5

Cold Plasma Techniques for Pharmaceutical and Biomedical Engineering

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1. Introduction

Plasmas can be defined as the state of ionized gas consisting of positively and negatively charged ions, free electrons and activated neutral species (excited and radical), and are generally classified into two types, thermal (or equilibrium) plasma and cold (or non-equilibrium) plasma, based on the difference in characteristics.

The thermal plasma is the state of fully ionized gas characterized by a high gas temperature and an approximate equality between the gas and electron temperature ($Tg \approx Te$) and can be generated under atmospheric pressure. The energetic of this plasma is very high enough to break any chemical bond, so that this type of plasma can be excluded from most of organic chemistry, let alone from the field of pahramceutical science.

In contrast, the cold plasma is most characterized by a low gas temperature and a high electron temperature ($Tg << Te$), and easily generated by electric discharges under reduced pressure. The field of plasma chemistry deals with occurrence of chemical reactions in the cold plasma including atmosphere pressure glow discharge plasma.

One of the characteristics of surface treatment by cold plasma irradiation 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.

In recent years, biomedical applications of cold plasma are rapidly growing due to the fact that the use of cold plasmas is very useful to treat heat-sensitive objects such as polymeric materials and biological samples. The demonstrations of plasma technology in the biomedical field have created a new field at the intersection of plasma science and technology with biology and medicine, called "Plasma Medicine". (Fridman et al., 2008)

When the cold plasma is irradiated onto polymeric materials, the 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 such plasma-induced radicals, study of the resulting radicals is of utmost importance for understanding of the nature of plasma treatment. 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 application works. (Kuzuya et al., 2001a, 2005, 2009)
This contribution focuses on our plasma techniques for pharmaceutical and biomedical engineering on the basis of findings from a series of studies on plasma-induced surface reactions in variety of polymers. For the pharmaceutical engineering field, the controlled drug release technology by using plasma-induced cross-linking and/or degradation of polymer was developed for the preparation of rate- and time-controlled drug release tablet. Furthermore, this technique was used to develop the advanced DDS such as gastric floating drug delivery system (FDDS) possessing gastric retention capabilities and patient-tailored DDS for large intestine-specific drug delivery. For the biomedical engineering fields, the durable surface hydrophilicity and lubricity on hydrophobic biomedical polymers were fabricated by plasma-assisted immobilization of carboxyl group-containing polymer onto the surface. The surfaces thus prepared were further used for the covalent immobilization of biomolecules for developing biomedical devices such as cell culture substrate, biosensing system and blood-compatible material.

2. Nature of plasma-induced polymer radicals

Plasma induced radicals on polymer surface permit reactions for surface modification in several different ways such as CASING (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. However, research has essentially been phenomenological, and detailed studies of such plasma-induced surface radicals of polymer have not been reported.
Over the years, 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. (Kuzuya et al., 1991a-c, 1992a-c, 1993ab, 1994, 1995, 1996a, 1997a, 1998ab, 1999ab) 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. 1. 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.

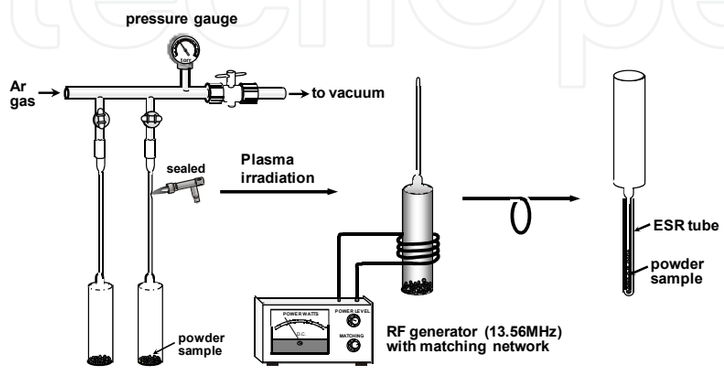

Fig. 1. Schematic representation for plasma irradiation and ESR spectral measurement

Figure 2 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 radical structures have been identified.

From 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.

All kinds of plasma-irradiated polymers are eventually exposed to air for their practical use, so the studies of the auto-oxidation process are also important for plasma-irradiated polymer. Figure 3 shows a reaction scheme for the formation of peroxy 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.

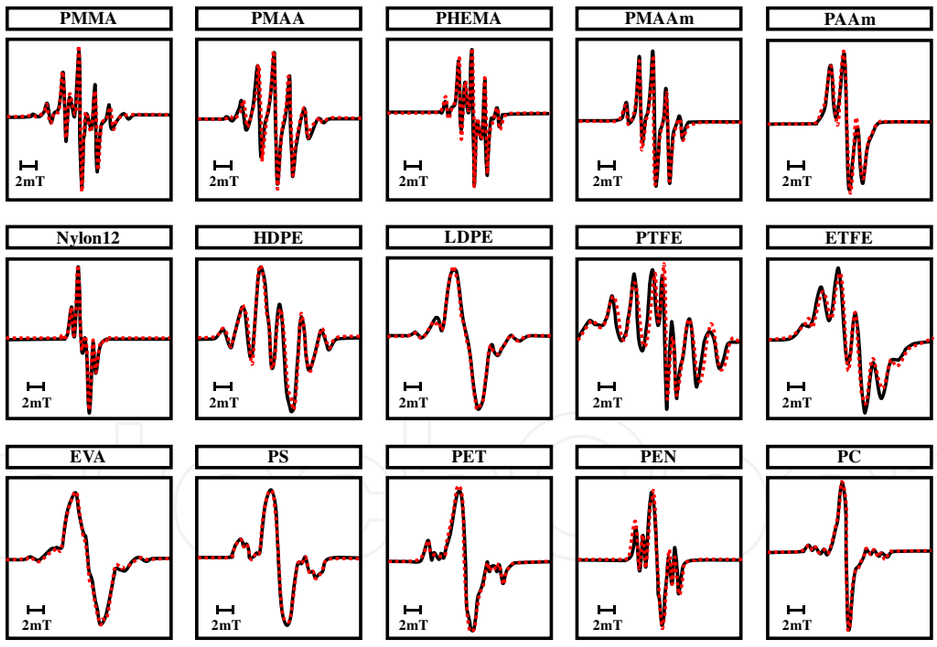

Fig. 2. Room temperature ESR spectra of plasma-induced radicals in organic polymers, together with the simulated spectra shown as dotted lines. Plasma conditions: 40W, Ar 0.5 Torr, 3 min. PMMA: polymethylmethacrylate, PMAA: polymethacrylic acid, PHEMA: poly-(2-hydroxyethyl) methacrylate, PMAAm: polymethacrylamide, PAAm: polyacrylamide, HDPE: high density polyethylene, LDPE: low density polyethylene, PTFE: polytetrafluoroethylene, ETFE: (ethylene-tetrafluoroethylene) copolymer, EVA: (ethylene-vinylacetate) copolymer, PS: polystyrene, PET: polyethyleneterephtalate, PEN: polyethylenenaphthalate, PC: polycarbonate

Figure 4 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. 2, as well as the simulated spectra as shown in dotted lines. 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.

