Protein adsorption on poly(methacrylic acid) modified silicon nanowire arrays

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Statement of Purpose: Controlled protein adsorption and desorption by manipulation of environmental conditions for biomedical and biotechnological applications including controlled drug delivery, protein separation, and biosensors are of great importance. Persistent efforts have been made to design such smart materials, especially by surface modification with stimuli-responsive polymers. Among them, poly(methacrylic acid) (poly(MAA)) is a typical weak polyacid, and it has been reported that for surfaces grafted with poly(MAA) change of pH leads to change of conformation and surface wettability,[1] which in turn influences protein-surface interactions. The introduction of nanostructure is believed to be an effective way to enhance the stimuli-responsiveness of surface properties.[2] Accordingly, we have designed a new and effective system for immobilization of proteins and pH-responsive protein adsorption and desorption.

Methods: The silicon nanowire arrays (SiNWAs) investigated in this study were prepared by chemical etching of silicon wafer in $AgNO_3$/HF aqueous solution.[3] Then the initiator was immobilized on the SiNWAs (with "apparent" surface area of 0.5 cm^2 for each disc) followed the procedures reported previously.[4] SI-ATRP grafting of tBMA was carried out on SiNWAs to produce SiNWAs-poly(MAA). Protein adsorption from PBS solution (1 mg/mL) was determined by radiolabeling with ^{125}I using a Wizard 3" 1480 Automatic Gamma Counter.

Results: Protein adsorption on the materials was detected by using two proteins, fibrinogen and lysozyme, with pI at 5.5 and 11.0 respectively, showing much different charge characteristics in physiological environment. It was found that both proteins had a very high binding amount (about 100 μg/disc, or 200μg/cm^2) on SiNWAs-poly(MAA), and the amounts of both fibrinogen and lysozyme adsorbed on SiNWAs-poly(MAA) at pH9.0 were less than 5% of those adsorbed at pH4.0. (Figure 1). The results suggested that SiNWAs-poly(MAA) had high capacity binding of proteins at pH4.0 and lower adsorption at pH9.0 regardless of the charges they carried. Such pH-sensitivity of protein adsorption on these materials may be mainly due to hydrophobic interactions and hydrogen bonding as the conformation of the polymer chains transitions other than electrostatic interactions.

To investigate whether the protein adsorbed at pH4.0 could be released by change of pH to 9.0, and if the pH-sensitivity of this material maintained during the change of pH buffers, SiNWAs-poly(MAA) samples after adsorbing lysozyme at pH 4.0 were incubated in PBS at pH 9.0 for 3 h and the quantity remaining adsorbed was measured. It was found that nearly 90 % adsorbed lysozyme was released from substrate into solution by simply increasing the pH. As shown in Figure 2, the process of adsorption and release by pH change can be repeated over cycles. This behavior may be of interest for immobilized enzyme systems.[5]

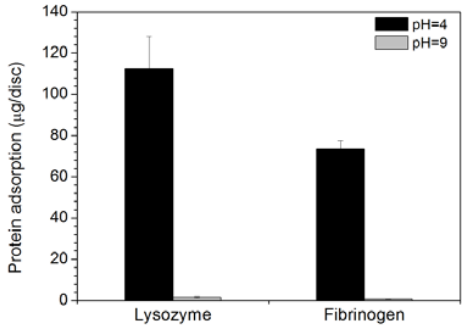

Figure 1. Lysozyme and fibrinogen adsorption on SiNWAs-poly(MAA) surface. Data are means ± standard error (n=3).

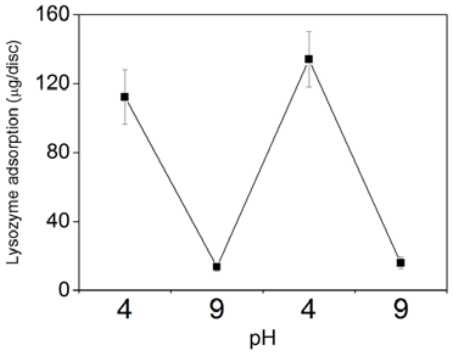

Figure 2. Reversible binding of lysozyme on SiNWAs-poly(MAA) surface via pH switching. Data are means ± standard error (n=3).

Conclusions: In summary, a new material based on poly(MAA) modified nanoscale substrates for pH-controlled reversible immobilization of high amount of proteins is demonstrated. Combining the pH-sensitivity of poly(MAA) chains and an enhanced local topographic effect of the 3D nanostructured SiNWAs, such pH-controlled reversible binding of proteins is of great importance for many biomedical and biotechnology applications including bioseparations and biosensors.

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