Nature of Protein Adsorption in Artificial Tear Solution on Plasma-Irradiated Polymethylmethacrylate Surface

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To evaluate the effect of plasma treatment on protein adsorption onto a polymer surface, we carried out Ar plasma irradiation on polymethylmethacrylate (PMMA) plate and examined the adsorption behavior of protein after immersion of plasma treated PMMA plate in the artificial tear solution containing lysozyme, albumin and γ-globulin. It was found that the total adsorption of protein in the artificial tear solution to PMMA is suppressed by the Ar plasma treatment. But the adsorption of each component protein differs according to the surface condition of polymers and obviously corresponds to the change with time in the surface wettability. It is concluded that introduction of a hydrophilic group into the PMMA surface by plasma treatment and coming off of it are associated with the adsorption behavior of proteins. Since the adsorption of protein to the plasma-treated PMMA surface changes over time, care should be exercised in determining it, when an attempt is made to clarify the effect of plasma irradiation on the adsorption to the plasma-irradiated polymer surface.

Keywords plasma treatment; polymethylmethacrylate; protein adsorption; artificial tear; hydrophilicity; hydrophobicity

Controlling the protein adsorption on a polymer surface is important not only for basic research in chemistry at an interface but also for the development of biocompatible polymer materials for medical use, and in the fields of drug engineering such as drug delivery systems (DDS) and clinical pharmacy where it relates to plastic containers for medical use. Improvement in adsorption also leads to improvement in the immunity determination method using enzyme immobilization.[1)]

Studies on the control of protein adsorption on a polymer surface include the surface grafting of hydrophilic polymers,[2)] chemical hydrophilization of a polymer surface, and polymer coating by microphase separation.[3)] Closely related to protein adsorption to polymers, cell adhesion reportedly decreases with an increase in the wettability of the polymer surface[4)] or *vice versa*.[5)] Assuming that this contradiction was attributable to differences in the chemical structure of polymers, Ikada *et al.* have recently conducted a study using various polymers and reported the effects of plasma irradiation on them.[6)]

We have reported a number of electron spin resonance (ESR) studies on plasma-induced surface radicals of polymers to elucidate the molecular mechanism of the plasma surface treatment,[7)] and based on the findings obtained we have also reported preparation of multilayered particles applicable for DDS.[8)]

As part of our work on pharmaceutical application of plasma surface treatment, we have made an extended study on the nature of protein adsorption characteristics of the plasma-irradiated surfaces using artificial tear solutions. We selected polymethylmethacrylate (PMMA) (acryl resin), which is in wide use as a polymer for medical purposes including pharmaceutical additives and hard contact lenses.

Materials and Methods

Methylmethacrylate (MMA) monomer is commercially available and was purified by distillation before use. The PMMA plate was made by plate-polymerization of MMA with azobisisobutyronitrile (AIBN) as a polymerization initiator using a gasket (*ca.* 1 mm thick) and a glass plate. The cross-linked PMMA plate was likewise obtained by plate-polymerization with a prescribed amount of ethyleneglycol-dimethacrylate (EDMA) as a cross-linking agent. The plate was cut to a fixed size before the protein adsorption test.

Plasma irradiation was carried out using the apparatus illustrated in Fig. 1, and is essentially the same as reported earlier.[8)] The plasma was generated by a radio frequency discharge of inductive coupling using a

