Pharmaceutical and Biomedical Engineering by Plasma Techniques

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The nature of plasma-induced surface radicals formed on a variety of organic polymers have been studied by electron spin resonance (ESR), making it possible to provide a sound basis for future experimental design of polymer surface processing using plasma treatment. On the basis of the findings from such studies, several novel bio-applications in the field of drug- and biomedical-engineering have been developed. Applications for drug engineering include the preparation of reservoir-type drug delivery system (DDS) of sustained- and delayed-release, and floating drug delivery system (FDDS) possessing gastric retention capabilities, followed by preparation of “Patient-Tailored DDS”. Furthermore, the preparation of composite powders applicable to matrix-type DDS was developed by making a mechanical application to the surface radical-containing polymer powders with drug powders. In applications for biomedical engineering, the novel method to introduce the durable surface hydrophilicity and lubricity on hydrophobic biomedical polymers was developed by plasma-assisted immobilization of carboxyl group-containing polymer on the polymer substrate. The surfaces thus prepared were further used for the covalent immobilization of oligo-nucleotides (DNA) onto the polymer surfaces applicable to constructing DNA diagnosis system, and also plasma-assisted preparation of functionalized chemo-embolic agent of vinyl alcohol-sodium acrylate copolymer (PVA-PAANa).

Key words: plasma treatment; electron spin resonance (ESR); surface radical; drug delivery system (DDS); composite powder; biocompatible surface; biomolecular immobilization; chemo-embolic agent.

1. Introduction
The cold plasma is most characterized by a low gas temperature and a high electron temperature, and easily generated by electric discharges under reduced pressure. The field of plasma chemistry deals with occurrence of chemical reactions in the cold plasma.

The cold plasmas are being utilized for ever-increasing number of applications. These techniques are environmental friendly process owing to dry chemistry, thus, they do not produce contaminated aqueous wasted water. One of the characteristics by plasma treatment is the fact that it is surface limited (ca. 500-1000 Å) so that only the surface properties can be changed without affecting the bulk properties.

It is known that cold plasma of inert gas emits intense UV and/or VUV ray to cause an effective...
energy transfer to solid surface and gives rise to a large amount of stable free radicals on the polymer surface. In view of the fact that surface reactions of plasma treatment are initiated by plasma-induced radicals, study of the resulting radicals is of utmost importance for understanding the nature of plasma treatment. However, the detailed studies of such plasma-induced surface radicals had not been worked out. Thus, we have undertaken plasma-irradiation of a wide variety of polymers, synthetic and natural, and the surface radicals formed were studied in detail by electron spin resonance (ESR) coupled with the aid of systematic computer simulations. On the basis of the findings from a series of such studies, we were able to open up novel plasma-assisted bio-application works.

In the present article, our novel bio-application works especially in the field of pharmaceutical engineering by plasma techniques are described, which include: (1) For the field of drug engineering, preparations of double-compressed (DC) tablets applicable to reservoir-type DDS of sustained and time-controlled release, the development of intragastric FDDS for oral controlled-release dosage forms possessing gastric retention capabilities, and all these devices leading to novel “Patient-Tailored DDS” administered under consideration of individual variation of the environment (pH and transit time, etc.) in gastrointestinal (GI) tract, and functionalized composite powders applicable to matrix-type DDS by mechanical applications of plasma-irradiated polymer powder. Figure 1 shows conceptual illustration for the preparation of these devices. (2)

For the field of the biomedical engineering, introduction of durable hydrophilicity and lubricity onto hydrophobic biomedical polymer for improvement of the surface biocompatibility, and the immobilization of bio-molecules on the surface thus prepared for fabrication of the biomedical devices such as bio-chips, as well as brief overviews of ESR studies on plasma-induced surface radicals of several organic polymers relevant to the present study.

2. Nature of Plasma-Induced Polymer Radicals [1-17]
Plasma induced surface radicals permit reactions for surface modification in several different ways such as CASEING (cross-linking by activated species of inert gas), surface graft and/or block copolymerization, and incorporation of functional groups. All these techniques are referred to as plasma techniques.

