

POLYMER BRUSHES FOR BIOAMTERIALS

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Abstract - Surface-initiated living radical polymerization has been used to graft well-defined polymers with an extremely high graft density, and such densely grafted polymers have been classified as the “concentrated” polymer brush (CPB), which exhibits characteristic structure and properties (Scheme 1). One of the most interesting and promising applications of the CPB is believed to be as a biointerface to tune the interactions between material surfaces and our body (e.g., body fluids or tissue). Especially, it is important to prevent non-specific protein adsorption on biomaterials, because the adsorbed proteins often trigger subsequent biofouling, namely, cell adhesion. Therefore, first, I elucidated the mechanism of interactions between proteins and concentrated brushes. Then, I confirmed the excellent bioinertness of the concentrated polymer brushes. In addition, I developed bioactive surface combining the bioinert CPBs and biofunctional moieties. In this presentation, I will provide an overview of my research on CPB-based biointerface.

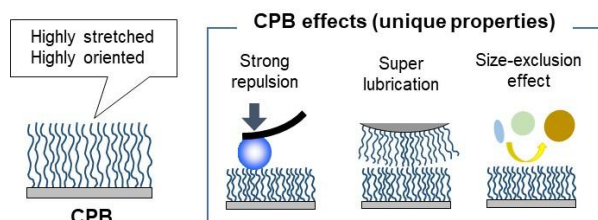
Keywords: Concentrated polymer brushes, living radical polymerization, biointerface, biomaterials.

Introduction

The control of interactions that occur at the interface between materials and their biological environments is of outstanding importance in a broad range of biomedical applications, ranging from biosensors to implantable medical devices. Therefore, a variety of polymer coating techniques have been extensively studied.

Recently, living radical polymerizations have been successfully applied to graft polymerization, leading to the development so-called “concentrated” polymer brushes (CPBs). In a good solvent, the CPBs are highly extended nearly to their full contour length because of their high-dense grafting. As a result, the swollen, extended CPBs exhibit unique properties such as high strong repulsion, super lubrication and size-exclusion effect, which have never been observed on semi-dilute brushes (SDPBs).

Focusing on the unique structure and properties of the CPBs (Scheme 1), I aimed to develop a novel biointerface which can reduce or prevent biofouling (bioinert property)²⁻⁵ and to selectively bind a target biomolecule (bioactive property)⁶. In this presentation, I will present an overview of my current research.



Scheme 1. Structure and properties of concentrated Polymer brush (CPB).

Experimental

Preparation of CPBs on Si wafers.

First, fixed initiators (BPE) for atom transfer radical polymerization (ATRP) were immobilized on the surface of silicon wafers ($1 \times 1 \text{ cm}^2$). Next, SI-ATRP of biocompatible monomers such as HEMA and PEGMA were carried out as previously reported.²⁻⁴ A representative SI-ATRP reaction of HEMA is described as follows. A methanol solution containing HEMA (4.5 M), Cu(I)Br (25 mM), 2,2'-bipyridine (bpy) (63 mM), and the free initiator EBIB (22.5 mM) was prepared in a glove box purged with nitrogen. The polymerization solution and BPE fixed silicon wafer were placed in a glass tube with a stopcock and heated in a shaking oil bath at 30°C for a prescribed time.

Protein adsorption test.

Protein adsorptions on the CPBs grafted on Si wafers were quantified by quartz crystal microbalance (QCM).

Cell adsorption test.

Cells were harvested according to the manufacturer protocol. Then, cells were seeded on the CPBs grafted on silicon wafers. After incubation for the prescribed time, actin filaments and nuclei of the adherent cells were stained by Phalloidin and DAPI coloring in red and blue, respectively.

Results and Discussion

Protein repellency on the CPBs.