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.

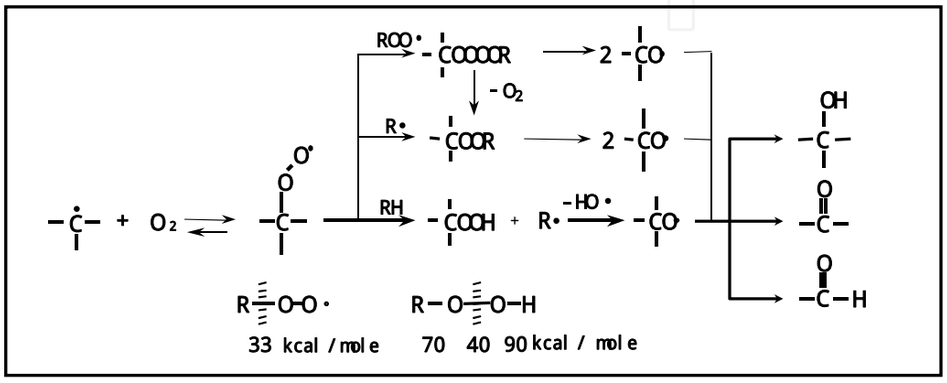

Fig. 3. Peroxy radical formation from carbon-centered radical with molecular oxygen and its reaction, resulted in introduction of oxygen functional groups on polymer surface

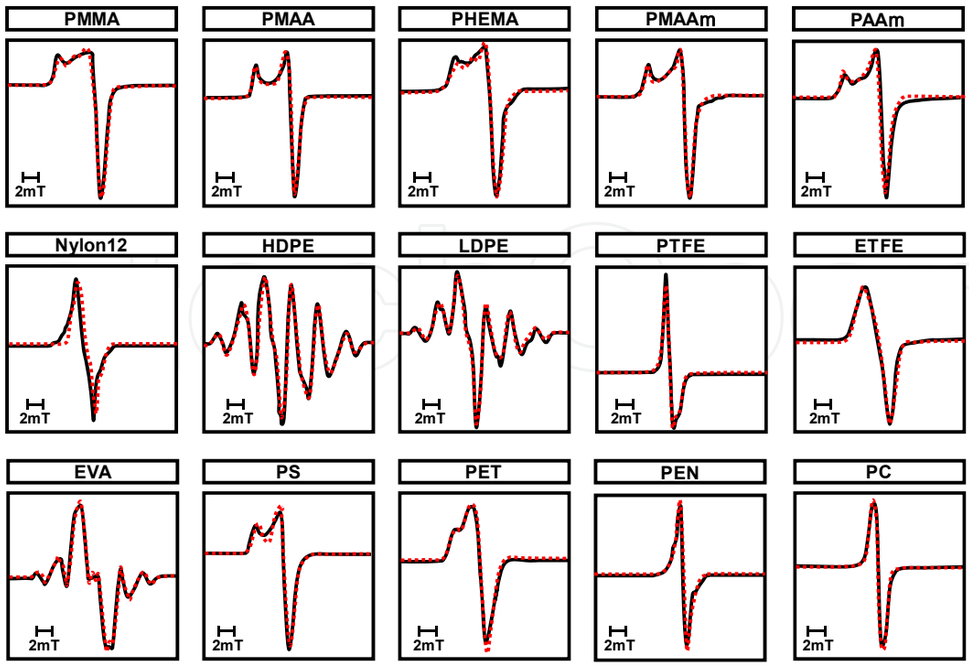

Fig. 4. Room temperature ESR spectra of various plasma-irradiated polymers after exposure to air

As shown in Fig. 5, exposure of plasma-irradiated HDPE 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. The extraordinary instability of HDPE 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 sp^3 carbons in HDPE. 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. 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.

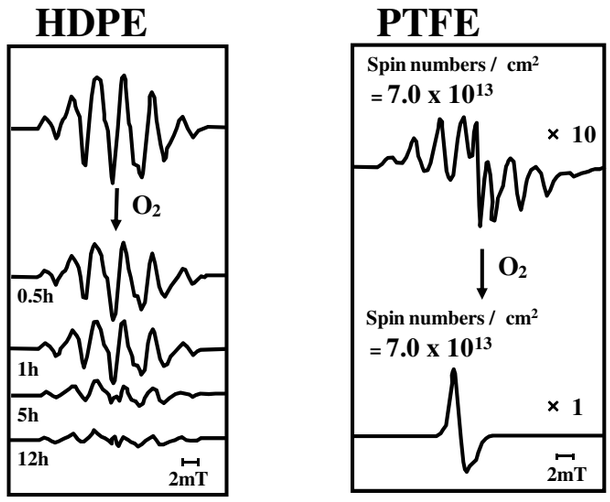

Fig. 5. Difference in free radical reactivity with oxygen between HDPE and PTFE

3. DDS preparation by plasma techniques

A drug delivery system (DDS) is a formulation or device that safely brings a therapeutic agent to a specific body site at a certain rate to achieve concentration at the site of drug action. Development of more "patient-friendly" DDS improves drug efficiency and patient compliance. 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.

As an application of plasma techniques to DDS technologies, the encapsulation of drug particle by plasma-polymerized thin film was reported. (Susut & Timmons, 2005). In this case, however, the drug molecule is exposed by plasma to cause the undesirable degradation of drug molecules. On the other hand, we have developed plasma-assisted preparation of multi-layered tablets (Fig.6). In this method, plasma is irradiated on the outermost layer of double-compressed (DC) tablets so that the direct exposure of plasma to

drug molecules in the core table can be avoided. Figures 7 and 8 illustrate the schematic representation for preparation of DC tablet and the experimental setup for plasma-irradiation on the tablets, respectively.