In order to elucidate the nature of plasma surface treatment, we have been working on the structural identifications of plasma-induced surface radicals of various kinds of organic polymers as studied by electron spin resonance (ESR) spectra coupled with the systematic computer simulations. One of the advantages of plasma-irradiation over other types of radiations for the study of the polymer radicals is that the radical formation can be achieved with a brief plasma-duration by a simple experimental apparatus such as those we have devised. The experimental setup for the plasma-irradiation and ESR spectral measurement is schematically shown in Fig. 2. This method makes it possible not only to study the polymer radicals without a significant change of polymer morphology but also to follow readily the ESR kinetics for the radical formation, so that we can carry out systematic computer simulations with a higher credibility.
Fig. 2. Schematic representation for plasma irradiation and ESR spectral measurement.

Figure 3 shows the observed ESR spectra of plasma-induced surface radicals formed on several selected polymers relevant to the present study, together with the corresponding simulated spectra shown as dotted lines. Based on the systematic computer simulations, all the observed spectra in addition to those shown here were deconvoluted and the component radial structures have been identified.

Based on a series of this work, we were able to establish the general relationship between the structure of radicals formed and the polymer structural features. Crosslinkable polymers give the mid-chain alkyl radical as a major component radical, while degradable polymers give the end-chain alkyl radical as a major component radical, and if polymers are of branched structure or contain the aromatic ring, the cross-link reactions occur preferentially on these moieties. And, one of the common features is that dangling-bond sites (DBS) is more or less formed in all plasma-irradiated polymers resulted from occurrence of CASING.

Since all plasma-irradiated polymers are eventually exposed to air for their practical use, we have to understand the nature of oxygenation, i.e. the auto-oxidation.

Figure 4 shows a reaction scheme for the formation of proxy radical and its ensuing process (hydroperoxide, alkoxyl radicals formation) demonstrating how auto-oxidation ends up with introduction of oxygen-containing functional groups such as hydroxyl groups, carboxyl groups and so on, and dissipation of the surface radical formed.

Therefore, we have studied the nature of peroxy radical formation as an initial process of auto-oxidation.

Fig. 3. Room temperature ESR spectra of plasma-induced radicals in organic polymers, together with the simulated spectra shown as dotted lines. Plasma conditions: 40W, Ar 0.5 Torr, 3 min.


Fig. 4. Peroxy radical formation from carbon-centered radical with molecular oxygen and its reaction, resulted in introduction of oxygen functional groups on polymer surface

Figure 5 shows several examples of ESR spectra of peroxy radicals formed immediately after exposure of the plasma-irradiated polymers to air, which correspond to those shown in the previous Fig. 3, as well as the simulated spectra as shown in dotted lines. [14] It can be seen that in some polymers, the spectral pattern remained unchanged with only lowering the intensity, and in other polymers, the spectra have been completely converted to the one exhibiting a typical spectral pattern of peroxy radical.

Fig. 5. Room Temperature ESR Spectra of Various Plasma-Irradiated Polymers after Exposure to Air
Note that, in most polymers, such an intensity of peroxy radicals usually decreases to less than 30-40% of the original carbon-centered radicals even immediately after exposure to air, except for polytetrafluoroethylene (PTFE), which can be best discussed on its comparison with that of high density polyethylene (HDPE) to understand the nature of auto-oxidation in more detail.

As shown in Figure 6, exposure of plasma-irradiated PE to air at room temperature did not give the ESR spectra of peroxy radicals, but the ESR spectra did show only the decrease in the spectral intensity. On the other hand, the peroxy radicals of PTFE are extremely stable for a long period of time at room temperature. The spectral intensity, therefore, is nearly the same as that of the original radicals. [2]

The extraordinary instability of PE peroxy radical can be ascribed to the rapid chain termination reaction through the hydroperoxide consuming several moles of molecular oxygen, due to the presence of abundant hydrogen atoms bonded to sp³ carbons in PE, while the exceptional stability of PTFE peroxy radicals can be attributed to the absence of any abstractable hydrogen in PTFE to undergo the chain termination reactions. Because of occurrence of this type of oxygenation reaction, plasma treatment by inert gas plasmolysis has a tendency to result in the introduction of surface wettability in many polymers. It should be noted, however, that such a fact does not hamper our development of DDS preparation as demonstrated in drug release tests, which will be described in the following sections.

