QUANTITATIVE EVALUATION OF DYNAMIC COATING ON PLASTIC MICROCHIPS FOR PREVENTING PROTEIN ADSORPTION

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ABSTRACT

In the current research we studied coating efficiency of series of water soluble polymers with different hydrophilicity on microchip made of Poly(methyl methacrylate) (PMMA). Various characteristics of the dynamic coatings such as the efficiency for suppression of protein adsorption and electroosmotic flow (EOF), improving hydrophilicity of the microchannel surface and resolution for separation of protein samples were studied. The results give a better understanding of the interaction of protein samples to the PMMA surface.

KEYWORDS: Dynamic coating, Microchip electrophoresis, Poly(methyl methacrylate)

INTRODUCTION

Polymeric microchips have tremendous potential as disposable devices; however microchip electrophoresis of proteins presents a serious problem when using untreated polymeric microchips due to protein adsorption onto the wall of the microchannels. Adsorption of protein to the microchannel in turn will cause several problems such as peak broadening, non-uniform ζ–potential over the length of the microchannel and unstable EOF. To avoid these negative effects, the surface in the most commonly used polymeric microchip must be modified and dynamic modification of the microchannel surface is a simple and reproducible method. Hydrophobic polymers such as methylcellulose (MC) or polysaccharides which do not adsorb strongly to the normal capillary surfaces are very well adapted to hydrophobic surfaces of polymeric microchips [1, 2]. Comparison of the efficiency of different dynamic coatings can lead to a better understanding of the mechanism of protein adsorption and will help to chose an appropriate coating [3].

EXPERIMENTAL

In this research, first we measured the amount of fluorescent protein which adsorbed to uncoated PMMA microchips. The adsorbed protein was recovered from the chip surface using a solution of 10% SDS. Then the amount of protein adsorbed to the microchannel in presence of 0.2% of MC, Hydroxypropyl methylcellulose (HPMC), Polyvinyl alcohol (PVA), polyvinylphenol (PVP) and co-coating by MC-Tween-20 were measured. Further characterization of the dynamic coating on the PMMA surface was done by measuring water contact angle and EOF. The details of these methods could be found in our previous publications [1, 2].

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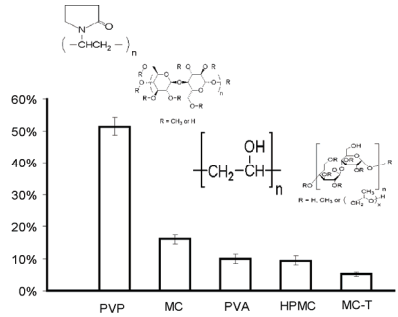

Figure 1. Comparison of adsorbed protein to the PMMA microchannels in presence of different dynamic coatings. The amount of the protein adsorbed to uncoated microchannels was considered as 100% adsorption. (n=5).

RESULTS AND DISCUSSION

The results in figure 1 show that coating using MC-Tween 20 suppresses 95% of protein adsorption and this suppression is 90% for solutions of HPMC and PVA. Hydrophilicity of the PMMA surface was checked by measuring water contact angle. The results in figure 2-a show that a rather hydrophilic surface was achieved using HPMC, MC-Tween-20 and PVA solutions. The EOF mobility for the uncoated PMMA microchannel was $(9.3\pm0.4) \times 10^{-5}$ $cm^2.V^{-1}.s^{-1}$. The results in figure 2-b show that using 0.2% PVA solution, the EOF has almost the same value as uncoated microchip $(8.2\pm0.3 \times 10^{-5}$ $cm^2.V^{-1}.s^{-1})$. But the EOF in presence of 0.2 % of PVP $(1.1\pm0.1\times10^{-5}$ $cm^2.V^{-1}.s^{-1})$, HPMC $(8.0\pm0.4 \times 10^{-6}$ $cm^2.V^{-1}.s^{-1})$, MC $(6.9\pm0.6 \times 10^{-6}$ $cm^2.V^{-1}.s^{-1})$ decreased by nearly one order of magnitude.

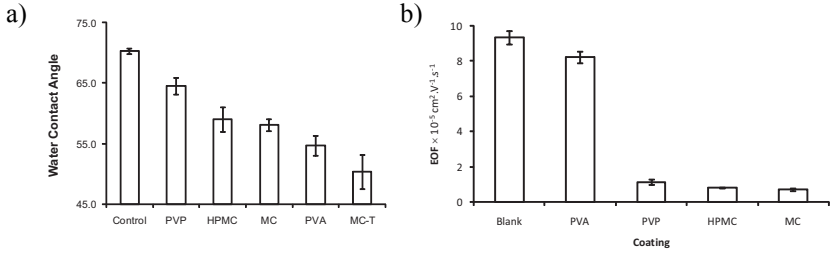

Figure 2. Part a shows measurement of water contact angle on coated PMMA surface in comparison with uncoated PMMA microchip as a control (n= 5). Part b shows EOF measurement in the PMMA microchannel. 450 v was applied in 3.5cm microchannel. The measurements were done in TBE 1x buffer (pH= 8.3). (n = 5).

Figure 3 shows a conclusion from three basic tests for evaluation of a dynamic coating. Using this scheme one can predict that MC-Tween 20 and HPMC are the best choice for suppression of protein adsorption. However the electropherograms in figure 4 show that a better resolution was achieved using PVA solution which has the highest EOF in these series of polymers.

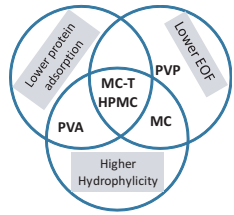

Figure 3. This scheme is concluded from previous two figures. The polymers which have been tested in this paper are categorized based on three most important characteristics of the dynamic coatings.

CONCLUSIONS

The results show that the polymers which have both hydrophobic and hydrophilic parts in their structures are more efficient for suppression of protein adsorption in PMMA microchips. Polymers with more hydrophilic groups such as PVA which may adsorb to the PMMA surface trough their hydrophobic part has less effect for decreasing EOF but can be efficient for suppressing the protein adsorption.

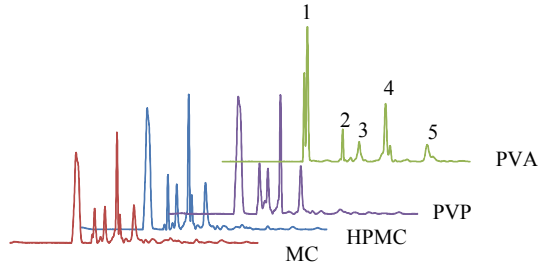

Figure 4. Microchip electrophoresis of FITC-trypsin inhibitor was done under non-denaturing condition in 0.2% of different polymer solutions (Peak 1 free is for FITC, peaks 2 to 5 are different modifications of trypsin inhibitor).

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