The adsorption of proteins on poly(2-hydroxyethyl methacrylate) (HEMA) brushes was systematically investigated by QCM as a function of graft density (the graft density $\sigma = 0.01, 0.1$, and 0.7 chains/nm^2) and protein size (2 - 13 nm in an effective diameter $2R_g$).² As shown in Figure 1, despite the same polymer was used, the CPB much reduced protein adsorption compared with the SDPB. I also investigated protein adsorptions on PPEGMA and PHEA brushes. Importantly,

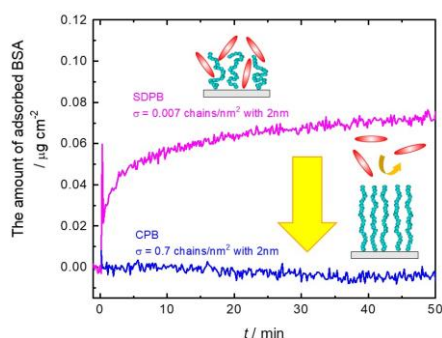


Figure 1. Amount of adsorbed BSA on PHEMA CPB (blue line) and SDPB (pink line).

independent of chemical structures, CPBs prevented protein adsorptions much lower than SDPBs.⁴

The mechanism of interaction between proteins and CPBs (size-exclusion effect of CPBs).

Even if proteins are completely excluded from the brush layer, they may still interact with the outermost surface of the brush. By its principle, QCM may fail to correctly detect a rather weak affinity interaction with the swollen brush. In order to clarify this issue, I made a chromatographic study using a double-pored silica monolithic column (with mesopores of ca 80 nm) modified with a well-defined, concentrated PHEMA brush.³ The modified monolithic column was characterized by eluting pullulans with different molecular weights, clearly showing the additional size-exclusion mode by the concentrated brushes (figure 2(b)). This mode gave a sharp separation with a critical molecular weight (a size exclusion limit) of about 1000, the size of which was comparable to the distance between the nearest-neighbor graft points. Then, the elution behavior of proteins with different molecular weights suggested that the interaction of the concentrated PHEMA brush with proteins is undetectably low at the outermost surface but significant inside (open circles in figure 2). These results indicate that the size exclusion of the concentrated brushes plays an important role in protein repellency, namely, bioinertness.

Prevention of cell attachment on the CPBs.

The first biological event at interface between material and body fluids is non-specific protein adsorption, which typically triggers cell adhesion and subsequent biological reactions. Since CPBs showed excellent protein repellency, they are expected to suppress cell adhesions. To confirm this, I seeded human umbilical vein endothelial cells (HUVEC) and L929 fibroblast cells on CPBs and SDPBs of PHEMA, poly(2-hydroxyethyl acrylate) (PHEA) and poly{(polyethylene glycol monomethyl ether) methacrylate} (PPEGMA). After the incubation for 1, 4 and 7 days, the attached cells were fixed on the brush samples and counted. Cells significantly adhered to all

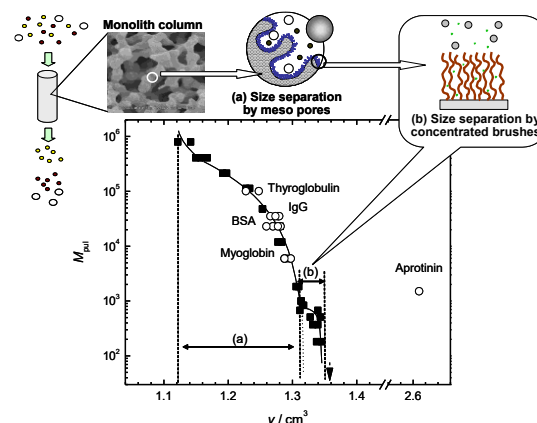


Figure 2. Plot of molecular weight M_{pul} vs elution volume v for pullulans (filled squares and solid curve) and proteins (open circles) eluted through the PHEMA-grafted monolithic columns. The molecular weights of the proteins are the reduced value independently determined by pullulan-calibrated GPC. The arrow heads show the so-called ghost peaks of the eluent. The flow rate was 0.2 ml/min with PBS as eluent at room temperature. The inset shows a cartoon illustrating the size-exclusion mode by the brush.

the SDPBs at similar levels as on the cell culture PS dish. In contrast, all the CPBs, regardless of polymer types, showed suppression of cell adhesions. This was attributed to the excellent protein repellency of the CPBs.

Conclusions

Due to space constraints, I was unable to provide details; however, I also demonstrated *in vitro* and *in vivo* blood compatibility of CPBs.⁵ In addition, I developed a highly sensitive diagnostic immunoassay (for selective capture of the target antigen) by combining bioinert CPB and antibody.⁶

Given the wide range possibility for designing CPB architecture and their unique properties, I believe that bioinert and bioactive CPBs would be useful in biomedical device applications.

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