For the most suitable therapy, development of controlled-release systems for drug delivery is one of the most active areas today in the entire field of drug research. A wide variety of approaches of controlled-release DDS have been thus far investigated for oral application. Oral drug delivery is the most desirable and preferred method of administrating therapeutic agents for their systematic effects such as convenience in administration, cost-effective manufacturing, and high patient compliance compared with several other routes. We have developed plasma-assisted preparation of multi-layered tablets applicable to oral DDS. Figure 7 illustrates the schematic representation for preparation of double-compressed (DC) tablets and drug dissolution test including the experimental setup for plasma-irradiation on the tablets.

Fig. 6. Difference in Free Radical Reactivity with Oxygen between HDPE and PTFE

3. Pharmaceutical Engineering for DDS preparation by Plasma Techniques [18-37]

3-1. Preparation of Sustained-Release DDS from Plasma-Irradiated DC Tablets [21-27]

The development of new active pharmaceutical ingredient (API) is often hampered or even blocked due to side effects of these new APIs. Some of the severe side effects may be caused by the early and high peak blood plasma concentration of APIs just after oral-administration. This problem can be overcome by altering the blood plasma concentration profile so that a more gradual absorption rate is obtained. In that case, sustained-release DDS that drug is slowly released over a prolonged period of time is an ideal therapeutic system.

When oxygen plasma was irradiated to the
outermost layer of the DC tablet, which consists of a drug as a core material and a mixture of plasma-crosslinkable and plasma-degradable polymer powders as a wall materials, plasma degradable polymers could be selectively eliminated and simultaneously the crosslinkable polymer undergoes the rapid cross-link reaction to result in the formation of the porous outer layer of the tablet. As a result, the drugs could be released from the tablet through the resulting micropore [22-27].

Figure 8 shows the effect of oxygen plasma duration on theophylline release from the DC tablet as the representative example of the release test. As shown in Fig. 8A, when a mixed powder of polystyrene (PS) and polyoxymethylene (POM) for the outer layer is used, it is seen that the release rate of theophylline increases as plasma duration increases, while the blank tablet did not exhibit any appreciable release of theophylline even with longer dissolution time [21]. Thus, the release profile of theophylline from DC tablet can readily be controlled by the selection of plasma operational tunings. Based on the fact that the value of weight loss shown in parentheses increases as the plasma duration increases, it is apparent that plasma degradable polymer, POM, could be selectively eliminated by oxygen plasma-irradiation, while plasma-crosslinkable PS undergoes the cross-link reaction, to result in the formation of the porous outer layer of the tablet (Figure 9). Then, theophylline could be released from the tablet through the resulting micropore evidenced by the scanning electron micrographs (SEM) pictures.

![Fig. 8. Effect of oxygen plasma-irradiation on theophylline release from DC tablet. The values shown in parentheses denote the weight loss of the tablets after plasma-irradiation. Core tablet: 100mg (Theophylline). For (A) Outer layer : 80mg (PS:POM=1:1), Plasma conditions : Power: 50W, Pressure: 0.5 Torr, O₂ 50ml/min.
For (B) Outer layer: 80mg (PLA:POM=1:3, 1:1, 3:1), Plasma conditions : Duration: 2h, Power: 6W, Pressure: 0.5 Torr, O2 50ml/min.]

Similar work has included the preparation of the controlled release tablet by using bioerodible polylactic acid (PLA) in place of PS. The sustained release tablet was similarly obtained based on the theophylline release test as shown in Fig. 8B. Furthermore, DC tablet containing an insulin-PLA matrix tablet as a core material was prepared and the changes in blood glucose levels after the subcutaneous implantation of the DC tablet in diabetic rats was examined (Fig.10) [23]. As the result, normal blood glucose levels were maintained for 10 days in the plasma-irradiated DC tablet and the release rate of insulin in the steady state from the plasma-irradiated DC tablet was 5 IU/h which was calculated from the data from 4 to 34 h. These results indicated that DC tablet consisting of PLA and POM as the outer layer can be applied to an implantable dosage form in the subcutaneous tissue.

![Fig. 9. Scanning electron micrograph (SEM) of DC tablet using PS/POM (1:1) as outer layer before and after plasma irradiation. Plasma conditions: 50 W, O₂ 0.5 Torr, 50 mL/min.]