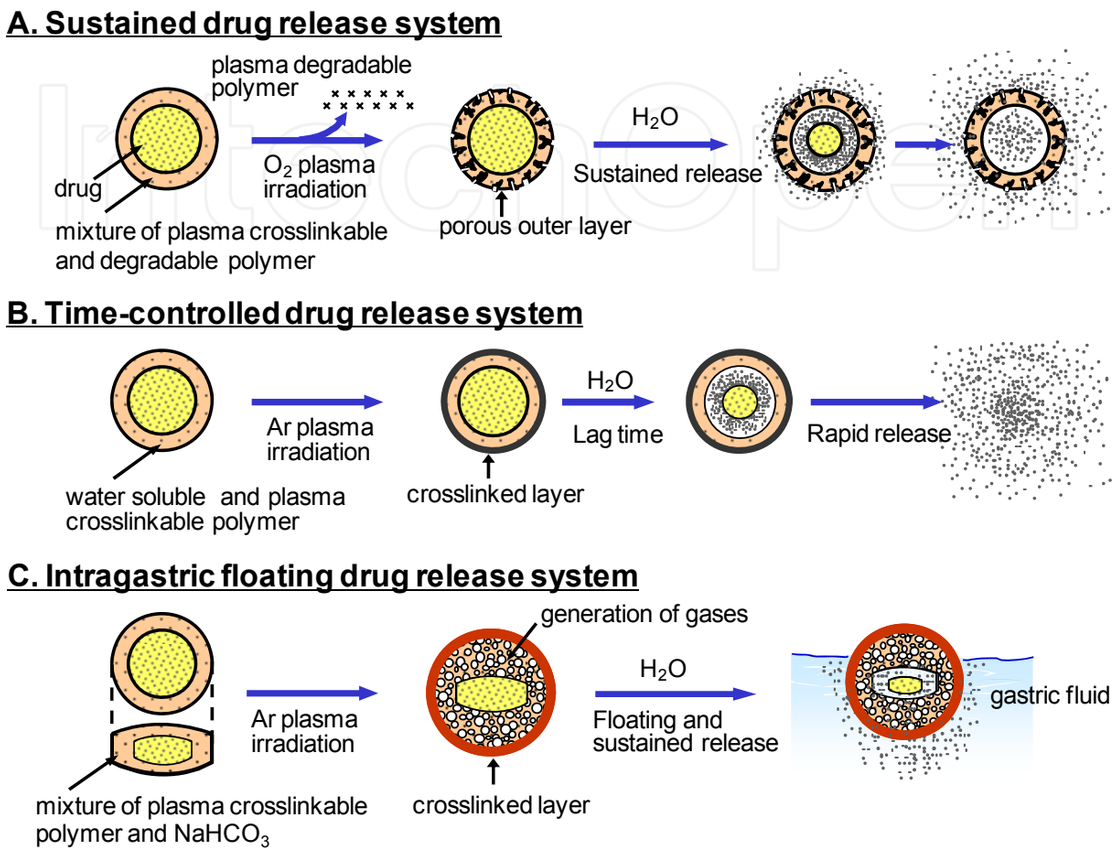

Fig. 6. Conceptual illustration for preparation of DDS for controlled drug release by plasma techniques

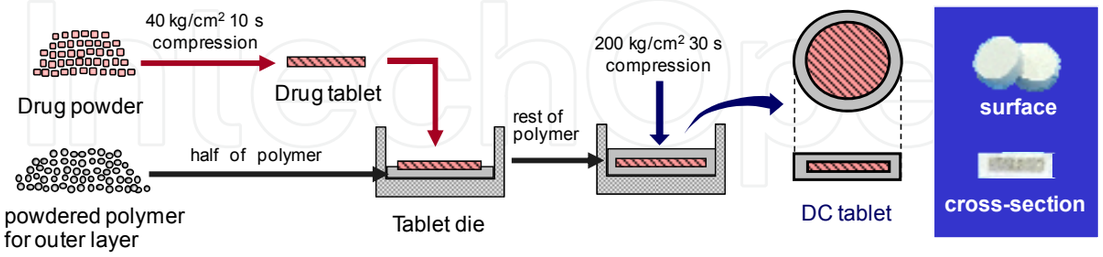

Fig. 7. Schematics for preparation of double-compressed tablets

3.1 Preparation of sustained drug release system by plasma techniques

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.

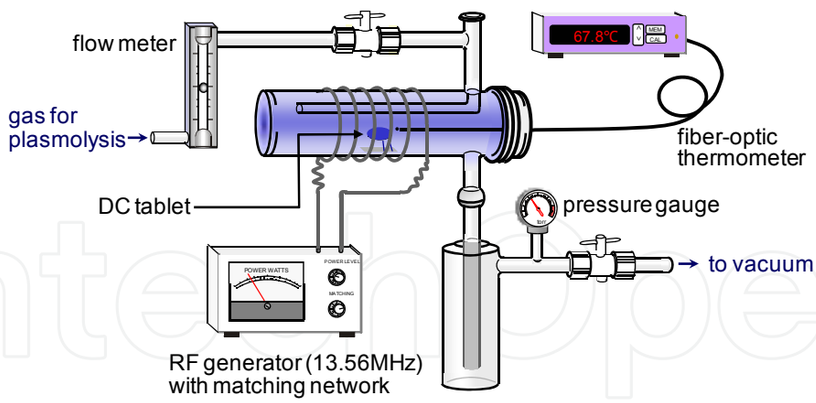

Fig. 8. Experimental setup for plasma-irradiation on DC tablet

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. (Fig. 6A) (Kuzuya et al, 1991de, 1996b; Ishikawa et al., 1993, 1995, 1996; Yamakawa et al., 1993)

Figure 9 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. 9, when a mixed powder of plasma crosslinkable polymer, polystyrene (PS), and plasma degradable polymer, 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. (Kuzuya et al. 1991d) 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 (Fig. 10). Then, theophylline could be released from the tablet through the resulting micropore evidenced by the scanning electron micrographs (SEM) pictures.

Similar work has included the preparation of the inplantable controlled release tablet by using bioerodible polylactic acid (PLA) in place of PS. 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. 11).

(Yamakawa et al., 1993) The 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 a long-acting implantable dosage form in the subcutaneous tissue.

