Surface modification and characterization of a cyclic olefin copolymer for magnetic bead-based stop-flow microfluidic ELISA

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ABSTRACT

The Enzyme-Linked Immuno-Sorbent Assay (ELISA) is the current standard for biomarkers' quantification. Adapting ELISA into a microfluidic format can further reduce the volume of reagent, duration and cost required for the completion of the assay. An innovative platform was developed for the realization of simultaneous microfluidic ELISA in stop-flow conditions. Magnetic micro-beads were used to overcome the problem of transport limitation. The design and signal detection method were tested in PDMS chips, allowing for the quantification of anti-streptavidin with low picomolar sensitivity. Nevertheless, the non-specific adsorption (NSA) of protein on PDMS has so far limited the platform to the off-chip preparation of the reactive beads. Consequently, thermoplastic materials are being investigated to fabricate a next generation of chip with increased resistance to NSA. Primary results relative to the surface characterization of a cyclic olefin copolymer (Zeonor 750R) following exposure to various duration of oxygen plasma or UV-ozone are presented in this report. Both treatments were found to reduce the hydrophobicity of the native polymer although with different effects on the physical and optical properties of the treated material.

Keywords: microfluidic ELISA, magnetic beads, thermoplastic, non-specific adsorption of protein, surface modification and characterization.

1 INTRODUCTION

The Enzyme-Linked Immuno-Sorbent Assay (ELISA) is the current standard for biomarkers' quantification. This method, although very sensitive, requires large volumes of reagents as well as long incubation times due to the high volume-to-surface ratio. The use of microfluidic to perform sandwich immunoassays permits to specifically address these drawbacks through the reduction of volumes and diffusion distances.

Although the number of reported microfluidic platforms for immunoassay is growing very fast, most systems are limited by the difficulty to control the fluidic flow, the increase in volumes used due to the poor exploitation rate of the analyte and by inadequate surface properties of the channel walls. Consequently, we developed a platform for microfluidic ELISA featuring an original design for the realization of multiple simultaneous assays in stop-flow conditions. The use of magnetic micro-beads allows to increase the active surface area and to generate an internal mixing, thus accelerating the analyte exploitation in a transport-limited regime.[1] Working in stop-flow conditions rather than in continuous flow eliminates the necessity for the precise control of extremely slow flow rates and lowers the volumes of reagent required for the assay.

The first generation of the microfluidic structure was fabricated in Poly(dimethylsiloxane) (PDMS) in order to test the detection procedure.[2] Reactive streptavidin-coated magnetic micro-beads were prepared by incubating them first with various concentrations of anti-streptavidin antibodies (analyte), followed by incubation with Alkaline Phosphatase coupled-secondary antibodies (fig. 1).

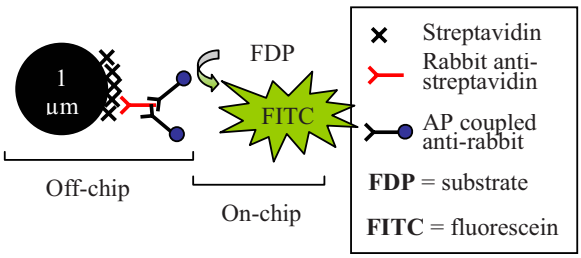

Figure 1: Schematic of the analytical concept for the quantification of anti-streptavidin antibodies.

The beads were then injected and captured into the reaction chambers by an external rare earth magnet. Next, the channels were rapidly filled with a solution of FDP (enzymatic substrate). After a short incubation period with magnetically induced mixing, the reactions were stopped simultaneously by moving the solution away from the reactive magnetic beads into the downstream detection area. Using this procedure, anti-streptavidin antibodies were quantified with low picomolar sensitivity (0.1 – 6.7 pM), a linear range of about 2 order of magnitude and good reproducibility (fig. 2). However, the strong noise

generated by the non-specific adsorption (NSA) of proteins on the PDMS channels has so far limited the platform to the off-chip preparation of the reactive beads.

