Abstract

（論 文 の 内 容 の 要 旨）

論文題目　Surface Design of Polymer Biomaterials to Control Protein Adsorption by Nano-force Analysis of Molecular Interactions
（分子間相互作用力のナノ解析を基盤としたタンパク質吸着を規定する生体材料の表面設計）

氏　　名　坂田　翔

Biological responses induced at interfaces between artificial material surfaces and biological environments should be precisely understood and highly regulated for the further development of the fields of biomaterials engineering, cell engineering, regenerative medicine, and medical industry based on these sciences. In particular, protein adsorption onto the materials surface is induced in the very early stage of a series of biological responses, and the property of adsorbed protein layer is a significant factor that determines subsequent biological reactions. Therefore, it is significant to understand the characteristics of adsorbed protein layer. Protein adsorption phenomena proceed through several steps, and various interaction forces such as surface-protein interactions and protein-protein interactions are closely related to the protein adsorption phenomena, and determine the all behaviors on each step. Based on the above, the objective of this study is to elucidate the protein adsorption phenomena based on analysis of the molecular interaction forces. This research contributes to develop the novel surface design of polymer biomaterials to highly regulate the protein adsorption through the nano-force analysis of various molecular interactions. In order to achieve the purpose and the reach the final goal, the strategy integrating the two elemental technologies was developed initially, that is, (1) Nano-fabrication of well-characterized polymer brush surfaces, and (2) Nano-force analysis by atomic force microscopy. Based on the strategy, this thesis is divided into six chapters: (Chapter 1) the general introduction of the relative materials and techniques those will be used in this study, (Chapter 2) the detail design, preparation, and characterization of model surface, (Chapter 3) the nano-force analysis of various molecular interactions, (Chapter 4) the quantitative evaluation of the protein adsorption behaviors, (Chapter 5) the constructive analysis applying the established system, and (Chapter 6) the conclusion of the research and the further perspective.

1. General introduction

Chapter 1 is the general introduction of total thesis, describing the significance of the comprehension and the advanced control of protein adsorption, and the analytical technique and the platform surface to understand protein adsorption from the perspective of the molecular interaction forces. First, the significance of protein adsorption phenomena and the characteristics of protein adsorbed layers in the biomaterials fields were summarized. Next, the utility of the novel analytical methodology by atomic force microscopy (AFM) to understand the nano-scale molecular interaction forces dominating the protein adsorption behavior on the materials surfaces was introduced. Furthermore, the importance of the well-defined model surface and the fabrication of well-characterized polymer brush surfaces with precise structure and controlled properties for the nano-force analysis were described and emphasized. The significance of the surface design to control the protein adsorption through the nano-force analysis of the molecular interactions was clarified.

2. Preparation of polymer brush surfaces as well-characterized model surfaces

Chapter 2 describes the design of well-characterized polymer brush surfaces as platform moel surfaces for refined understanding of the relationship between the nano-scale molecular interaction forces and the protein adsorption behavior at the molecular level. The zwitterionic poly(2-methacryloyloxyethyl phosphorylcholine (MPC)) brush surface, the cationic poly(2-trimethylammonium methacrylate (TMAEMA)) brush surface, the anionic poly(3-sulfopropyl methacrylate (SPMA)) brush surface, and the hydrophobic poly(*n*-butyl methacrylate (BMA)) brush surface were systematically prepared by surface-initiated atom transfer radical polymerization (SI-ATRP) method, and characterized. The results of surface structure analysis indicated that the highly dense and considerably homogeneous polymer brush layers composed of each monomer unit were constructed. The polymer brush surfaces exhibited the intended physicochemical properties (the hydrophilicity/hydrophobicity and the surface potential) reflecting the chemical structure of each monomer unit. Thus, a series of well-characterized polymer brush surfaces with the precisely regulated structure and the widely controlled properties were successfully fabricated. It was concluded that these polymer brush surface systems would provide a systematic understanding about various molecular interaction forces and the protein adsorption behaviors.

