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Procedia Engineering 25 (2011) 292 – 295

Procedia Engineering

www.elsevier.com/locate/procedia

Proc. Eurosensors XXV, September 4-7, 2011, Athens, Greece

# Selective immobilization of proteins guided by photo-patterned poly(vinyl alcohol) structures

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**Abstract**

The development of "protein resistant" materials is challenging since protein physisorption takes place on most surfaces due to van der Waals interactions, hydrogen bonding and entropy effects. In this work a new process for converting a surface resistant to protein adsorption is presented by using a photo-patternable poly(vinyl alcohol) (PVA) based film. This material minimizes effectively protein physisorption and it can be patterned through photolithography on top of any substrate. Herein the PVA-based film is patterned on top of a poly(styrene) (PS) film, in order to achieve selective protein patterning on the PS film and demonstrate the resistance of the PVA-based material to protein physisorption. The proposed methodology is expected to facilitate the fabrication of sensors and bioelectronic devices since it provides a patterning route with alignment capabilities for protein resistant-surfaces and it is based on an easy to implement process.



Keywords: photolithography; non-fouling materials; poly(vinyl alcohol); 12-phosphotungstic acid

## 1. Introduction

One of the most important requirements in microarrays applications, and in general in biodevices fabrication and use for analytical purposes, is the patterning of "protein resistant" regions in order to minimize background noise and to avoid false positive signals. This is extremely difficult to be achieved

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1877-7058 
doi:10.1016/j.proeng.2011.12.072

since most solid substrates tend to adsorb proteins in a non specific manner, due to the several protein active groups that can interact with the substrate [1].

Poly(ethylene glycol) (PEG) based materials are commonly used to inhibit non-specific protein adsorption. Their function is attributed to the high hydrophilicity and free movement of attached PEG molecules. Thus, the passivation of sites with PEG silanes is reported in literature in order the functional layer to be surrounded with non reactive sites [2, 3]. The patterning of protein resistant surfaces is highly desirable to facilitate device fabrication and for this reason numerous techniques have been proposed for patterning PEG-based materials. The deposition of PEG silane on specific regions of a poly(*N*-hydroxysuccinimidyl methacrylate) film by microcontact printing has been reported [4]. Tetraglyme (tetraethylene glycol dimethyl ether) deposited by plasma polymerization, can be patterned by standard photolithography and lift-off processes [5, 6]. Atomic force microscopy has been also used for patterning PEG monolayer [7]. Moreover, the coverage of hyperbranched polymeric films with PEG has been reported by Crooks and his co-workers [8, 9].

In this work, a photo-patternable PVA-based material is proposed as a "protein resistant" surface. Particularly the material is based on a PVA film with dispersed molecules of the Keggin-type 12-phosphotungstic acid. This material can be easily spin-coated to any substrate and patterned by photolithography. The proposed methodology is expected to find wide applications in protein patterning since the materials used in the proposed procedures are commercially available, the films of photo-patterned PVA can be easily prepared by spin-coating to any substrate and the patterning is achieved through photolithographic processes that can be easily implemented in microsystems fabrication laboratories, using already available equipment.

## 2. Materials and Methods

12-Phosphotungstic acid hydrate $H_3PW_{12}O_{40}{}^{x}H_2O$, PVA (Mw 13,000-23,000 98% hydrolyzed) and PS (Mw 280,000) were obtained from Aldrich. The 12-phosphotungstic acid is a polyoxometalate and it will be referred as POM. The poly(styrene) solution contained 2% (w/w) PS in methyl isobutyl ketone. The film of the PS was applied after the modification of the silicon surface by hexamethyldisilazane, which is used as an adhesion layer. The PS film was spin-coated at 2000 rpm for 1 min and thermally treated at 120$^{o}$C for 5 min.

The PVA-based photo-patternable material contained 4.3% (w/w) PVA and 14.4% (w/w) POM. The solution of the PVA-based material was spin-coated on modified silicon surfaces at 4000 rpm for 30s and thermally treated at 70$^{o}$C for 1 min (Post Applied Bake, PAB). Then, the films were exposed to deep UV light through a broadband 254nm filter (with 50nm bandwidth) by using a DUV Oriel Hg-Xe lamp. During the photolithrographic process, the films were exposed in a contact-printing mode. Afterwards the films were thermally treated at 88$^{o}$C for 3 min (Post Exposure Bake, PEB). Finally the development of the films was performed in a mixture of methanol and water in order to completely remove the unexposed regions and the POM molecules from the final film as well. Always fresh solutions were used in order to avoid problems due to hydrolysis of PVA in an aqueous environment or stability problems of the PVA solutions.

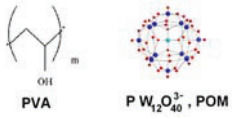


Fig. 1. The components of the photoresist.