![Fig. 10. Change in blood glucose levels with time after the subcutaneous implantation of the DC tablet in rats. Outer layer: a mixed powder of PLA 10000 and POM (3/1), Core tablet: a mixed powder of PLAS5000 and insulin (1/1). Plasma conditions: 6 W, O₂ 0.5 Torr, 50 mL/min, 3h.]

When a water-soluble polymer is used as a wall material of the DC tablet, the drug release rate...
from the tablet would be dependent on the solubility of the polymer used. In fact, the rapid release from a DC tablet containing theophylline with the water soluble polymer, polymethacrylic acid (PMAA) or polyacrylamide (PAAm), used for a wall material was suppressed by argon plasma-induced cross-link reactions and changed into the slow release with a sigmoid release pattern due to decrease in the solubility of water-soluble polymers [26].

3-2. Preparation of Time-Controlled Drug Release System by Plasma Techniques [28-31]

Today, the therapy based on the factor of biorhythmic time is becoming more and more important in the progress toward an aging society in many countries, in addition to customary controlled-release systems. Time-controlled release system has a function of timer, so that main technical point for the development of this system is how to control a lag time and a drug release after lag time.

It is well known that methacrylic-acrylic acid copolymers including their derivatives with various combinations and composition ratios of the monomers have been used as pharmaceutical aids for enteric coating agents commercially known as a series of Eudragits. These Eudragit polymers turn to be water-soluble in a certain specific pH solution, and they show a different dissolution rate. The structures and the dissoluble pH values of several Eudragit polymers are shown in Fig. 11.

![Fig. 11. Structures and dissoluble pH values of several commercial Eudragit polymers used for enteric coating agents.](image)

Since plasma-crosslinkable acrylic monomers are one of the component polymers in Eudragits L100-55, argon plasma-irradiation would lead to the suppression of Eudragit L100-55 solubility even in a dissoluble pH-value solution (pH>5.5) due to the occurrence of the surface cross-link reactions. Thus, when Eudragit L100-55 is used as a wall material of the DC tablet, the initial drug release could be completely sustained for a certain period of time.

With this expectation in mind, we have undertaken argon plasma-irradiation to examine the possibility of a rapid-release DC tablet of Eudragit L100-55, being converted into a delayed-release tablet, i.e. the time-controlled DDS.

Figures 12 and 13 show the effect of argon plasma irradiation on the theophylline release profiles in pH 6.5 buffer solution and the SEM pictures of the surface of Eudragit L100-55 tablet before and after argon plasma-irradiation, respectively. It is seen that the Eudragit L100-55 tablets plasma-irradiated for 3 min and 5 min have shown to produce prolongation of lag-time for theophylline release.

The SEM pictures demonstrated that the tablet surface with 5 min-irradiation has converted into the rather smooth surface with clogging the crack presenting at particle-particle interfaces by softening of Eudragit L100-55, and into the porous outer layer with 10 min irradiation. It is considered that the porous layer was formed not only by the effect of plasma irradiation but also by physical actions such as evolved gas scattering accompanied by softening of the Eudragit L100-55 due to the plasma heat fusion.

![Fig. 12. Effect of plasma duration on Theophylline release from plasma-irradiated double-compressed tablets of Eudragit L100-55 in pH 6.5 buffer solution. Plasma conditions: 50W, Ar 0.5Torr, 50mL/min.](image)
3-3. Preparation of Intragastric FDDS by Plasma Techniques [32-34]

Intragastric FDDS has been noted as orally applicable systems for the prolongation of the gastric emptying time [35,36]. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment. It has applications also for local drug delivery to the stomach and proximal small intestines. Gastro retention helps to provide better availability of new products with new therapeutic possibilities and substantial benefits for patients.

In the course of our study on plasma-assisted DDS preparation, we found that carbon dioxide was trapped in the tablet when argon plasma was irradiated onto the surface of DC tablet composed of plasma-crosslinkable polymers possessing carboxyl group as an outer layer. It was considered that such tablets could be applicable to FDDS.

