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Computer Modeling of Vroman Effect in Protein Adsorption upon Polyurethane Surface

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단백질흡착에서의 브로만효과에 대한 컴퓨터 모델링

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Introduction

Protein adsorption is known to be the first stage of blood-material interaction. Among hundreds of plasma proteins, especially the three proteins such as albumin, fibrinogen and immunoglobulin (IgG) have particularly been focused in this research because of their richness in blood and preference to adsorb on the artificial surface. Polymeric surfaces also have various complex characteristics according to their composition and fabrication methods. There are many papers which reported the adsorption of these three plasma proteins on various polymeric surfaces [1-3]. The Vroman effect is one of the most potential phenomena affecting the protein adsorption upon the polymeric surface. This effect is normally represented by fibrinogen adsorption kinetics. The amount of adsorbed fibrinogen has a peak with respect to incubation time as shown in Figure 1. This phenomena is dependent upon the material characteristics of the adsorbing surface.

We developed a new microscopic computer model for the fibrinogen adsorption upon the polyurethane surface[4]. In this paper, we expanded our model in order to simulate the Vroman effect.

Numerical Scheme

According to the chemical composition of polyurethane, its surface was modelled by an 150x150 square lattice which was composed of two segment types; hydrophobic (H-) and hydrophilic (H+) domains. Each point in the 150x150 lattice represents a H+ or a H-, which was distributed randomly as shown in Figure 2. The adsorption of proteins and latexes on flat uniform surfaces can often be described by a random sequential adsorption (RSA) process. Such a process requires irreversible adsorption and no diffusion of the particles on the surface after they are adsorbed. An occupied site remains occupied, no site may be occupied by more than one square, corresponding to the conditions of irreversible adsorption and no overlapping between particles, is assumed as descrived ·in respectively . The hydrophobic surface in the conventional RSA process was modified by the randomly mixed surface of hydrophobic and hydrophilic domains. This mixed surface represents the hard and soft segments of the polyurethane surface. This modified random sequential protein adsorption process was performed as follows ;

1. Select a random position in the 150x150 lattice which is made of randomly mixed hydrophobic and hydrophilic points at a given ratio.
2. Select the specise of the plasma protein by random sampling from the protein pool where three kinds of plasma protein were mixed in ta given ratio.
3. Count the number of hydrophobic points in the pxp protein box which is centered at the position selected in the first procedure.
4. Determine the success or failure of protein adsorption by comparing the number of the counted hydrophobic number in the second procedure with the adsorption threshold.
5. Equilibrium state of protein adsorption was checked by unabsorbed trials during above three procedures.

6. Calculate the total numbers of each adsorbed protein molecules after the equiliburium state is arrived.

The fibrinogen box was first modelled as a square of 3x3 i.e. p=3, and Albumin and IgG are p=2. The threshold of protein adsorption was 5 of 9 points in the fibrinogen box, which means that the hydrophobic interaction is strong enough to adsorb on the polyurethane surface. With various thresholds of the protein adsorption, several simulations were performed in order to find the appropriate value of the adsorption threshold. Equilibrium state of the protein adsorption is one of the important parameters in this simulation. The amount of each adsorbed protein is not changed at the equilibrium state. Thus, we could detect the equilbrium state of the adsorption process by checking the accumulating number of the successive adsorption failures. We tried to find the appropriate equilibrium condition by changing the equilibrium state threshold in the simulation.

Plasma Proteins Adsorbed on Polyurethane Beads

Competitive ^{14}C-labelled protein adsorption studies were performed with diluted bovine plasma. Fresh bovine whole blood was collected in 3.8 % sodium citrate anticoagulant solution (final dilution 9:1, v/v). The blood was then carefully transferred to polystyrene tubes, centrifuged at 2,500 g at 4 °C to prepare platelet-poor plasma (PPP). Concentrations of proteins in bovine plasma were determined by standard methods. Control plasma was found to have contained 2.66 mg/ml of fibrinogen, 30 mg/ml of albumin, and 9.25 mg/ml of IgG. Protein solution for adsorption experiment from plasma were prepared by adding tracer amounts of radiolabelled protein in amounts corresponding to approximately 0.5 -10 % of protein concentration to freshly prepared bovine plasma. For experiment, plasma was diluted with phosphate buffered saline (PBS, pH 7.4). Quantities of surface modified PU beads (800 mg, surface area of 14 cm^2) were carefully weighed into plastic disposable 10 ml syringes and equilibrated with 5 ml of PBS overnight. Prior to adsorption studies, the buffer was removed and 1.6 ml of protein solution introduced into the syringe system. Sets of syringes were arranged for various adsorption time intervals (1-120 minutes) as well as different concentrations (0.2-10 %) of protein solution to produce adsorption kinetics and isotherm data. Adsorption kinetics were constructed for different adsorption times at a fixed plasma concentration and adsorption isotherms for each concentration at a fixed adsorption time. After adsorption at each time and concentration, the plasma was expelled from the syringe, leaving the beads inside the syringe. The syringe was filled with PBS and washed extensively with PBS until no further activity was detected in the eluent, which was confirmed by the fact that the absorbance at 280 nm of the eluent returned to zero. Finally, a 2 %(w/v) solution of sodium dodecyl sulfate (SDS) in PBS was added to the syringes and agitated for 48 hours to dissolve any bound proteins. Aliquots of the solution after SDS elution were assayed for radioactivity and compared to the eluent and stock solutions for quantifiable depletion of radioactive species.

Results and Discussion

Figure 3 shows the result of the computer simulation in which the fibrinogen kinetic curve has a peak. We could show the feasibility of our computer model for simulation of the Vroman effect. Effects of each parameters in our computer model, which represents various physical factors, on the Vroman effect will be determined through further simulations.

References

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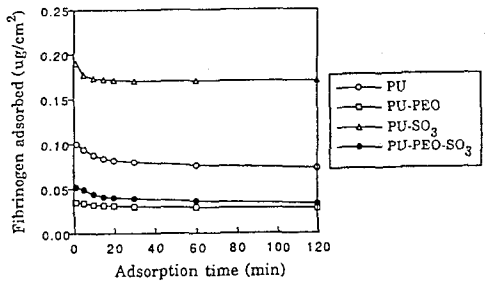

Figure Adsorption kinetics for fibrinogen from 1% plasma on different PU surfaces.

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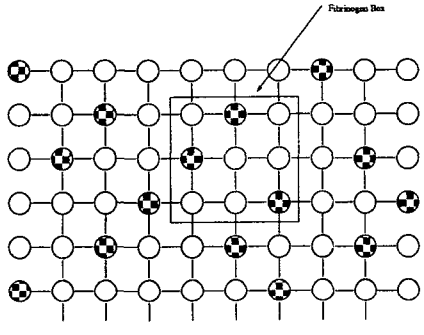

Figure 2. Schematic diagram of the polyurethane lattice space

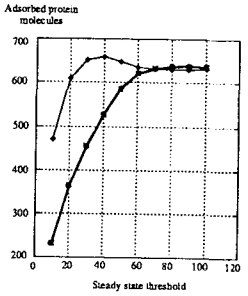

Figure 3. Adsorption Kinetics of the Plasma Proetin onto Polyurethane