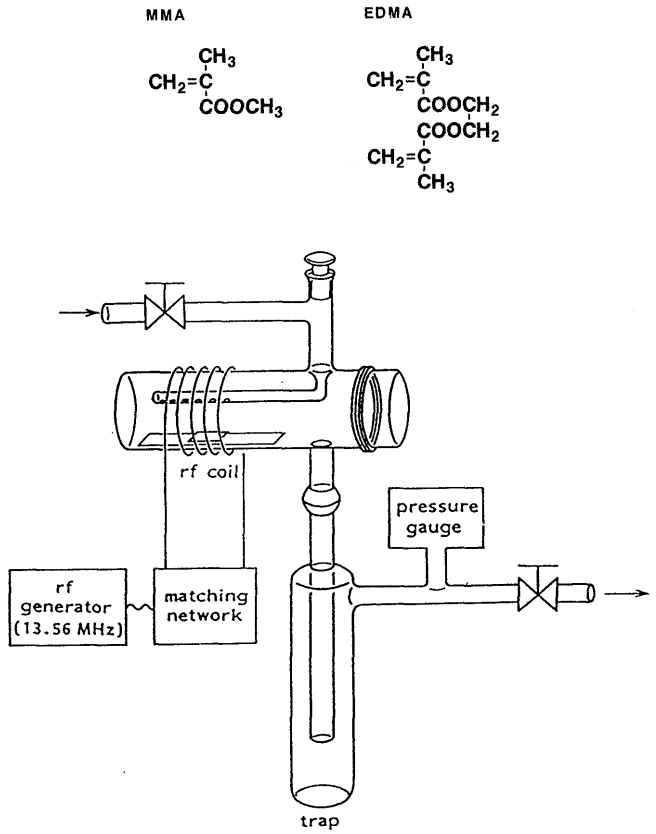

Fig. 1. Experimental Setup for Plasma Treatment of PMMA Plate
rf, radio frequency.

TABLE I. Formula of Artificial Tear Solution

Ingredient	Concentration (w/v %)
Lysozyme	0.120
Albumin	0.388
γ-Globulin	0.161
NaCl	0.900
$CaCl_2 \cdot 2H_2O$	0.015
$NaH_2PO_4 \cdot 2H_2O$	0.104

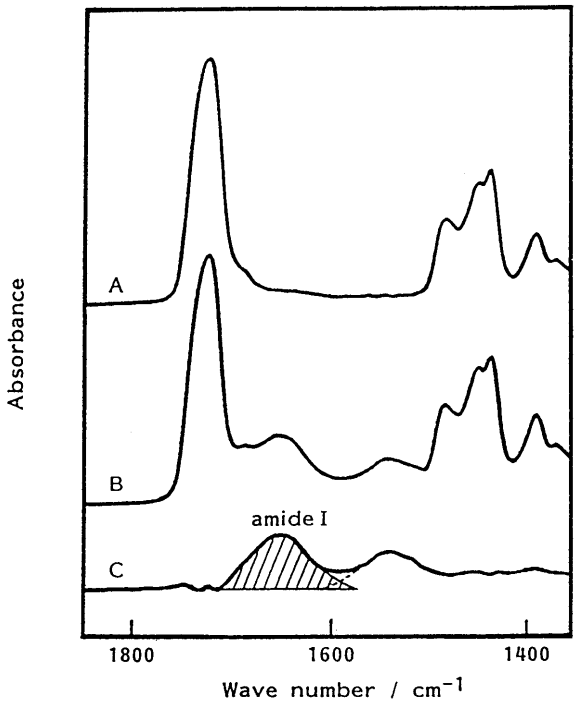

Fig. 2. Illustration for Determination of Amide I Peak Intensity of Adsorbed Proteins in Artificial Tear Solution on PMMA Plate

Band intensities are normalized on the basis of C=O band of PMMA (1735 cm^{-1}). A, B: before (A) and after (B) treatment with artificial tear solution. C: amide I intensity (C) in difference spectrum.

four-loop antenna at 13.56 MHz, and with the PMMA plate placed in the center of the antenna coil, Ar plasma was irradiated for 5 min in a closed system.

Regarding the protein adsorption, an artificial tear solution,[9] a mixed solution of lysozyme, albumin and γ-globulin (Table I)or a single solution of each protein was used, and the PMMA plate was immersed in the protein solution for a fixed time at 37 °C for adsorption. The plate was then rinsed with a phosphate buffer solution (pH 7.0), dried under reduced pressure at room temperature and the amount adsorbed was assessed.

Assessment of the amount of protein adsorbed was made by the fourier-transform-IR-attenuated total reflection (FT-IR-ATR) method as reported by Matsui *et al.*[10] Briefly, the difference between the spectrum for the sample and the spectrum for the control after the protein adsorption treatment was calculated, and then the amount of protein adsorbed was determined from the area strength of the characteristic peak of amide I in protein observed in the vicinity of 1650 cm^{-1} as illustrated in Fig. 2. The contact angle was measured by the drop-on-plate method.