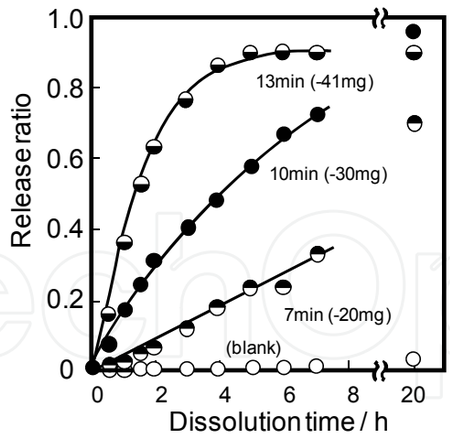

Fig. 9. Effect of oxygen plasma-irradiation on theophylline release from DC tablet (A). The values shown in parentheses denote the weight loss of the tablets after plasma-irradiation. Core tablet: 100mg (Theophylline). Outer layer : 80mg (PS: POM=1:1). Plasma conditions : Power: 50W, Pressure: 0.5 Torr, O_2 50ml/min

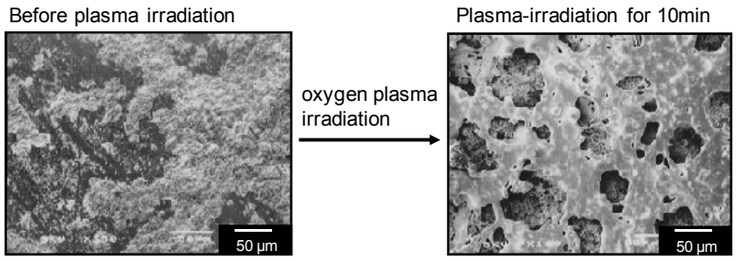

Fig. 10. Scanning electron micrograph (SEM) of DC tablet using PS/POM (1:1) as outer layer before and after oxygen plasma irradiation

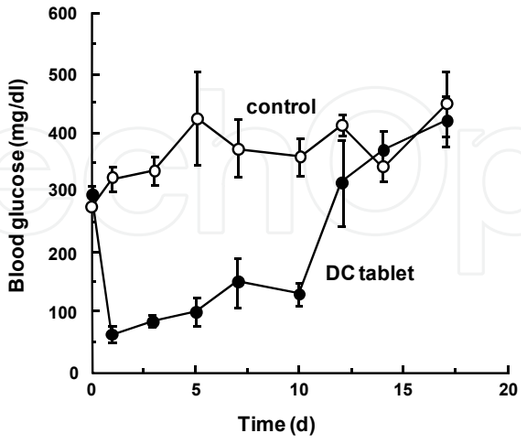

Fig. 11. Change in blood glucose levels with time after the subcutaneous implantation of the DC tablet in rats. Outer layer: a mixed powder of PLA and POM (3/1), Core tablet: a mixed powder of PLA and insulin (1/1).

Plasma conditions: 6 W, O_2 0.5 Torr, 50 mL/min, 3h

3.2 Preparation of time-controlled drug release system by plasma techniques

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. Thus, the time-controlled drug release system has been noted as orally applicable DDS that is useful for the drug delivery to the specific site of gastrointesrinal tract.

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. 12.

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. (Kuzuya et al., 2001b) (Fig. 6B)

Figures 13 and 14 show the effect of argon plasma irradiation on 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.

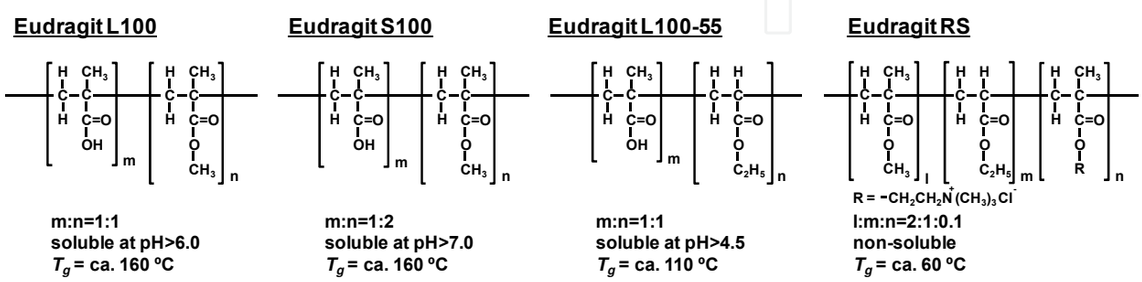

Fig. 12. Structures and dissoluble pH values of several commercial Eudragit polymers used for enteric coating agents

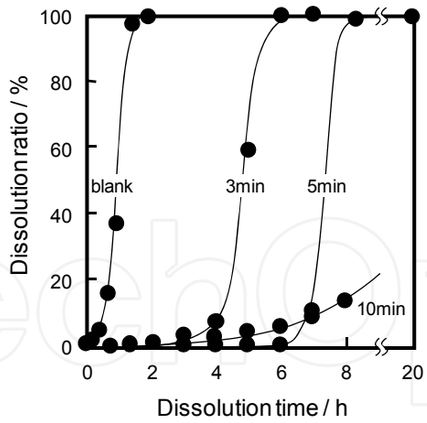

Fig. 13. Effect of plasma duration on Theophylline release from plasma-irradiated DC tablets of Eudragit L100-55 in pH 6.5 buffer solution

Plasma conditions: 50W, Ar 0.5Torr, 50mL/min.

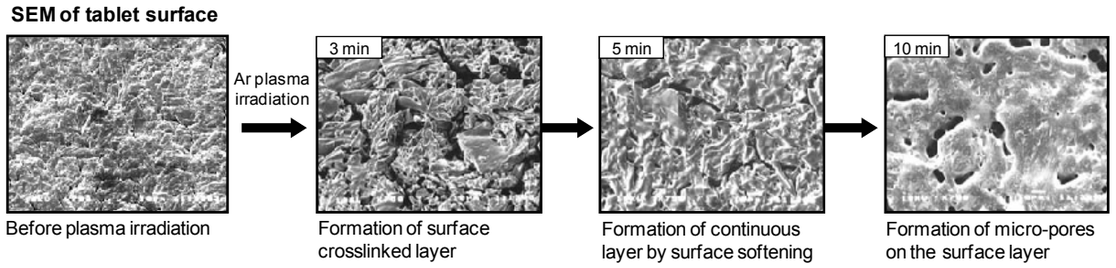

Fig. 14. SEM pictures of Eudragit L100-55 tablet before and after plasma irradiation

Plasma conditions: 50 W, Ar 0.5 Torr, 50 mL/min.