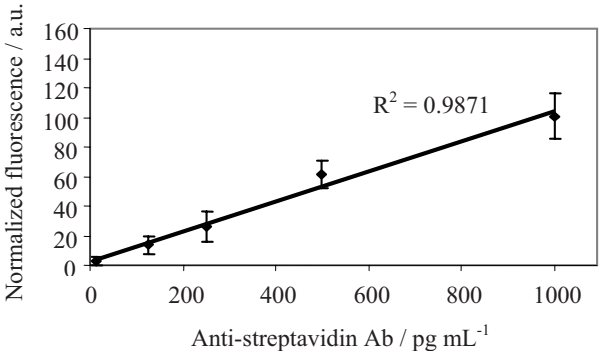

Figure 2: Quantification of anti-streptavidin antibodies for concentrations ranging from 12.5 pg mL^{-1} (0.1 pM) to 1000 pg mL^{-1} (6.7 pM).

In micro-channels, the surface-to-volume ratio is much larger than in typical macroscopic systems. The importance of interactions between the walls, the solution, and any molecules present in this solution is thus greatly amplified. In particular, the non-specific adsoption of protein on the channel walls can cause an important loss of sensitivity in immunoassays. Therefore the choice of the material for the fabrication of the microfluidic device is of primary importance. Biochips have been previously realized in hard materials such as glass or silicon.[3,4] The trend is however to use polymers since they are less expensive and easier to fabricate using soft-lithography techniques. In particular, PDMS has been widely used for microfluidics since the technique of replica molding has been introduced by Duffy *et al.*[5] Since then, many techniques have been reported to decrease the adsoption of proteins on PDMS.[6-8] Although suitable for most applications, the lack of reproducibility and stability of modified PDMS limits its use for sensitive ELISA, where both the specific and unspecific signals are amplified.

Thermoplastics are inexpensive polymers that can be rapidly micro-fabricated by hot-embossing[9] and chemically modified following the appropriate surface treatment.[10] Therefore, these polymers are currently being investigated to fabricate a next generation of microfluidic ELISA chip with increased resistance to NSA. Cyclic olefin copolymers (COC) appeared to be good candidates for this application due to their suitable optical properties and strong chemical resistance.[11,12] In this study, Contact angle measurement, infra-red spectroscopy, atomic force microscopy and fluorescence microscopy were used to characterize the effects of oxygen plasma and UV-ozone treatments on the commercially available polycycloolefin Zeonor 750R.

2 MATERIALS AND METHODS

2.1 Surface treatment of Zeonor 750R

Samples of 1 cm^2, 1 mm thick, were cut from Zeonor 750R (Z750R, Zeon Corporation) disks produced by injection molding. The pieces were rinsed with methanol and acetone and dried under nitrogen. Plasma oxidation was performed in a Plasmalab 80 Plus (Oxford Instruments) at 30 sccm, 60 mbar O_2 and 150W.[12] UV-ozone treatments were realized in a UV TipCleaner (Bioforce Nanosciences).

2.2 Contact angle measurement

Surface wettability was determined by measuring the static contact angles of deionized water (DI) using a surface analysis system equipped with image analyzer software (VCA Optima 2500, AST Products). An auto pipette was used to ensure a uniform volume of DI water droplet (0.5µl). Each drop was allowed to equilibrate on the surface for 20 seconds before the picture was acquired. The experiments were run at room temperature on 2 identical samples at 6 different positions on the sample surface.

2.3 Fourier Transform IR Spectroscopy

Fourier Transform Infra-Red (FTIR) measurements were realized on a Spectrum One FT-IR Spectrometer equipped with the Universal ATR Sampling Accessory (Perkin Elmer). Each spectrum resulted from the average of 16 repeated scans with a resolution of 4 cm^{-1}.

2.4 Atomic Force Microscopy

Atomic Force Microscopy (AFM) analyses were performed using a Nanoscope IIIa (Digital Instruments) employing tapping mode in air. The images of 1 µm x 1 µm were obtained at a scan frequency of 1 Hz and analyzed with Nanoscope 5.12r5.

2.5 Fluorescence imaging

An inverted microscope (TE2000-U, Nikon) equipped with a high pressure mercury lamp (C-SHG1, Nikon) and a digital camera (DXM1200F, Nikon) operated with the ACT-1 software were used to acquire the fluorescent pictures. The intensities of fluorescence were measured with ImageJ (software for image analysis in Java).