Results and Discussion

Protein Adsorption from Artificial Tear Solution on Ar Plasma Treated PMMA Surface The adsorption behavior of protein after immersion of the Ar plasma-treated PMMA plate in the artificial tear solution, together with that in the plasma-untreated PMMA plate, is illustrated in Fig. 3. When the plate was immersed immediately after the plasma treatment, the amount adsorbed at the beginning of immersion was larger than that of the untreated case and showed a maximum about 2 h later. However, the amount decreased gradually with prolongation of the immersion time, fell off to about 60% of the untreated case and became constant.

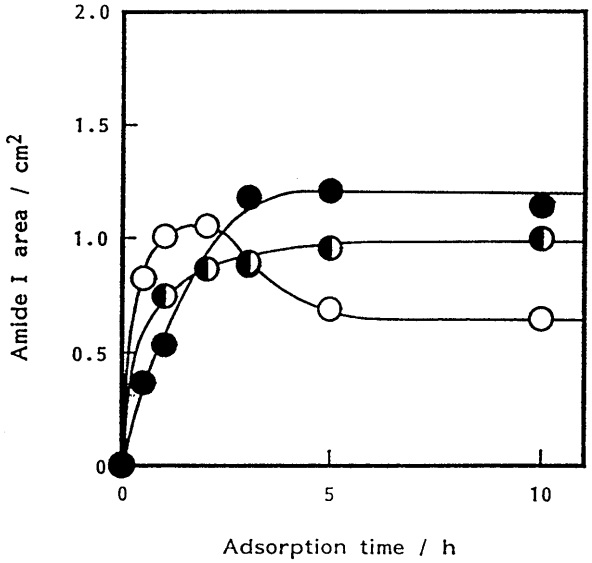

Fig. 3. Progressive Changes of Protein Adsorption in Artificial Tear Solution on PMMA Plates

●, blank; ○, adsorption on PMMA plate immediately after Ar plasma-irradiation, ◐, adsorption on PMMA plate left to stand for 24 h after Ar plasma-irradiation. plasma condition: 0.5 Torr, 50 W, 5 min.

Protein adsorption on the PMMA plate which had been kept dry under reduced pressure for more than 24 h after the plasma treatment showed the same behavior as in the plasma-untreated case; the amount adsorbed increased with immersion time and became constant at about 80% of the plasma-untreated case about 5 h later without showing a maximum in adsorption.

As above, the Ar plasma treatment showed a difference in the characteristics of adsorption between the sample immediately after the treatment and the sample that was preserved for a fixed time after the treatment. However, a fixed inhibitory effect on the protein adsorption in the artificial tear solution was noted in either case.

Adsorption of Protein Component of Artificial Tear Solution on the Plasma-Irradiated PMMA Surface The amount adsorbed of each component protein in the artificial tear solution was likewise examined to clarify details of the specific adsorption pattern of the sample immediately after the plasma treatment.

Figure 4 shows the results of adsorption of each component protein to plasma-untreated PMMA plate. As is clearly seen, the adsorbed amount of γ-globulin, a hydrophobic protein, is the largest among three kinds of protein, followed by albumin and lysozyme in this order. Figure 5 shows the adsorption on the plasma-treated PMMA surface. A sharp decrease in the amount of γ-globulin adsorbed and an increase in the amount of albumin adsorbed, a hydrophilic protein were recognized in comparison with the plasma-untreated case. Adsorption characteristics of γ-globulin and albumin were similar to

