論文の内容の要旨

Abstract of Dissertation

Preparation of Nonbiofouling Biointerfaces on Polymeric Substrates by Phospholipid and Their Microchip Applications
(リン脂質ポリマーによる高分子基板への生物非汚染界面の創製とマイクロチップへの応用)

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Changing the biofuoling nature of polymeric substrates which are commonly used for fabrication of microchip devices is of an hugely important issue to be addressed since the microchip devices have been extensively researched and widely used in many application in handling biological entities such as DNA separation, immunoassay, cell sorting, biosensors and enzymatic assays due to the potential offered by miniaturizing including high throughput of analysis, low cost of fabrication, multiplex functionality, and portability. The higher surface-to-volume ratio in the devices, the more the surfaces is available for interacting with biomolecules. The nonspecific protein and cell adsorption is one of the common problem in them need to be eliminated. Therefore, I took the concept of cell-membrane-biomimicry interface based on phospholipid to be created on the polymeric substrates by taking two approach which are by coating with MPC copolymers and brush type poly(MPC).

Firstly, I demonstrated the construction of the nonbiofouling interface by one- step-simple dip coating process using poly(2-methacryloyloxylethyl phosphorylcholine(MPC)-*co*-*n*-butyl methacrylate)(PMB) and poly(MPC-*co*-2-ethylhexyl methacrylate-*co*-2-(dimethyamino)ethyl methacrylate)　(PMED). The amphiphilic MPC copolymers anchored strongly on the substrates and provided excellent nonspecific protein adsorption resistance. The hydrophilic property and the surface mobility of the modified surfaces were greatly improved compared to the unmodified ones. In term of surface charge, the MPC copolymers assure the ζ-potential to the zero level. The protein adsorption on the polymer materials with and without the surface modification was evaluated using a protein mixture of human plasma fibrinogen and serum albumin and the reduction of the nonspecific protein adsorption was excellently improved ranging form 56% to 90% to the level of 0.2 μg/cm^2. This generic modification can be a promising method for improving protein resistant property of hydrophobic substrates. Furthermore, by choosing the good solvent, surface modification of PDMS substrate by both copolymers can be successfully conducted. Moreover, the PMED is more effective for modification of PDMS substrate.

Secondly, I studied another versatile approach to prepare nonbiofouling surface bearing brush type poly(MPC) on polymeric substrates based on surface-initiated living radical graft polymerization with keeping in mind that this type of surface could repress nonspecific protein adsorption to the nano level (ng/cm^2). Thus, macrophotoiniferter composed of ethyl hexyl methacrylate (EHMA) and vinyl benzyl *N,N`*-diethyldithiocarbamate (VBDC) was synthesized. The chain density was controlled by changing the composition of *N,N`*-ditethyldithiocarbamate on the photomacrophotoiniferter while the chain length was controlled by changing photoirradiation time. The characterization of the poly(MPC) modified surfaces was conducted by static and dynamic water contact angle, x-ray spectroscopy (XPS), ATR-IR, atomic force microscope (AFM), and ellipsometry. The nonspecific protein adsorption test was conducted by contacting with single protein solutions and binary protein solution of fibrinogen and albumin and the amount of adsorbed protein was determined by using micro BCA method. The dry thickness and molecular weight of poly(MPC) grafted on the surfaces increased according to photoirradiation time although the livingness of the process could only be maintained up to 1 hour photoirradiaition time. The hydrophilicity of the modified surfaces was greatly improved as both water static and dynamic contact angle showed very low contact angles due to the complete covering of the surface by poly(MPC) chains especially in the higher chain density (high composition of photoiniferter). The morphology of the surfaces varies depending on the photoirradiation time and chain density where the smooth surfaces were generally generated on medium chain density and chain length of poly(MPC). Total nonspecific protein adsorption was effectively suppressed on moderate to high poly(MPC) chains, moderate chain length and smooth surfaces to the level of 0.2 μg/cm2. The mechanism of the protein adsorption from single solutions and binary solutions of fibrinogen and albumin was briefly studied based on the protein adsorption mechanism proposed by some group of researches.

This approach can also be applied for modification of many kinds of polymeric substrates although the compatibility of solvent to the macrophotoiniferter and substrates must be carefully taken into account.

The cell adhesion tests revealed that all the poly(MPC)-modified surfaces excellently inhibit cell adhesion of fibroblast-like cell (L929) assuring the nonbiofouling properties of the modified surfaces.

For application in microchip devices, modification of microfluidic devices for inhibiting nonspecific protein adsorption (biofouling) have been conducted by the two approaches; by simple coating with MPC copolymer (PMB and PMED) and by brush type poly(MPC). The simple coating method have offer a one step modification for PDMS-based microchannel device as the protein adsorption resistance was greatly improved. I found also that PMED was much more effective in covering the nature of PDMS. Further, nonbiofouling interface in PET-based

microchannel was also created by constructiong brush type poly(MPC). The best conditions of modification in this microchannel were seemingly different with the ones in modification plat substrates due to the UV light intensity and the thickness of PET substrate. Thus, the best conditions for PET-based microchannel interface modification need to be addressed. Further, construction of micropatterned nonbiofouling and biorecognition interface can be done easily in ambient condition.

In the future, the mechanism of protein adsorption on the rough surface should be done in odder to get the clear insight of the fact that the nonspecific protein adsorption was still severe on the rough interface. Creating dual function surface which has nonbiofouling part and biorecognition part using the living radical polymerization based on dithiocarbamate can be easily conducted on the polymeric substrates. Therefore, the ability to perform immunoassay-based microarray biosensors (immunobiosensors) could be conducted and should be studied. The challenge to perform biosensors inside the microchannel also needs to be addressed.