3.3 Preparation of intragastric FDDS by plasma techniques

Intragastric FDDS has been noted as orally applicable systems for the prolongation of the gastric emptying time (GET). (Singh & Kim, 2000; Streubel et al., 2006). The bulk density of FDDS is lower than that of gastric fluids and thus it remains buoyant on stomach contents for a long time in the drug releasing process. Prolonged gastric retention improves bioavailability, reduces drug waste, and improves solubility for drugs that are less soluble in a high pH environment like intestinal environment. It has applications also for local drug delivery to the stomach and proximal small intestines.

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. Based on such findings, 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. (Fig. 6C) (Kuzuya et al., 2002a; Kondo et al., 2004; Nakagawa et al., 2006) Figures 15-17 show the floating property of the DC tablet 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 $NaHCO_3$ as an outer layer. As shown in Fig. 16, the plasma heat flux caused the thermal decomposition of

$NaHCO_3$ 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. 17). (Nakagawa et al., 2006)

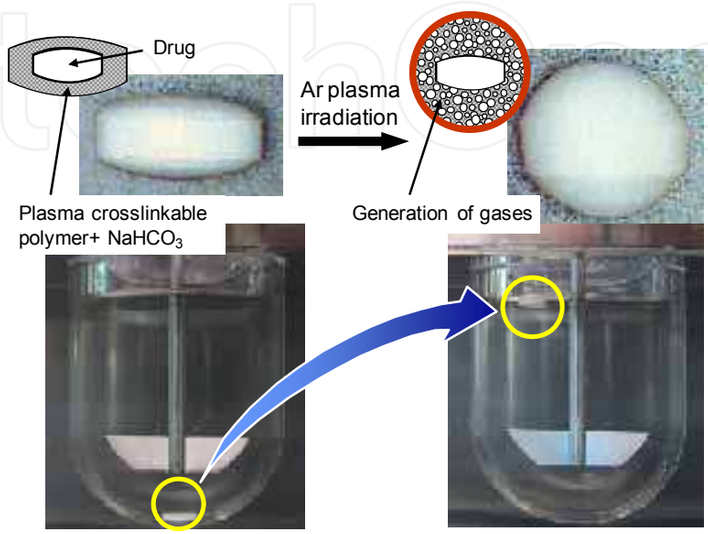

Fig. 15. Photos of DC tablet for FDDS before and after plasma irradiation

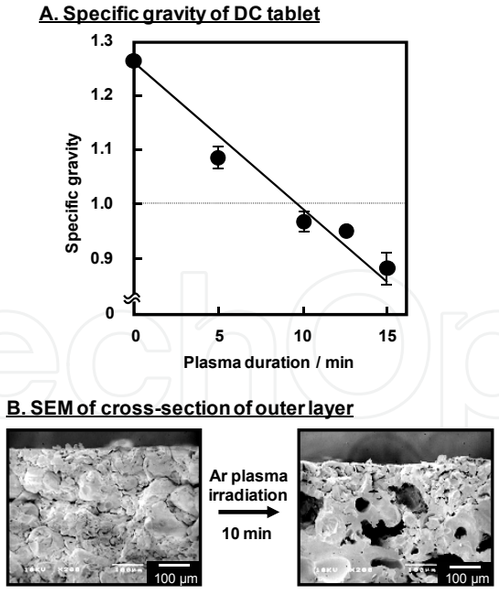

Fig. 16. Effect of plasma irradiation on the specific gravity of DC tablet (A) and SEM pictures of cross-section of DC tablet before and after plasma-irradiation. Outer layer: a mixed powder of Povidone, Eudragit L100-55 and $NaHCO_3$ (68/17/15), Core tablet: 5-fluorouracil

Plasma conditions: 20Hz pulse frequency (on/off cycle = 35ms/15ms), 100 W, Ar 0.5 Torr, 50ml/min.

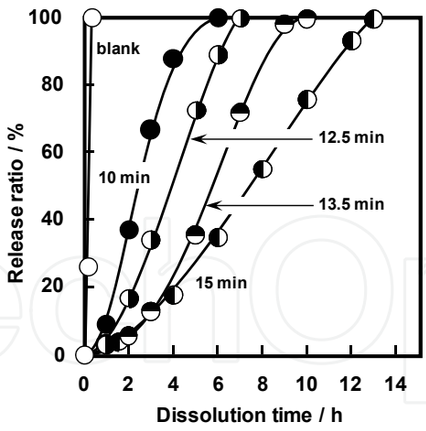

Fig. 17. Effect of pulsed plasma duration on drug release from plasma-irradiated DC tablet. Outer layer: a mixed powder of Povidone, Eudragit L100-55 and $NaHCO_3$ (68/17/15), Core tablet: 5-fluorouracil

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 targeting

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.

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 18 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. (Sasai et al., 2004) 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

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.

We have reported the occurrence of mechanically induced surface radical recombination of plasma-irradiated polymers. (Kuzuya et al., 1996a) As shown in Fig. 19, plasma-irradiated