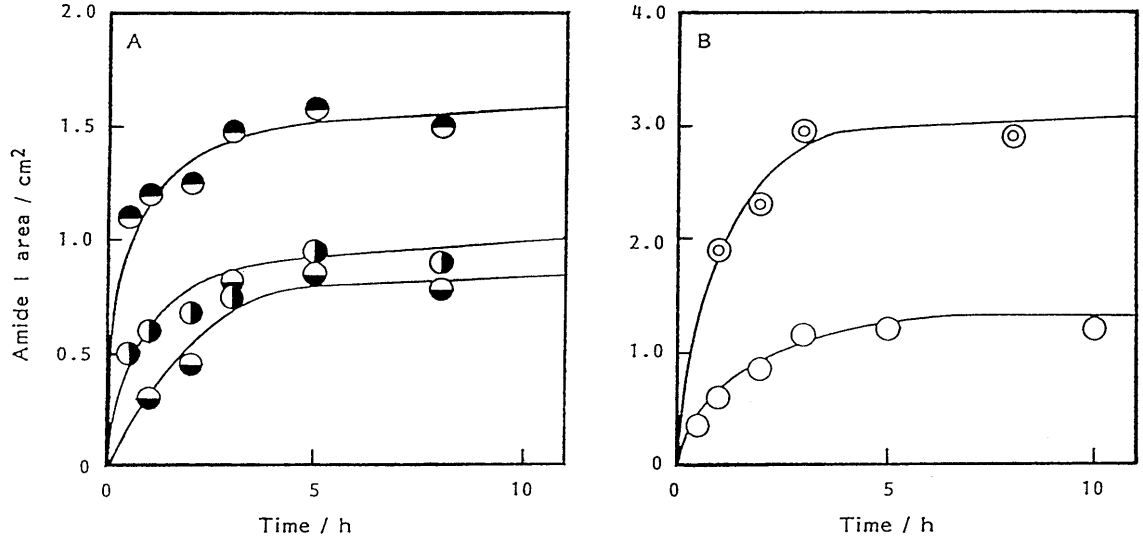

Fig. 4. Progressive Changes of Component Protein Adsorption on Virgin PMMA Plate

A: ◒, lysozyme; ◑, albumin; ◓, γ-globulin. B: ◎, sum of three component proteins; ○, artificial tear solution.

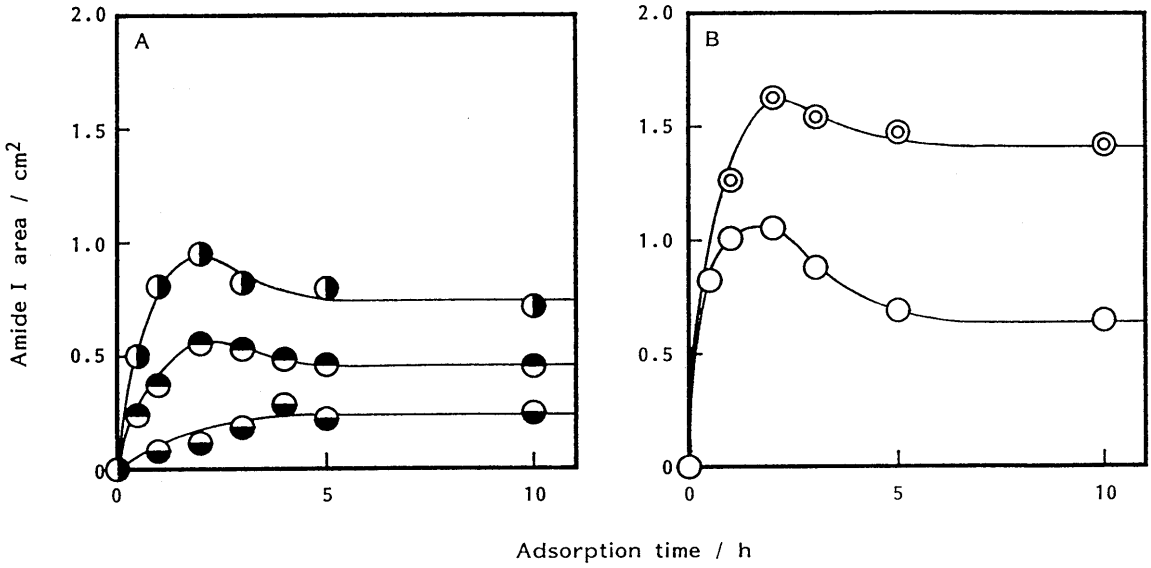

Fig. 5. Progressive Changes of Component Protein Adsorption on Ar Plasma Treated PMMA Plate

A: ◒, lysozyme; ◑, albumin; ◓, γ-globulin. B: ◎, sum of three component proteins, ○, artificial tear solution.

those of artificial tear solution, and the adsorption became constant after showing a maximum. When the sum of adsorption from various protein solutions was compared with the adsorption from the artificial tear solution, the latter was smaller regardless of whether or not the plasma treatment had been made. This may be attributable to the adsorbability having been lowered by a competitive adsorption of various proteins to the adsorption site on the PMMA surface because of the artificial tear being a mixed solution of proteins, and also by the interaction between protein molecules because of the total protein concentration having risen.