3 RESULTS AND DISCUSSION

Flat surfaces of Zeonor 750R have been exposed to various durations of either oxygen plasma or UV-ozone. Both treatments have induced drastic changes in the surface wettability of the material. The resulting contact angles with water are listed in table 1.

Untreated		70.2 +/- 4.7		
Oxygen plasma	15''	30''	60''	-
	12.1 +/- 3.5	10.0 +/- 3.0	9.8 +/- 3.6	-
UV-ozone	5'	15'	30'	60'
	48.9 +/- 5.7	34.2 +/- 2.1	21.0 +/- 3.7	20.1 +/- 4.8

Table 1: Static contact angles with deionized water on untreated and treated Zeonor 750R.

The contact angle of native Z750R is about 70°, which demonstrate the rather hydrophobic nature of the polymer. After treatment with only 15 seconds of oxygen plasma, the contact angle dropped to approximately 12°. Longer plasma treatments did not yield to significantly lower values. Treatments with UV-ozone decreased the contact angle to about 20° after 30 minutes, displaying a lesser effect on surface wettability than the oxygen plasma.

The AFM study of samples following the various treatments revealed that the surface topology of UV-ozone treated samples did not exhibit significant change as compared to the untreated control (fig. 3). Conversely, oxygen plasma treated samples displayed an increase in surface roughness, which extent depended on the duration of the treatment. Polymer pieces exposed for 15 and 30 seconds exhibited very similar topography, whereas the surface roughness was further increased after a 1 minute exposure. Based on the AFM data, and considering the similar wettability of the oxygen plasma treated surfaces, the exposure time used for the subsequent experiments was chosen to be 30 seconds.

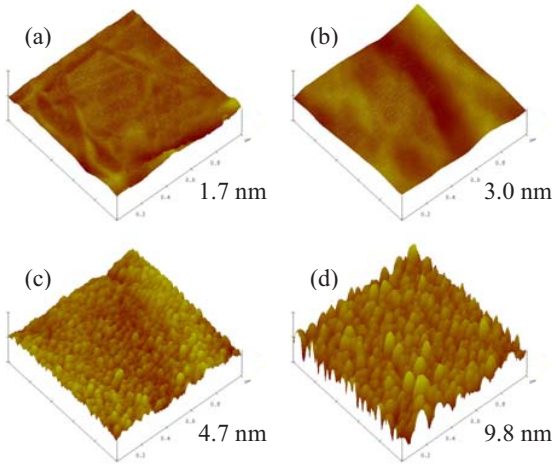

Figure 3: 3D-AFM images and surface roughness (rms) before and after treatment a) untreated, b) UV 30', c) plasma 30'' and d) plasma 60''. Images are 1 x 1 μm with 50 nm units on the z axis.

Interestingly, the treatment with UV-ozone was found to generate a strong auto-fluorescence of the material (fig. 4), which represents an important drawback of this technique for its application in fluorescent immunoassays. One of the main reasons for using COC among other thermoplastics is indeed their suitable optical properties and lack of auto-fluorescence.[11] Further investigations with infra-red spectroscopy revealed a change in the chemical composition of the UV-ozone treated samples, which might cause the observed auto-fluorescence (fig. 5).

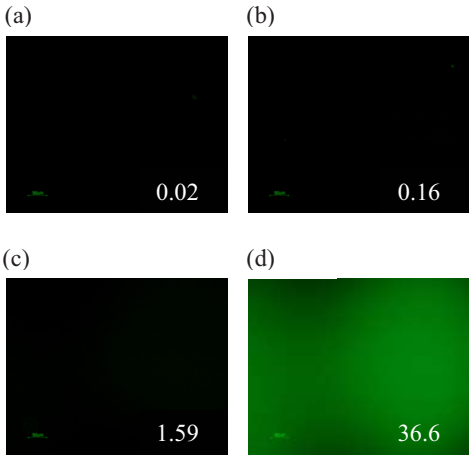

Figure 4: Auto-fluorescence of Z750R for FITC before and after treatment a) untreated, b) plasma 30'', c) UV 5' and d) UV 30'. The average fluorescence intensities were calculated with ImageJ on the entire pictures.