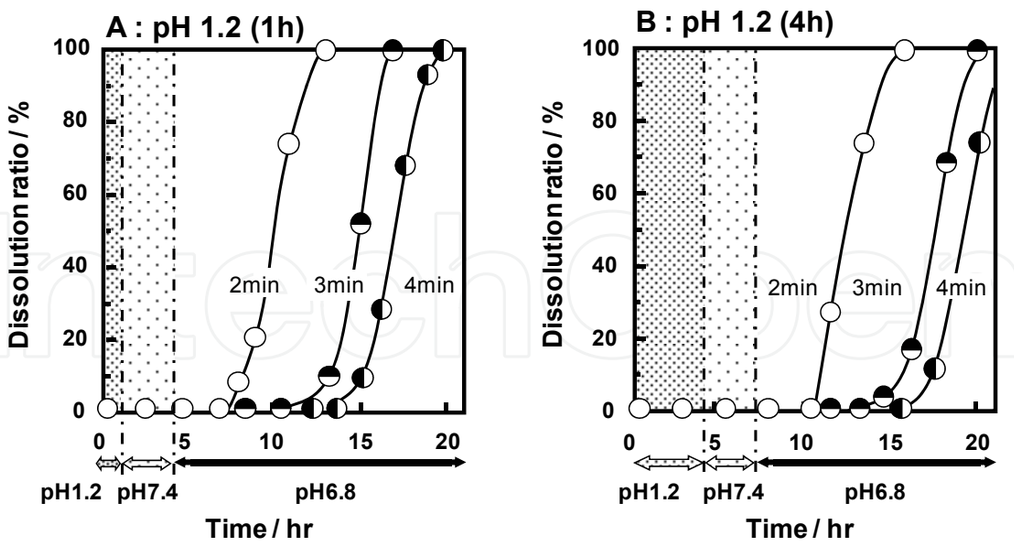

Fig. 18. Release property of theophylline from helium plasma-irradiated DC tablet in the GI tract-simulated dissolution test. (A) for 1h in pH 1.2; (B) for 4h in pH 1.2. Outer layer: a mixed powder of Eudragit L100-55 and Eudragit RS (7/3), Core tablet: theophylline

Plasma conditions: 30W, He 0.5 Torr, 50mL/min.

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.

As shown in Fig. 20, 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.

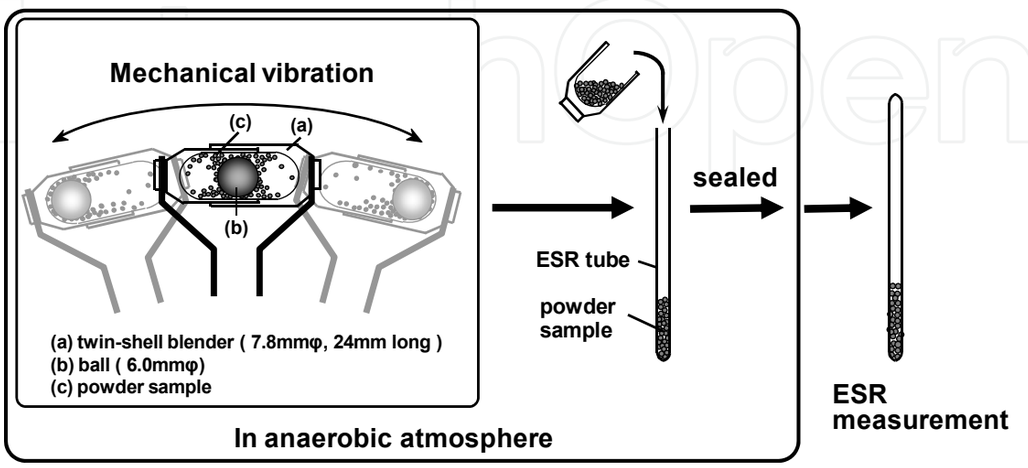

Fig. 19. Schematic representation for mechanical vibration and ESR measurement

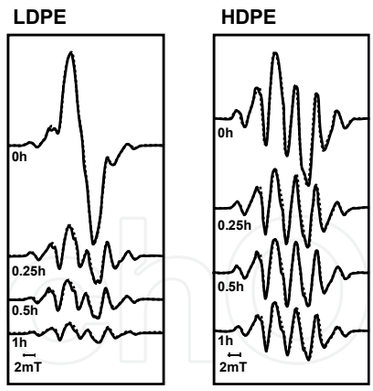

Fig. 20. Progressive changes in observed ESR spectra of 10 min plasma-irradiated LDPE and HDPE powders on mechanical vibration (60 Hz) in Teflon twin-shell blender, together with the simulated spectra shown as dotted lines

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. Figure 21 shows the conceptual illustration for matrix-type DDS preparation using plasma irradiated polymer powder. Examples of the theophylline release from the resulting composite powders of LDPE and HDPE are shown in Fig. 22. It is seen that the theophylline

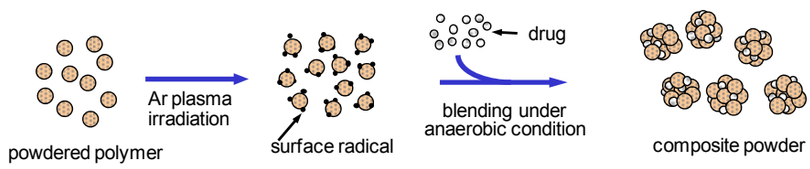

Fig. 21. Conceptual illustration for matrix-DDS for sustained drug release

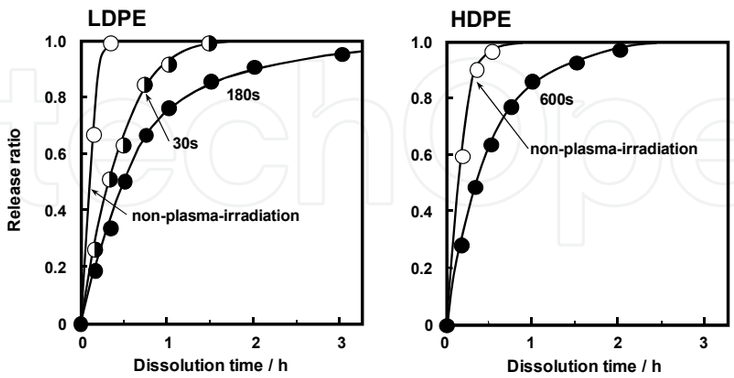

Fig. 22. Theophylline release profiles from the composite powder composed of Theophylline and Ar plasma- irradiated PE, LDPE and HDPE

LDPE plasma-irradiated for 60s: 0.5 x 10^{18} spin/g, for 180 s: 1.0 x10^{18} spin/g.
HDPE plasma-irradiated for 60s: 1.0 x 10^{18} spin/g.
Plasma conditions : 40W, Ar 0.5 Torr, 1 min.

release is apparently suppressed from each of plasma-irradiated PE powders, being proportional to the spin number of the surface radicals, due to trapping theophylline powder into the PE matrix. (Kuzuya et al., 2002b) It should be noted here that the theophylline release is further retarded from the tablet prepared by compressing the above composite PE powders.

4. Biomedical engineering by plasma techniques

Various polymers are extensively used in biomedical applications. However, most of polymers commonly used in industrial field do not always possess surface properties required/desired for biomaterials. Cold plasma irradiation has been widely used for surface treatment of biomaterials.