In fact, we have obtained the intragastric FDDS by plasma-irradiation when the DC tablet was prepared using the outer layer so as to trap evolved carbon dioxide. Figure 14 shows the floating property on the simulated gastric fluid and the release property of 5-fluorouracil (5-FU) from argon pulsed plasma-irradiated DC tablet using a mixture composed of a 68/17/15 weight ratio of Povidone, Eudragit L100-55 and NaHCO₃ as an outer layer. As shown in Fig. 15, the plasma heat flux caused the thermal decomposition of NaHCO₃ to generate carbon dioxide and resultant gases were trapped in bulk phase of outer layer, so that the tablets turned to have a lower density than the gastric contents and remained buoyant in simulated gastric fluid for a prolonged period of time. In addition, the release of 5-FU from the tablet is sustained by occurrence of plasma-induced crosslink reaction on the outer layer of tablet and the release rate of 5-FU can be well controlled by plasma operational conditions (Fig. 16) [34].

**Fig. 13.** SEM pictures of Eudragit L100-55 tablet before and after plasma irradiation. Plasma conditions: 50 W, Ar 0.5 Torr, 50 mL/min.

**Fig. 14.** Photos of DC tablet for FDDS before and after plasma irradiation.

**Fig. 15.** Effect of plasma irradiation on the specific gravity of DC tablet (A) and SEM pictures of cross-section of DC tablet before and after plasma-irradiation. Outer layer: a mixed powder of Povidone, Eudragit L100-55 and NaHCO₃ (68/17/15), Core tablet: 5-fluorouracil. Plasma condition: 20Hz pulse frequency (on/off cycle = 35ms/15ms), 100 W, Ar 0.5 Torr, 50ml/min.
3-4. Patient-Tailored DDS for Large Intestine Targeted-Release Preparations [37]

With most of today's oral DDS devices, it is difficult for all patients to obtain the expected therapeutic effects of drugs administered, because of the individual difference in the environment such as pH value and the transit time in gastrointestinal (GI) tract, which causes the slippage of time-related and positional timing of drug release. From a viewpoint of the real optimization of drug therapy, in order to fulfill the specific requirements on drug release at the appropriate sites in GI tract, the “Patient-Tailored DDS” (Tailor-Made DDS) should be administered based on the diagnosis of each patient's GI environment, which can be obtained by direct monitoring using a diagnostic system of the pH sensitive radio telemetry capsule, so called “pH-chip”.

We have fabricated an experimental setup for the simulated GI tract for large intestine targeting, the dissolution test solution being changed in pH value corresponding to stomach (pH 1.2), small intestine (pH 7.4) and large intestine (pH 6.8), and examined the drug release test of plasma-irradiated double compressed tablet in the simulated GI tract.

Figure 17 has shown the preliminary result of theophylline dissolution test in pH 6.8 test solution on the DC tablets using a mixture of Eudragits L100-55/ RSPO (7: 3) as outer layer. It is seen that the lag-time has increased with the extension of plasma irradiation time. The lag-time has not been largely affected by treatment in pH 1.2 and pH 7.4 test solutions, which indicated the possibility for the development of the “Patient-Tailored DDS” targeting the large intestine such as colon. We are now elaborating these initial studies aiming at more rapid drug release right after the drug preparations reached the prescribed pH value of the large intestine due to contents of semi-solid nature in large intestine.

3-5. Preparation of Functionalized Composite Powders Applicable to Matrix-Type DDS [38]

The recombination of solid-state radicals is significantly suppressed due to the restriction of their mobilities, unlike radicals in the liquid or gas phase. Interactions between radicals at solid-solid interfaces do not occur under a normal condition [8].

We have reported the occurrence of mechanically induced surface radical recombination of plasma-irradiated polymers. As shown in Fig. 18, plasma-irradiated polyethylene (PE) powder, low-density polyethylene (LDPE) and high-density polyethylene (HDPE), was applied to mechanical vibration in a Teflon twin-shell blender for the prescribed period of time at room temperature under strictly anaerobic conditions, and submitted to ESR measurement.
plasma-irradiated PE powders, being proportional to the spin number of the surface radicals, due to trapping theophylline powder into the PE matrix [38]. It should be noted here that the theophylline release is further retarded from the tablet prepared by compressing the above composite PE powders.

Fig. 18. Schematic representation for mechanical vibration and ESR measurement.

As shown in Fig. 19, the spectral intensity gradually decreased, with change of the spectral pattern for the case of LDPE, as the duration of mechanical vibration increased. This clearly indicated that plasma-induced surface radicals of PE underwent effectively the solid-state radical recombination in intra- and inter-particle fashion on its mechanical vibration, since the spectral intensity did not appreciably decrease on standing at room temperature, so long as it is kept under anaerobic conditions.

