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Evaluation of physical and chemical structures required for anti-biofouling self-assembled monolayers and polymer brush films

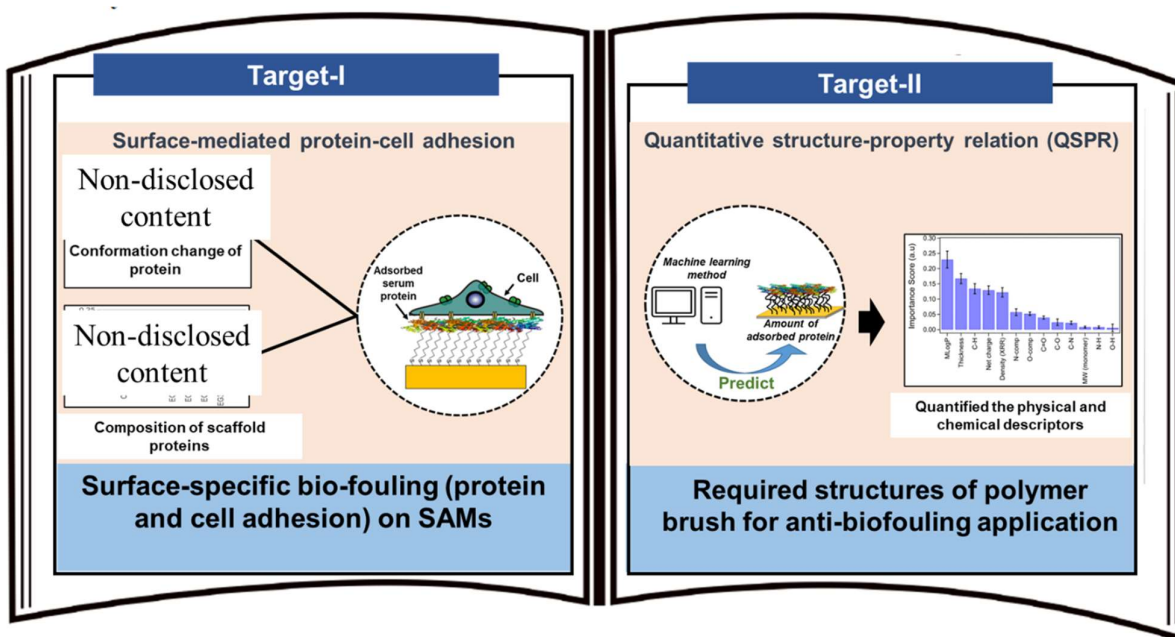
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Thesis Outline



Chapter I: Introduction

Understanding the impact of the material's physicochemical properties on protein and cell adhesion is important in developing new anti-biofouling materials. The adsorption of serum proteins onto the material surface is the initiating event for most physiological responses. The adsorbed serum proteins provide a scaffold layer that contains specific cell receptor-ligand binding sites where cells adhere. Therefore, surface-mediated protein adsorption and protein-cell interaction studies clarify the required structures for designing the anti-biofouling surface. For over a decade, self-assembled monolayers (SAMs) have been used to investigate the influence of physicochemical properties on the fundamental biomolecular interaction due to their well-defined structure and easily customizable terminal groups. It is clarified that the interactions between water molecules and the monolayer surfaces play a crucial role in the anti-biofouling mechanism of SAMs. Compared to SAMs, thin polymer films have a better anti-biofouling property due to steric or volume exclusion effect and chemical stability.¹⁻⁴

Chapter II: Survey of related literature

Previous studies primarily focused on investigating material's chemical and physical structures using single proteins or mixtures of a few proteins to develop anti-biofouling materials for in vivo applications. In those works, under single protein environment it was clarified that surface-mediated protein's conformation change and scaffold protein's composition are vital factors for surface-mediated cell adhesion. However, actual biological environments, such as blood serum or body fluids, are complex mixtures of various biomolecules, rendering evaluation strategies interestingly complicated.^{1,5,6}

Chapter III: Methodologies used in this study

In my thesis work, I systematically investigate the protein and cell adhesion by dividing the overall complex process into two targets, where I used SAMs as simple model organic surface and polymer brush films as actually used coating for anti-biofouling applications. Specifically, using SAMs, I investigated (1) the effect of physical and chemical properties of biomaterial surface on serum protein adsorption, denaturation, and cellular response on simple monolayer organic surfaces and (2) the serum adsorption onto complex polymer brush films and quantitatively evaluated the physicochemical properties required for anti-biofouling application using informatics.