Relationship between Adsorbability of Proteins and Wettability on PMMA Surface It is generally known that the polymer surface is made efficiently hydrophilic by plasma irradiation.[11] Figure 6 shows changes in the contact angle with time in the Ar plasma-irradiated PMMA surface when the sample was kept in water (37 °C) and in dry air under reduced pressure, on which we reported previously.[12] As is apparent from this figure, the contact angle of the PMMA surface decreases considerably with the Ar plasma treatment, but thereafter gradually increases and becomes constant about 5 h later whether it is kept in water or in dry air under reduced pressure. The contact angle that has become constant is more greatly increased when kept in water.

Judging from the fact that the time required for the protein adsorption on the plasma-treated PMMA surface to become constant coincides with the time for this increase in the surface contact angle (decay of wettability) to become constant, the adsorption behavior specific to the plasma-irradiated PMMA surface shown by albumin, a hydrophilic protein, appears closely related to the changes with time in the wettability on this surface.

As mentioned, we earlier elucidated the radicals produced on the plasma-irradiated polymer surface which were studied by ESR with the aid of systematic simulation.[7] On the basis of the findings obtained, we also clarified the mechanism for hydrophilization of the plasma-treated polymer surface and also that for the decay of the wettability over time.[12] That is, PMMA being a typical degradable polymer produces an end-chain (terminating) alkyl radical upon the Ar plasma treatment, unlike crosslinkable polymers such as polyethylene and polystyrene.[7,8] Therefore, PMMA shows considerable etching property upon plasma irradiation. This means that lowering the molecular weight of surface polymers proceeds in plasma irradiation onto the plasma-degradable polymers such as PMMA. Thus, a considerable decrease of the contact angle (hydrophilicity given) immediately after plasma irradiation includes introduction of a hydrophilic group (oxygen functional group) into lower-molecular-weight species on the surface as well. The moieties with lower-molecular-weight, however, gradually detach from the polymer surface and consequently the hydrophilicity of the plasma-treated surface decays with time.

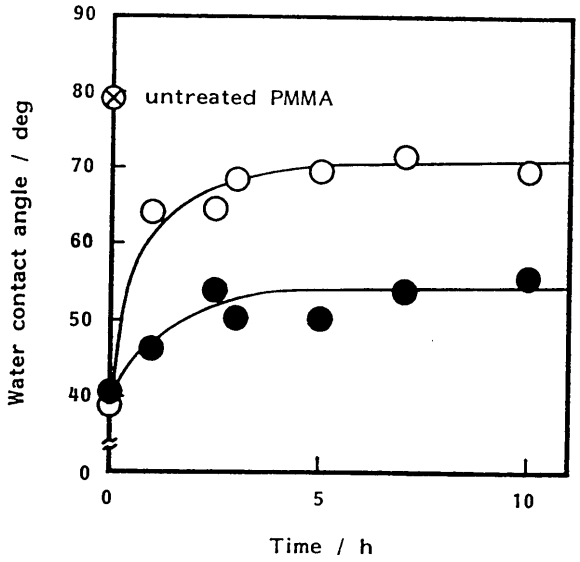

Fig. 6. Progressive Changes of Water Contact Angle of Ar Plasma-Exposed PMMA Plate

●, aging in dry air; ○, aging in water. Plasma condition: 0.5 Torr, 50 W, 5 min.