Fig. 19. Progressive changes in observed ESR spectra of 10 min plasma-irradiated LDPE and HDPE powders on mechanical vibration (60 Hz) in Teflon twin-shell blender, together with the simulated spectra shown as dotted lines.

Plasma conditions : 40W, Ar 0.5 Torr, 10 min.

For the matrix-type DDS preparation, the mechanical vibration of plasma-irradiated PE powder was carried out in the presence of theophylline powder so as to immobilize the theophylline powder into PE matrix formed by inter-particle linkage of PE powder. Examples of the theophylline release from the resulting composite powders of LDPE and HDPE are shown in Fig. 20. It is seen that the theophylline release is apparently suppressed from each of the composites with the vibration.

Fig. 20. Theophylline release profiles from the composite powder composed of Theophylline and Ar plasma-irradiated polyethylenes, LDPE and HDPE. LDPE plasma-irradiated for 60s: 0.5 x 1018 spin/g, for 180 s: 1.0 x1018 spin/g. HDPE plasma-irradiated for 60s: 1.0 x 1018 spin/g.

Plasma conditions : 40W, Ar 0.5 Torr, 1 min.

4. Biomedical Engineering by Plasma Techniques

The wettability of polymer surface is an important characteristics relating to the biocompatibility for biomaterials. It is known, however, that the wettability introduced by plasma treatment decays with time after treatment. The mechanism has been ascribed to several reasons such as the overturn of hydrophilic groups into the bulk phase for crosslinkable polymers, and detachment of the hydrophilic lower-molecular weight species from the surface for degradable polymers. We have reported a novel method to introduce a durable surface wettability and minimize its decay with time on several hydrophobic polymers (poly-ethylene-naphthalate (PEN), low-density polyethylene (LDPE), Nylon-12 and polystyrene (PS)) [39-42]. The method involves a sorption of vinylmethylether-maleic anhydride copolymer (VEMA) into the surface layer and its immobilization by plasma-induced cross-link reaction, followed by hydrolysis of maleic anhydride linkage in VEMA to generate durable hydrophilic carboxyl groups (VEMAC) on the surface (Fig. 21). The present method was applied to preparation of functionalized polyurethane-made catheter with durable surface lubricity.
4-1. Preparation of Clinical Catheter with Durable Surface Lubricity

One of the most important requirements of clinical catheters is the durability of the surface lubricity to diminish the patient pain in use. Figure 22 shows the representative data of measurement of surface slipperiness as a function of the number of repeated rubbing of the treated catheter against silicon rubber. [39]

In can be seen that the resistance of the catheter containing VEMA without Ar plasma-irradiation and of the commercial catheter starts to gradually increase after moving the catheter back and forth around 20-30 number of times in both cases, while that of catheter containing VEMA Ar plasma irradiated for 30 s and 60 s remained low up to around 130-150 number of times. Prolonged plasma irradiation such as for 300 s and 600 s duration, however, did show very poor durability of slipperiness, probably due to the formation of too highly crosslinked surface. Thus, the result shows clearly much higher functionality in terms of durability of surface lubricity.

4-2. Improvement of Cell Adhesion by Immobilizing VEMAC on Polymer Surface

In most types of cell, the adhesion to some substrates is a key primary process for the developments such as proliferation, survival, migration and differentiation. Polystyrene (PS) has been commonly used in a substrate for the in vitro cell culture due to excellent durability, low production cost, optical transparency in visible range and non-toxicity. However, PS must be subjected to a surface treatment for biomedical use because it is a very hydrophobic polymer.

In order to improve the cell adhesion properties of PS dish, VEMAC was immobilized on the surface using essentially the same method shown in Figure 21. Figure 23 shows the microscopic images of LNCap cells adhered on the VEMAC-immobilized PS dish (PS/VEMAC) after 6h in culture. As shown in Figure 23, a distinct difference in cell attachment and spreading of LNCap cells between on PS/VEMAC and on non-treated PS dish was observed. The PS/VEMAC surface showed much better adhesion and spreading properties, while the adhered cells were not observed on non-treated PS surface. This result indicates that the PS/VEMAC surfaces prepared by the present method have preferential culturing properties of LNCap cells.