Chapter IV: Results and discussion: Evaluation of anti-biofouling properties of SAMs based on protein-mediated cell adhesion in serum environment

In this work, I determine the mechanism behind the changes in protein conformation upon adsorption, the composition of ECM proteins, and the subsequent cell behavior on SAMs with different surface functionalities. Using Pearson correlation analysis, this study clarified that the adhered cell area and density positively associated with the amount of ECM proteins and the conformation change of the adsorbed proteins on different SAMs. It was found that the serum protein changed its conformation upon adsorption onto hydrophobic methyl-terminated SAM. Furthermore, a higher composition of the ECM proteins (fibronectin and vitronectin) was found and shown to promote subsequent human umbilical vein endothelial cell (HUVEC) adhesion and cell spreading. In contrast, the zwitterionic sulfobetaine SAM and the charged hydrophilic oligoether (EG3OMe) SAM were protein- and cell-resistant. On oligoether SAM, the protein adsorption gets suppressed by the range of 2-3 nm thick structured water layer, which was previously reported by Hayashi et al. As for the neutral hydrophilic oligo ethylene glycol

(OEG) SAMs, a trend in protein and cell resistance has been observed with changing the number of ethylene glycol terminal units. This trend could result from increasing numbers of polar groups which tightly bound water molecules in the interface found in past research. So it can be concluded that the amount of adsorbed scaffold protein and the conformational changes of scaffold proteins are the key parameter to control cell adhesion on SAMs in serum environment. However, the complete understanding of the effect of chain thickness on protein adsorption using SAMs is not possible as these are dense monolayer films.

Chapter V: Results and discussion: Investigation of protein adsorption on polymer brush films with structure-properties analysis

To clarify the impact the layer thickness combined with other chemical structures, here I used of polymer brush films (hydrophilic, charged hydrophilic and zwitterionic). This work focused on predicting the amount of adsorbed serum protein on different polymer brush films using a machine learning approach and evaluate the ranking of importance of the physicochemical parameters. Specifically, a quantitative evaluation of the polymer's chain thickness and density impact on serum protein adsorption was undertaken. It was found that the film thickness and density are two crucial physical factors that decide protein adsorption on polymer brush films. Using Pearson correlation analysis, it was further evaluated that thicker hydrophilic and zwitterionic polymer films are necessary for anti-biofouling applications, while charged hydrophilic polymer films must be thinner.

Chapter VI: Conclusions and Overall outlook

This thesis study reported the influence of the surface's physicochemical property on protein unfolding, the composition of ECM protein, and HUVEC cell adhesion in a complex serum environment. This study also reports on appropriate machine learning algorithm to evaluate complex serum protein adsorption on polymer brush films. Both studies in this thesis found that the charge neutrality of zwitterionic and neutral hydrophilic end groups and the thickness of polymers are essential for the design of anti-biofouling surfaces, which agrees with our general understanding and perception. Several implications have already been deduced on serum adsorption, scaffold layer formation, and cell adhesion based on the influence of the physicochemical properties and known anti-fouling mechanisms (steric repulsion and hydrodynamic interactions). However, further experiments must be performed to provide clear evidence on the protein unfolding and its effect on cell adhesion. Also, the influence of thickness on the anti-biofouling mechanism for polymer films needs to be established more clearly by using a broader range of thicknesses and collecting more data on different polymers. Finally, it is expected that this study's measurement techniques, machine learning approach, and the findings obtained using SAMs and polymer brush films, could be applied to develop anti-biofouling surfaces operating under biological environments. Our overall approach unveils the physicochemical structures required to design an anti-biofouling surface by addressing various surface-mediated biomolecular adhesion.

Major References

- (1) Hayashi, T.; Tanaka, Y.; Koide, Y.; Tanaka, M.; Hara, M. Mechanism Underlying Bioinertness of Self-Assembled Monolayers of Oligo(Ethyleneglycol)-Terminated Alkanethiols on Gold: Protein Adsorption, Platelet Adhesion, and Surface Forces. *Phys. Chem. Chem. Phys.* **2012**, *14* (29), 10196–10206.
- (2) Anderson, J. M.; Rodriguez, A.; Chang, D. T. Foreign Body Reaction to Biomaterials. *Semin. Immunol.* **2008**, *20* (2), 86–100.
- (3) Biran, R.; Pond, D. Heparin Coatings for Improving Blood Compatibility of Medical Devices. *Adv. Drug Deliv. Rev.* **2017**, *112*, 12–23.
- (4) Meyer, B. Approaches to Prevention, Removal and Killing of Biofilms. *Int. Biodeterior. Biodegradation* **2003**, *4* (51), 249–253.
- (5) Kushiro, K.; Lee, C.-H.; Takai, M. Simultaneous Characterization of Protein–Material and Cell–Protein Interactions Using Dynamic QCM-D Analysis on SAM Surfaces. *Biomater. Sci.* **2016**, *4* (6), 989–997.
- (6) Cantini, M.; Gomide, K.; Moulisova, V.; González-García, C.; Salmerón-Sánchez, M. Vitronectin as a Micromanager of Cell Response in Material-Driven Fibronectin Nanonetworks. *Adv. Biosyst.* **2017**, *1* (9), 1700047.