Analyses of Surface Interaction Between Endothelial Cells and Poly(2-methoxyethyl acrylate)

エムディ，アジズル，ハク

（様式２）

氏　　名　：Md Azizul Haque　エムディアジズル ハク

論 文 名　：

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（血管内皮細胞とポリ(2-メトキシエチルアクリレート)の表面相互作用の解析）

区　　分　：甲

論 文 内 容 の 要 旨

The cellular interaction with the biomaterials depends on several factors including protein adsorption, cell-substrate interaction, and cell-cell interaction. Cell adhesion strength to the substrate is a vital element to explain the biocompatibility of synthetic polymer biomaterials. In addition, cell attachment behavior on substrates also be regulated by the chemical and physical properties of the surface of biomaterials. Poly (2-methoxyethyl acrylate) (PMEA) is an FDA approved biocompatible polymer which is used as an antithrombogenic coating polymer in many sophisticated medical devices like artificial heart and lung, stents, catheter, and dialyzers etc. It is established that the water molecules interacting with PMEA can be classified into three types: free water, freezing-bound water (intermediate water), and non-freezing water. Intermediate water plays an important role to be an excellent biomaterial. PMEA can reduce the platelet adhesion by suppressing the adsorption and conformational change of fibrinogen. Recently, it was reported that non-blood cells can adhere to the coated surface of PMEA and its analogues through both integrin dependent and independent cell adhesion mechanism. However, there is no sufficient work of interaction analyses of endothelial cells and platelet PMEA analogues polymers. The present study was designed to investigate the interaction behavior of human umbilical vein endothelial cells (HUVECs) to the PMEA analogous polymer. Moreover, we have examined the HUVECs adhesion strength, HUVEC-HUVEC adhesion strength, platelet adhesion strength and number of platelet adhesion on both polymer and HUVECs monolayer, HUVEC migration analysis. We also observed the hydration state of the polymer-water interfaces. Based on our results, we indicated that the PMEA can be used as coating materials in construction of artificial small diameter blood vessel. We focus on how blood components and HUVECs interaction to biocompatible polymers in this thesis studies and whole the thesis work was separated in five following sections.

In **Chapter 1**, previous works and knowledges concerning biomaterials have been described in brief.

In **Chapter 2,** we stated HUVECs and platelet interaction analysis on PMEA analogous polymers.

In this study, we extensively investigated HUVEC–polymer and platelet–polymer interaction behavior by measuring the adhesion strength using single-cell force spectroscopy. Furthermore, the hydration layer of the polymer interface was observed using frequency-modulation atomic force microscopy. We found that endothelial cells can attach and spread on the PMEA surface with strong adhesion strength compared to other analogous polymers. We confirmed that HUVECs attachment and platelet adhesion are regulated by the amount of intermediate water. In contrast, we found that the hydration layers on the PMEA analogous polymers are closely related to their platelet adhesion behavior. Based on our results, it can be concluded that PMEA is a promising candidate for the construction of artificial small-diameter blood vessels owing to the presence of intermediate water and a hydration layer on the interface.

In **Chapter 3**, we reported that the confluent monolayer of HUVECs on PMEA plays a major role in mimicking the inner surface of native blood vessels. We extensively investigated the cell-polymer and cell-cell interactions by measuring adhesion strength using single-cell force spectroscopy. In addition, attachment, and migration of HUVECs on PMEA-analogous substrates were detected, and the migration rate was estimated. Moreover, the bilateral migration of HUVECs between two adjacent surfaces was observed. Furthermore, the outer surface of HUVEC was examined using frequency-modulation atomic force microscopy (FM-AFM). Hydration was found to be an indication of a healthy glycocalyx layer. The results were compared with the hydration states of individual PMEA-analogous polymers to understand the adhesion mechanism between the cells and substrates in the interface region. HUVECs could attach and spread on the PMEA surface with stronger adhesion strength than self-adhesion strength, and migration occurred over the surface of analogue polymers. We confirmed that platelets could not adhere to HUVEC monolayers cultured on the PMEA surface. Our findings show that PMEA can mimic original blood vessels through an antithrombogenic HUVEC monolayer and is thus suitable for the construction of artificial small-diameter blood vessels.

In **Chapter 4**, we have discussed the cell attachment behavior on biocompatible polymer brush surfaces rather the coating methods. We have investigated the HUVECs attachment ability, proliferation, and growth on grafted PMEA analogous brush system and bare gold surfaces. Immunocytochemical analysis reveals the cell morphology like cell area, circularity, aspect ratio and number of focal adhesions. In addition, HUVECs adhesion strength also measured. It was found that the polymer brush system increases the cell adhesion strength for some PMEA analogues compared to coating. We found that the elevation of adhesion strength comes due to the controlled height and grafting density (σ =0.11 chain/nm^2) of brush system and more focal adhesion formation of attached cells. Therefore, it can be said that polymer brush is an alternative of polymer coating in order to use it as a blood contacting surface.

In **Chapter 5**, the summary and future perspective of this study were described.