4-3. Plasma-Assisted Immobilization of Biomolecules onto Polymer Surfaces

Considerable interest has focused on the immobilization of several important classes of bio-molecules such as DNA, enzyme and protein, onto the water-insoluble supports. The development of DNA chips on which many kinds of oligo-DNA are immobilized, for example, has revolutionized the fields of genomics and...
bio-infomatics [43]. However, all the current biochips are disposable and lack of reusability, in part because the devices are not physically robust [44].

The method shown in Fig. 21 has further been extended to application for the covalent immobilization of single-stranded oligo-DNA onto VEMAC-immobilized LDPE (LDPE/VEMAC) sheet by the reaction of 5'-aminolinker oligo-DNA with a condensation reagent. [45, 46] The 5'-aminolinker oligo-DNA, which possesses an aminohexyl group as a 5'-terminal group of DNA is considered to be able to react with the carboxyl group on the surface of LDPE/VEMAC sheet. In fact, the resulting DNA-immobilized LDPE/VEMAC sheet was able to detect several complementary oligo-DNAs by effective hybridization.

To examine the reusability of DNA-immobilized LDPE/VEMAC sheet, we have repeatedly conducted the hybridization and dehybridization of fluorescence-labeled complementary oligo-DNA on the same DNA-immobilized LDPE/VEMAC sheet, according to the general procedure to remove bounded target DNA from the chip (washing with hot water (90 °C) for 5min). Figure 24 shows the result of reusability test based on the confocal laser microscope images of DNA-immobilized LDPE/VEMAC sheet. It can be seen that the fluorescence is observed nearly at the same level of intensity even after the several times repetition of the hybridization and dehybridization. The result indicated that the DNA-immobilized LDPE/VEMAC sheet obtained by the present method would be reusable.

Fig. 24. Scan image of the fluorescence intensity of LDPE-VEMAC-DNA sheet for reusability test . (A); Hybridization of complementary oligo-DNA, (B); After hot water rinse of sheet (A) for 5min. Rehybridization of complementary oligo-DNA on the same sheet (C); 2 times, (D); 5 times, (E); 7 times, (F); 8 times.

Furthermore, we used the LDPE/VEMAC surface for immobilization of enzyme [47, 48]. When the enzyme was immobilized covalently on solid surface, as is well known, the decrease in the enzyme activity has been commonly observed due to modifications in the tertiary structure of the catalytic sites. For the successful immobilization of enzymes on polymer substrate with retaining the activity, in this study, we prepared polyglycidylmethacrylate (pGMA) brushes on the LDPE/VEMAC sheet by atom transfer radical polymerization (ATRP) of GMA via carboxyl groups on the sheet. In the ATRP process, the polymerization degree of a monomer can be well-controlled and the resultant polymer has a narrow molecular weight distribution [49]. Figure 25 shows the reaction scheme for the functionalization of LDPE/VEMAC surface. The epoxy group of pGMA can react readily and irreversibly with nucleophilic groups like –NH₂ under mild conditions. In fact, we succeeded in the covalent immobilization of fibrinolytic enzyme, urokinase, as a model enzyme through the direct coupling with epoxy groups of GMA on the surface thus prepared. Table 1 shows the relative surface concentration of immobilized urokinase and its activity. As can be seen in Table 1, the relative surface concentration of immobilized urokinase increased with the polymerization time for the fabrication of pGMA brushes. On the other hand, the activity of immobilized urokinase also increased in the pGMA-grafted LDPE sheet prepared by ATRP up to 2 h but it then leveled off under the present experimental conditions. Therefore, the ratio of active urokinase on pGMA-grafted LDPE sheet decreased with the increase in polymerization time. These results indicate that the LDPE surface with high enzymatic activity can be obtained by controlling the structure of interfaces between the enzyme and the substrate using the present method.