Biotinylated BSA (25 μg/mL solution in 0.05 M phosphate buffer, pH 7.4) was immobilized for 1 h on PS-coated silicon wafers and patterned PVA films on top of them. Then, the wafers were washed

with blocking solution and immersed in blocking solution (0.1 g/L BSA in 0.05 M phosphate buffer, pH 7.4). After 1 h the samples were removed from the blocking solution, washed firstly with phosphate buffer and then with distilled water and dried on a $N_2$ stream. Afterwards, they were immersed in a 5 μg/mL AF® 546 labelled streptavidin solution in the blocking solution and incubated for 30 min. Finally, the wafers were washed extensively with 0.05 M phosphate buffer, pH 7.4, followed by washing with distilled water and dried on a $N_2$ stream.

Fluorescence images were taken using the Axioskop 2 plus epifluorescence microscope (Carl Zeiss, Germany) equipped with a Sony Cyber-shot digital camera. The fluorescence intensity on the biotinylated BSA coated areas was determined quantitatively using the Image ProPlus software (Media Cybenertics Co.) as the average fluorescence intensity per spot area. The net fluorescence value provided by the specific protein spots was determined by subtracting the values obtained by areas of the same wafer piece coated only with blocking protein.

## 3. Results

The cross-linking of PVA can be achieved through the photoreduction of POM caused by exposure at deep UV which provokes the oxidation of PVA. The oxidized PVA undergoes dehydration during PEB forming dienophiles and olefins, which are subsequently condensed via Diels-Alder reactions leading to PVA cross-linking (Figure 2a) as it has been reported in the past when a similar material had been proposed as a deep UV photoresist [10].

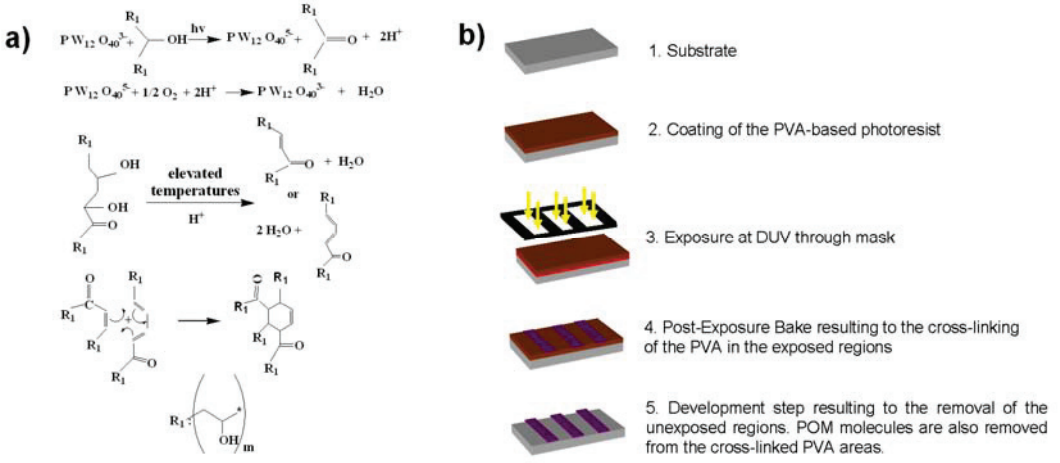


Fig. 2. a) Cross-linking of the PVA initiated by the photoreduction of POM and b) photolithographic scheme of the PVA process.

In the current investigation after the cross-linking of the PVA, which takes place during the exposure and the post-exposure bake steps, a modified development step is performed. In that step both the removal of the unexposed regions and the POM from the cross-linked polymer matrix take place. The lithographic process is schematically presented in Figure 2b.

The cross-linked PVA film obtained with the above process was evaluated regarding its use as protein resistant material. Therefore different coating times were tested. Complete absence of protein immobilization was observed at coating times higher than 30 min where equilibrium between the protein solution and the cross-linked PVA film interface has been restored. It is believed that the cross-linked

PVA films resist to protein physisortion in a way similar to PEG i.e. through the high hydrophilicity of the film containing a significant number of hydroxyl groups.

The photolithography of PVA was then performed on a PS film which favours protein adsorption. The patterned substrates containing PS and cross-linked PVA regions were evaluated for selective protein adsorption through the immobilization of biotinylated BSA. The immobilized biotinylated BSA was detected after reaction with streptavidin labeled with AlexaFluor 546 under a fluorescence microscope. As shown in Figure 3, biotinylated BSA was immobilized only on the PS regions. Furthermore the fluorescence signal from the cross-linked PVA structures was negligible proving the "protein resistant" capability of the proposed material.


Fig. 3. Patterning of biotinylated BSA to the polystyrene 40 um and 10um lines. The black regions correspond to the cross-linked PVA structures, where no fluorescence signal is detected.

## 4. Conclusions

Selective protein immobilization was achieved by creating structures of cross-linked PVA on top of a PS film. The components of the proposed materials are commercially available and the developed photolithographic process is simple, fast and is expected to facilitate applications requiring patterned protein resistant regions. Moreover, the cross-linked PVA film exhibits very good stability in aqueous solutions used for protein adsorption experiments. In addition, the proposed procedure can be applied on any substrate which favours protein adsorption.

## Acknowledgements

P. Pavli thanks the State Scholarships Foundation of Greece for her financial support.

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