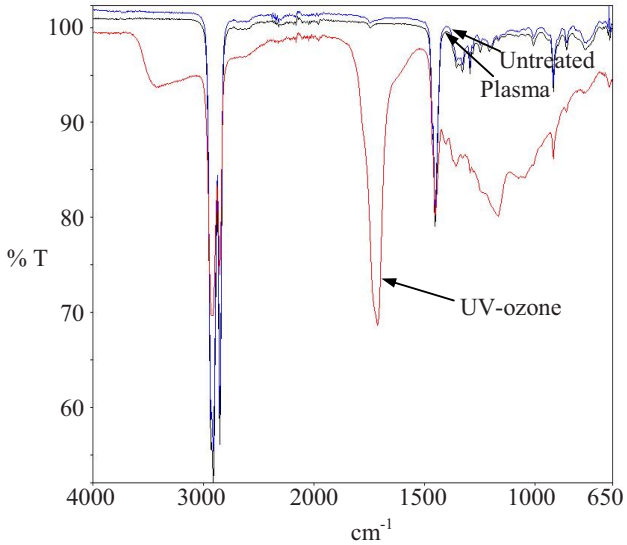

Figure 5: ATR-FTIR spectra for untreated, oxygen plasma and UV-ozone treated Zeonor 750R.

No variations of the spectra were observed between the untreated and the plasma treated samples. However, the spectrum of UV-ozone treated samples displayed three additional peaks respectively at 1170, 1715 and 3420 cm^{-1}. The peak at 1715 cm^{-1} has been correlated with hydrophilic carbonyl groups, which presence could explain the large enhancement of surface wettablity. Since, no such peaks have been detected in ATR-IR spectrum of samples exposed to oxygen plasma, the drastic loss of hydrophobicity could be essentially due to the increased roughness of the material. Yet, it is more likely that the formation of polar groups at the outermost surface, without sufficient penetration to be detected by IR spectrometry, also contributes significantly to this change. XPS analyses are currently undergone in order to determine with more precision the type and density of the functional groups that are generated at the surface of the treated polymer.[13]

These preliminary results demonstrate that both techniques are efficient in reducing the hydrophobicity of the native polycycloolefin. The achieved hydrophilic surface is highly desirable for microfluidic devices and strongly suggest the presence of functionalized groups that can be further utilized to graft protein resistant molecules such as poly-ethylene glycol (PEG).[14] Furthermore, it was found that exposure to UV-ozone can be a simple, affordable and efficient method to decrease significantly the hydrophobicity of thermoplastics for applications that does not use a fluorescent readout. When fluorescence is used for signal detection, exposure to oxygen plasma appeared to be a more appropriate technique, despite the induced roughness that slightly increases the surface area potentially subject to protein adsorption.

4 CONCLUSION

An innovative platform was developed to perform simultaneous microfluidic ELISA in stop-flow conditions by using magnetic micro-beads. The design and signal detection method were tested in PDMS chips, allowing for the quantification of anti-streptavidin with low picomolar sensitivity, a linear range of about 2 order of magnitude and good reproducibility. However, owing to the elevated non-specific adsorption of protein on the PDMS walls, other materials and surface modification methods were considered for the fabrication of the next generation of chips. The thermoplastic Zeonor 750R, a cyclic olefin copolymer, was used to investigate the effects of oxygen plasma and UV-ozone treatments on the properties of thermoplastic COC. Although both of them proved to be effective in reducing the hydrophobicity of the material, exposure to UV-ozone induced deeper changes that rendered the polymer strongly auto-fluorescent. Exposure to oxygen-plasma, on the other hand, generated an increased surface roughness. As a result, the type and duration of surface treatment have to be carefully selected with regards to the application and the readout used for the detection of the signal.

Ongoing work focuses on the determination of the nature and concentration of chemical groups at the surface of the treated polymer, which will be further utilized to graft protein resistant molecules such as PEG. Finally, the resistance to protein adsorption will be compared on non-treated, treated and PEG-grafted samples to determine the optimal surface treatment for a new generation of the microfluidic ELISA chip.

ACKNOWLEDGEMENTS

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