It is likely that the specific changes and decrease with time in adsorption of protein to the Ar plasma-treated PMMA surface is also governed by the same mechanism. That is, in virgin PMMA, the surface is hydrophobic and the hydrophobic region of γ-globulin turns in the direction of the polymer surface and adsorbs to it hydrophobically as illustrated schematically in Fig. 7A. Likewise, some albumin and lysozyme also adsorbs to the polymer surface. In the plasma-treated PMMA, the hydrophobic adsorption of γ-globulin is suppressed with hydrophilicity given to the PMMA surface and the adsorption of albumin, a hydrophilic protein, increases as illustrated in Fig. 7B. However, the hydrophilic region introduced into the

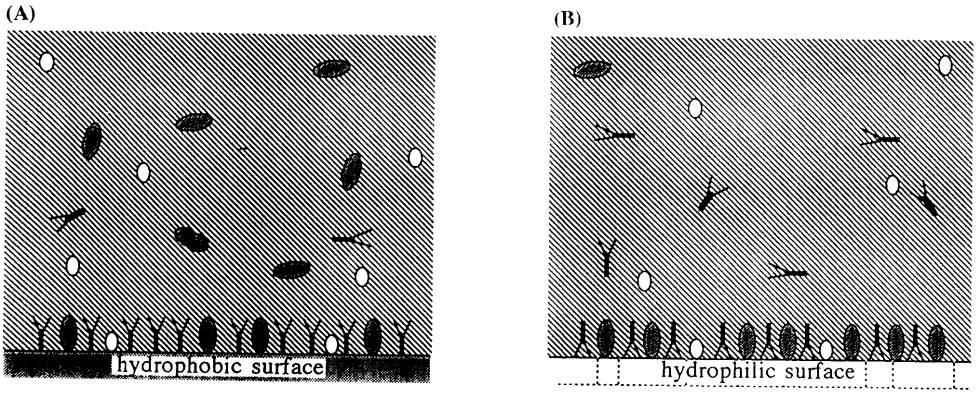

Fig. 7. Illustration of Protein Adsorption in Artificial Tear Solution to PMMA Surface before and after Plasma Treatment

(A) Virgin polymer surface; (B) Plasma-irradiated polymer surface. ○, lysozyme; ⬬, albumin; ⋖, γ-globulin.

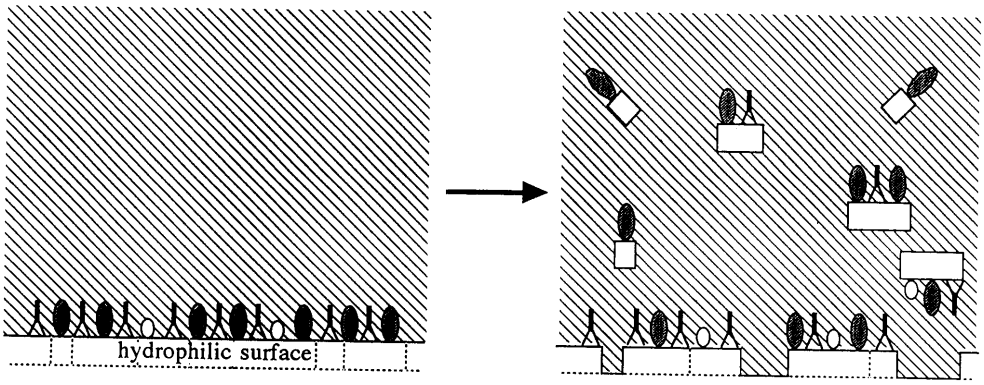

Fig. 8. Illustration of Mechanism for Protein Desorption with Time on Plasma-Irradiated PMMA Surface in Water

○, lysozyme; ⬬, albumin; ⋖; γ-globulin.

surface also includes introduction into the region of lower-molecular-weight species formed on the surface as mentioned above. It is therefore likely that adsorbed protein gradually comes off in concert with the lower-molecular-weight region which comes off gradually (Fig. 8). This is presumably the reason why the adsorption of protein, after gradually increasing, tends to decrease after reaching a maximum.

Protein Adsorption Behavior to Crosslinked PMMA Surface To confirm this mechanism, the adsorption behavior of protein from an artificial tear solution against several kinds of crosslinked PMMA differing in the degree of crosslinking was examined. The adsorption of protein to the crosslinked PMMA immediately after Ar plasma treatment showed the same behavior as in the case of the linear type PMMA surface regardless of the degree of crosslinking (Fig. 9). But the proportion of the decrease in the adsorption with time was shown to fall off simultaneously with a decrease in the maximum of the protein adsorption as the degree of crosslinking increases.