The wettability of polymer surface is an important characteristics relating to the biocompatibility of biomaterials. Plasma surface treatment is an effective method for hydrophilization of polymer surface. 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 (polyethylene-naphthalate (PEN), low-density polyethylene (LDPE), Nylon-12 and polystyrene (PS)). (Kuzuya et al., 1997b, 2001c, 2003; Sasai et al., 2008) 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 on the surface (Fig. 23). The surfaces thus prepared have been further applied to the substrate for covalent immobilization of biomolecules, fabrication of blood-compatible material and cell culture substrate.

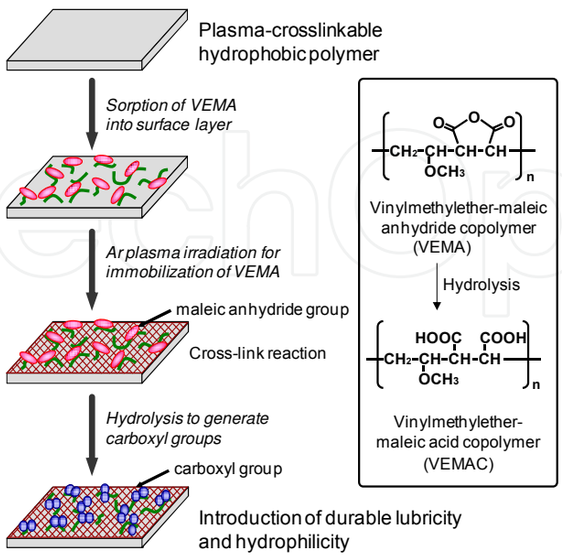

Fig. 23. Conceptual illustration for introduction of durable hydrophilicity onto the polymer surface by plasma techniques

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 24 shows the representative data of measurement of surface slipperiness as a function of the number form repeated rubbing of the treated catheter against silicon rubber. (Kuzuya et al., 1997b)

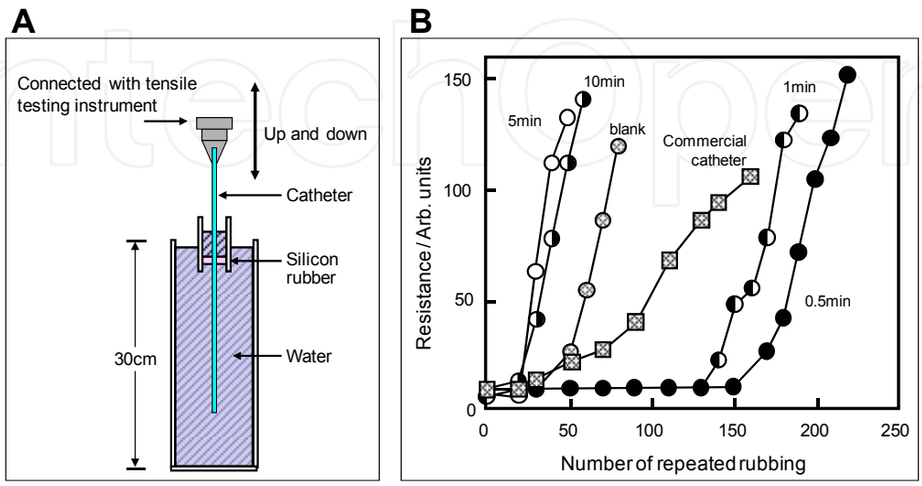

Fig. 24. Experimental setup for measurement of surface lubricity of plasma-irradiated polyurethane-made catheter (A) and durability of the surface lubricity of plasma-assisted VEMAC immobilized catheter in comparison with that of commercial catheter (B)

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 Cell culture application of VEMAC-immobilized substrate

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 Fig. 23. (Sasai et al., 2008) In addition, we also used VEMAC-immobilized PS (PS/VEMAC) as a substrate for immobilizing cell-adhesive peptide, Arginine-Glycine-Aspartic acid (RGD), to prepare the more cell-adhesive substrate. RGD containing peptide was immobilized on PS/VEMAC using EDC-NHS chemistry (1-Ethyl-3-(3-dimethylaminopropyl carbodiimide HCl and N-hydroxylsulfosuccinimide) through the surface carboxyl groups of PS/VEMAC. (Sasai et.

al., 2009) Figure 25 shows the microscopic images of mouse embryonic fibroblast, NIH3T3, adhered on each substrate after 2h in culture. As shown in Fig. 25, a distinct difference in cell attachment and spreading of NIH3T3 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 NIH3T3. Furthermore, cell adhesion and proliferation were significantly promoted by immobilizing RGD peptide on PS/VEMAC. The immobilized RGD peptide was specifically recognized by cell surface receptor proteins, integrins, so that the RGD-immobilized surface showed the cell adhesion properties even under the non-serum culture condition. (Sasai et al., 2010) These results indicate that PS/VEMAC is useful for not only a good cell culture substrate but also a substrate for immobilization of bioactive peptide for controlling cell behavior.

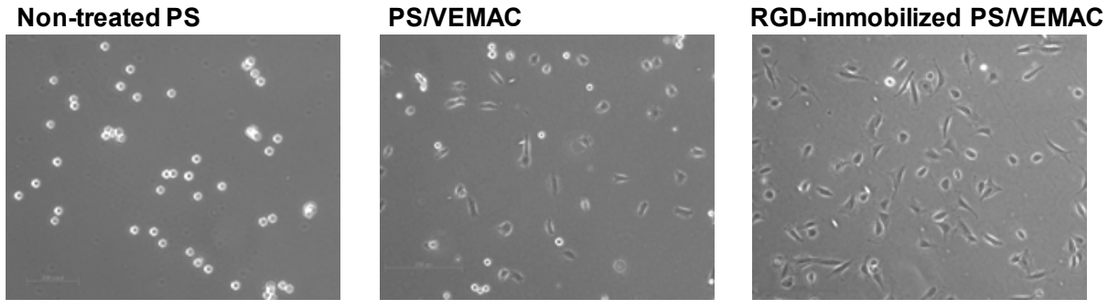

Fig. 25. Phase contrast light microscopic images of NIH3T3 on non-treated PS, PS/VEMAC and RGD peptide-immobilized PS/VEMAC after 2h in culture

The number of seeded cells: 1.0×10^5/dish
Culture medium: Dulbecco's modified Eagle medium supplemented with 10 % calf serum, 100 units/mL penicillin and 100 μg/mL streptomycin

4.3 Plasma-assisted immobilization of biomolecules onto polymer substrate

Considerable interest has focused on the immobilization of several important classes of biomolecules 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-informatics. However, all the current biochips are disposable and lack of reusability, in part because the devices are not physically robust.

