAC sheet.

Fig. 5 Change of contact angle on LDPE-VEMAC surface by washing with hot water (90 °C) for 5 min.

The immobilization of 5'-aminolinker-(dT)\(_n\) on the surface of LDPE-VEMAC sheet was carried out with DMTMM to obtain the LDPE-VEMAC-DNA sheet. Two kinds of oligo-DNAs labeled Cyanine-5, 5'-Cy5-(dA)\(_n\) (PM) and 5'-Cy5-(dT)(dA)\(_3\) (MM), were used for hybridization. The 0.1nmol/\(\mu\)L solution of oligo-DNAs labeled Cyanine-5 was dropped on the LDPE-VEMAC-DNA sheet and the sheet was kept at the room temperature for the 12-16 h. The LDPE-VEMAC-DNA sheet thus treated was washed with water 3 times and dried. Then the sheet was allowed to observe the fluorescence intensity with a confocal laser microscope.

Figure 4 is the confocal laser microscope images of LDPE-VEMAC-DNA sheet on which PM or MM was hybridized. The fluorescence was not observed in the LDPE-VEMAC-DNA sheet. (Fig. 4(A)) but was observed in the sheets hybridized with PM and MM (Fig. 4(B) and (C)) being much more intense in the sheet hybridized with PM than that with MM. The result shows that the present LDPE-VEMAC-DNA sheet can detect the complementary oligo-DNA.

It is generally known that the DNA chip is washed with hot water (90°C) for 5 min to remove bounded target DNA from probe. To examine the thermal stability of LDPE-VEMAC sheet, the contact angle of the sheet was measured after washing with hot water at 90°C for 5 min. It is seen from Fig. 5 that the contact angle value of LDPE-VEMAC sheet increased exponentially as the number of washing increased, and has been kept at the level nearly as low as the initial value up to 7 times washing. The result indicated that the VEMAC has been immobilized strongly enough to be reusable several times under the present conditions.
To confirm the reusability of LDPE-VEMAC-DNA sheet, we performed the hybridization and dehybridization of PM on this sheet repeatedly. The dehydration of PM was carried out by washing with hot water at 90°C for 5 min. Figure 6 shows the confocal laser microscope images of LDPE-VEMAC-DNA sheet for reusability test. The fluorescence was clearly observed by the hybridization of PM, and most of the fluorescence disappeared by the treatment of dehybridization as shown in Fig. 6(A) and (B). On repetition of the hybridization and dehybridization procedure conducted on the same sheet, the fluorescence intensity remained nearly unchanged, being close to the level initially acquired up to 7 times under the present conditions (Fig. 6(C)–(E)), although it was weakened steeply afterward (Fig. 6(F)). The present result suggests that the LDPE-VEMAC-DNA sheet can be reusable at least several times.

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