This is supported by the fact that the loss of weight due to the Ar plasma treatment is suppressed in crosslinked type PMMA (PEDMA) compared with the linear PMMA (Fig. 10). This is probably because the formation of lower-molecular-weight moieties induced by the formation of the end-chain alkyl radical was suppressed by crosslinking and also because the detachment of the introduced surface hydrophilic group was suppressed.

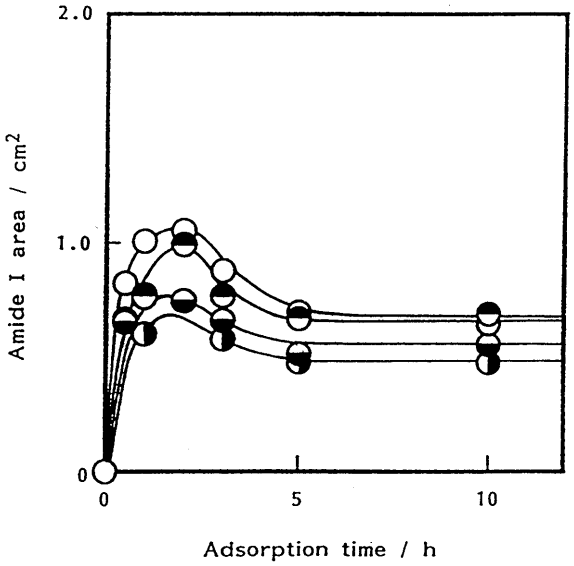

Fig. 9. Progressive Changes of Protein Adsorption on Cross-Linked PMMA Plate Immediately after Ar Plasma-Irradiation

Plasma condition: 0.5 Torr, 50 W, 5 min. MMA : EDMA = 1 : 0 (○), 20 : 1 (◓), 5 : 1 (◒), 1 : 1 (◑).

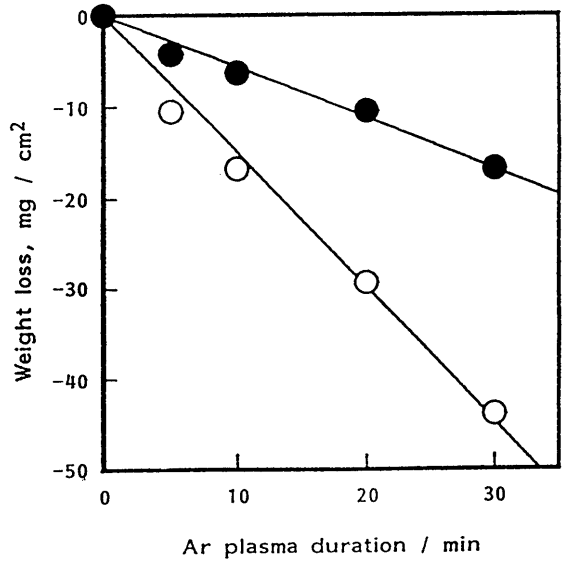

Fig. 10. Effect of Ar Plasma-Irradiation on Polymer Degradation

○, PMMA; ●, PEDMA.

Conclusion

It has been shown herein that the total adsorption of protein in an artificial tear solution to PMMA is suppressed by Ar plasma treatment. But the adsorption of each component protein differs according to the surface condition of the polymer and obviously corresponds to the change in surface wettability with time. It is concluded that introduction of a hydrophilic group onto the PMMA surface by the plasma treatment and its detachment is involved in the adsorption behavior of proteins. Since the adsorption of protein to the plasma-treated PMMA surface changes over time, care should be exercised in making a determination of amount. This is because there are many cases containing various proteins as in tears and cells in which the adsorption of protein to polymers for medical use poses a problem. The adsorption rate and changes in the adsorption of each protein with time should therefore be taken into consideration in any attempt to determine the effect of plasma irradiation on the adsorption to a plasma-irradiated polymer surface.

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