The method shown in Fig. 23 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. (Kondo et al., 2003, 2007) 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 de-hybridization of fluorescence-labeled complementary

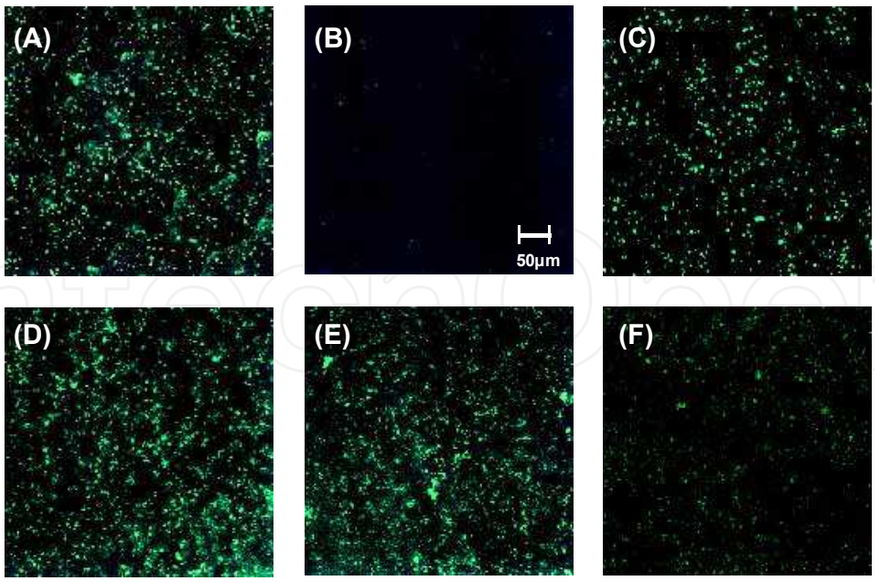

Fig. 26. Scan image of the fluorescence intensity of LDPE-VEMAC-DNA sheet for reusability test . (A); Hybridization of complementary oligo-DNA, (B); After hot water rinse of sheet (A) for 5min. Rehybridization of complementary oligo-DNA on the same sheet (C); 2 times, (D); 5 times, (E); 7 times, (F); 8 times

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 ^{o}C) for 5min). Figure 26 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.

Furthermore, we used the LDPE/VEMAC surface for immobilization of enzyme. (Sasai et al., 2006, 2007) 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. In fact, when an enzyme was directly immobilized on LDPE/VEMAC, the enzyme activity was really low. 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. (Patten et al., 1996) Figure 27 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 –NH_2 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.

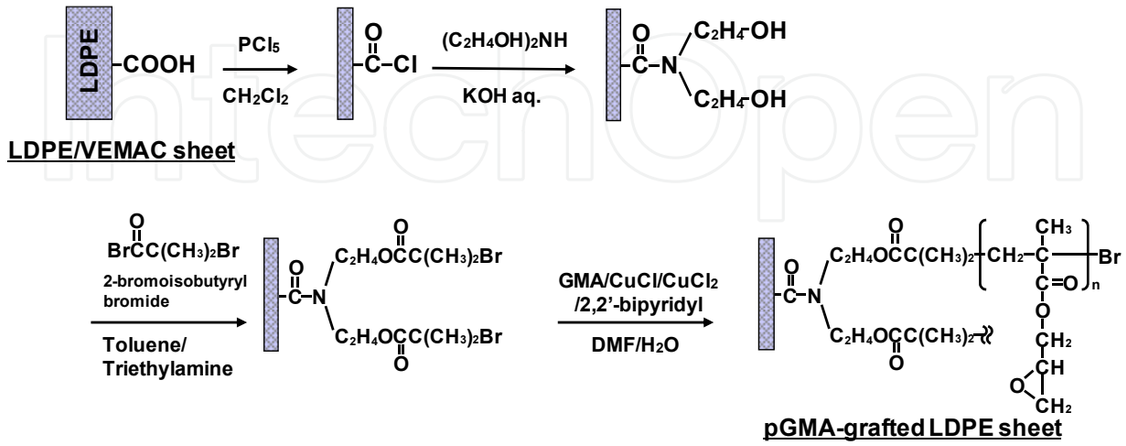

Fig. 27. Reaction scheme for fabrication of pGMA brushes on LDPE sheet by ATRP

pGMA grafted LDPE sheet	Immobilized UK (μg/cm²)(a)	Activity (IU/cm²)(b)	Ratio of active UK (%)
ATRP for 2h	0.44 ± 0.88	35.66 ± 2.77	101.3
ATRP for 4h	2.05 ± 0.88	31.34 ± 1.86	19.1
ATRP for 6h	4.53 ± 0.15	32.96 ± 4.63	9.1

(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.

Table 1. The amount of immobilized urokinase and its activity on LDPE sheet

5. Conclusion

On the basis of findings from a series of studies on the nature of plasma-induced radical formation on variety of organic polymers by ESR with the aid of systematic computer simulations, we were able to open up several pharmaceutical and biomedical applications by plasma techniques.

Plasma-assisted DDS preparations by our method contain several advantages; 1) solvent-free techniques, 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. It is hope that more precise insight into the scope and limitation will be gained in the course of study now in progress to establish the relationship between a drug releasing properties and plasma operational conditions.

For biomedical applications, we developed a novel method to introduce a durable surface wettability and minimize its decay with time on hydrophobic polymer substrate by plasma-assisted immobilization of carboxyl group-containing polymer, vinylmethylether maleic

acid copolymer (VEMAC). The surfaces thus prepared were potentially useful for not only the improvement of surface biocompatibility in biomaterials but also substrate for biomolecule immobilization due to the abundant surface carboxyl group.

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Biomedical Engineering, Trends in Materials Science

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Rapid technological developments in the last century have brought the field of biomedical engineering into a totally new realm. Breakthroughs in materials science, imaging, electronics and, more recently, the information age have improved our understanding of the human body. As a result, the field of biomedical engineering is thriving, with innovations that aim to improve the quality and reduce the cost of medical care. This book is the second in a series of three that will present recent trends in biomedical engineering, with a particular focus on materials science in biomedical engineering, including developments in alloys, nanomaterials and polymer technologies.

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