Fig. 25. Reaction scheme for fabrication of pGMA brushes on LDPE sheet by ATRP
Table 1  The amount of immobilized urokinase and its activity on LDPE sheet

<table>
<thead>
<tr>
<th>Sample sheet</th>
<th>Immobilized UK (µg/cm²) (a)</th>
<th>Activity (IU/cm²) (b)</th>
<th>Ratio of active urokinase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pGMA grafted LDPE (ATRP for 2 h)</td>
<td>0.44 ± 0.08</td>
<td>35.66 ± 2.77</td>
<td>101.3</td>
</tr>
<tr>
<td>pGMA grafted LDPE (ATRP for 4 h)</td>
<td>2.05 ± 0.08</td>
<td>31.34 ± 1.86</td>
<td>19.1</td>
</tr>
<tr>
<td>pGMA grafted LDPE (ATRP for 6 h)</td>
<td>4.53 ± 0.15</td>
<td>32.96 ± 4.63</td>
<td>9.1</td>
</tr>
</tbody>
</table>

(a) The amount of immobilized urokinase on the pGMA-g-LDPE sheet was determined by Bradford dye binding assay using bovine gamma globulin as the standard. (b) Activity of immobilized urokinase (IU/cm²) was assayed using Glu-Gly-L-Arg-MCA as the substrate.

4.4. Basic Study on Preparation of Functionalized PVA-PAANa Hydrogel for Chemo-embolic Agent

Vinyl alcohol-sodium acrylate copolymer (PVA-PAANa) is well known as non-crosslinked hydrogel (water absorbent polymer) due to the intense hydrogen bonding network among the hydroxyl groups of PVA moiety. The PVA-PAANa microsphere (ca.100µm) swells ca. 3.5 times in diameter larger than its original size in human serum within a few minutes and can pass through a microcatheter. Recently, its microsphere has been applied to the chemo-embolic agent used for Transcatheter Arterial Embolization (TAE) in clinical trials on patients [50-53]. The PVA-PAANa microsphere is shape-adjustable in nature according to the surrounding blood pressure because of the non-crosslinked structure, so that it has been shown to occlude the blood vessel much more effectively than any other conventional embolic agent such as gelatin sponge and lipiodol.

In order to seek for the possibility of further functionalization of PVA-PAANa such as a capability of controlling the ratio and rate of swelling by plasma processing, we have carried out argon plasma-irradiation onto the PVA-PAANa microsphere and the surface radicals formed were studied by ESR on its comparison with those of vinyl alcohol-acrylic acid copolymer (PVA-PAA) as well as its respective component homopolymer, PVA, PAA and its sodium salt (PAANa) [54]. In fact, it was found that the ESR spectra have shown the vast difference in pattern between PVA-PAANa and PVA-PAA, demonstrating the strong sodium salt effect on the nature of plasma-induced surface radical formation (Fig. 26). The systematic computer simulations of the ESR spectra revealed that the major spectral component was the radicals derived from PVA site for PVA-PAANa and the ones from PAA site for PVA-PAA. The SEM pictures indicated that the observed site-selectivity for the surface radical formation has been derived from the difference in the surface morphology between PVA-PAANa and PVA-PAA (Fig. 27).

PVA-PAANa forms the microphase separation structure with the condensed domain of PAANa site so as to reduce the effective surface area for the surface radical formation. The present result provides a basis for the future experimental design for giving an additional performance to PVA-PAANa, including the sustained drug-release function at the occluding site.

Fig. 26. Progressive changes in observed ESR spectra of plasma-irradiated PVA-PAANa and PVA-PAA together with the simulated spectra shown as dotted lines.

Fig. 27. SEM pictures of PVA-PAANa and PVA-PAA, and the conceptual illustration of microphase separation structure of PVA-PAANa.

5. Conclusion

The present results have clearly shown that one can prepare a variety of desired DDS devices, if one selects the tailored-polymers for wall materials
of double-compressed tablets as well as plasma operational conditions. And the method of plasma-assisted DDS preparations contains several advantages; 1) totally dry process, 2) polymer surface modification without affecting the bulk properties, 3) avoidance of direct plasma-exposure to drugs and 4) versatile control of drug release rates. Thus, it is hoped that more practical applications will be developed in the course of attempt now in progress. It should be noted, however, that we have restricted to use the organic polymers so as to manipulate the existing pharmaceutical aids licensed for practically patient use, since the pharmaceutical aids containing lower than 0.1% level impurity can be used without the structural identification, in accordance with the harmonized tripartite guideline in Japan, USA and EU. Otherwise it is very cost- and time-consuming to obtain the license for manufacturing new drug and quasi-drug substances, unlike the approval of industrial substances.

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