3. Nano-force analysis of molecular interactions

Chapter 3 describes the nano-force analysis of the various molecular interactions on the polymer brush surfaces via AFM. The three types of the force-versus-distance (*f*-*d*) curve measurements were performed using the AFM probes modified with (a) polymer brush layers,

(b) functional groups, and (c) proteins, respectively. The surface force analysis between the identical polymer brush surfaces revealed that the surface interaction forces were clearly separated by utilizing the systematically fabricated polymer brush surfaces system. That is, the cationic poly(TMAEMA) brush surface and the anionic poly(SPMA) brush surface generated only electrostatic interaction, and the hydrophobic poly(BMA) brush surface generated only hydrophobic interaction whereas no specific interaction existed on the zwitterionic poly(MPC) brush surface. The interaction forces at the functional group level were also quantitatively evaluated. Moreover, from the *f-d* curve measurements between surfaces and proteins, whereas the poly(MPC) brush surface exhibited no significant interaction force with any protein, the poly(TMAEMA), poly(SPMA), and poly(BMA) brush surfaces strongly interacted with proteins. Furthermore, it was clarified that the electrostatic interaction and the hydrophobic interaction played a role as the force that inhibit the spontaneous detachment of proteins from the surfaces in a physiological environment.

4. Quantitative analysis of protein adsorption behavior

Chapter 4 describes the quantitative evaluation of the protein adsorption behavior on the polymer brush surfaces, and discusses the relationship between the molecular interaction forces and the protein adsorption behaviors. The surface plasmon resonance (SPR) measurements showed that a large amount of proteins adsorbed on the cationic poly(TMAEMA), the anionic poly(SPMA), and the hydrophobic poly(BMA) brush surfaces, which generated the electrostatic or hydrophobic interaction, while the zwitterionic poly(MPC) brush dramatically reduced protein adsorption. Moreover, there were positive correlations between the strength of the direct surface-protein interaction forces measured by AFM and the whole amounts of adsorbed proteins measured by SPR. Besides, the circular dichroism (CD) measurement quantitatively demonstrated the conformational change of the protein adsorbed on the surface. From all of the experimental evidences, it was considered that the inhibition of the reversible and spontaneous detachment of proteins by the strong interaction forces would lead to the conformational change of the proteins on the surfaces, which resulted in high adsorption mass. Furthermore, from the perspective of the molecular interaction forces, it was concluded that the spontaneous and easy detachment of proteins from the surface, provided by the zwitterionic structure, would be an essential factor in effective suppression of protein adsorption and following biological responses at the biomaterials surface.

5. Analysis on mixed-charge copolymer brush surfaces

Chapter 5 describes the constructive analysis applying the system established in the above chapters. A series of binary copolymer brush surfaces composed of the cationic monomer unit

and the anionic monomer unit, poly(TMAEMA-*co*-SPMA) brush surfaces, were systematically prepared in various compositions by SI-ATRP for a detailed investigation of the contribution of the electrostatic interaction forces on the protein adsorption behavior. Surface characterization demonstrated that the highly dense copolymer brush surfaces with the even hydrophilic nature and the different surface potential reflecting the monomer unit compositions were successfully fabricated as a new well-characterized platform surfaces to quantitatively understand the electrostatic interactions. From the *f-d* curve measurements of AFM, it was quantitatively demonstrated the strength of the electrostatic interaction forces was widely controlled by utilizing these mixed-charge copolymer brush surface systems. Moreover, the direct surface-protein interaction forces and the protein adsorption mass corresponded to the magnitude of the electrostatic interaction forces of the surfaces and the charge properties of the proteins. Furthermore, the effectiveness of the balanced-charge (pseudo-zwitterionic) structure on protein repellent property was certified quantitatively from the perspective of the actual forces.

6. Concluding remarks

Chapter 6 gives the total conclusion of this thesis, including the significance and the future perspective of this study. In conclusion, a novel analytical system to elucidate the protein adsorption phenomena from the perspective of the nano-scale molecular interaction forces was established by integrating “the nano-fabrication of well-characterized polymer brush surfaces” (model platform surface) and “the nano-force analysis of various molecular interactions by AFM” (analytical method). This thesis clarified that this integrated system provides a new interpretation and insight for the protein adsorption phenomena on materials surfaces, and as one concept for the protein repellency at the biomaterials surface, it was concluded that the construction of the surfaces inducing no nano-level interactions and the spontaneous detachment of proteins from the surface would be an essential factor, as provided by the zwitterionic structure. The potency of the zwitterionic structure was also corroborated by the constructive analysis based on the established system. The analytical system proposed in this study is also versatile and applicable for other various interactions, which would lead to the further understanding of the whole protein adsorption process.