THERMORESPONSIVE POLYMER-BASED MICRODEVICE FOR NANO-LIQUID CHROMATOGRAPHY

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Abstract: We report here on the development of an integrated device for sample desalting and pre-concentration for nanoLC / ESI-MS analysis combining poly-(*N*-isopropyl acrylamide) (PNIPAM) grafted microbeads and the means to dynamically control their temperature. Thermoresponsive properties of PNIPAM induce switchable hydrophobic/hydrophilic surfaces on which peptides can reversibly adsorb and desorb. The device is fabricated on a glass or pyrex substrate with deposited Ti/Au electrodes serving as built-in resistive heating sources. Premolded microfluidic channels and reservoirs made in PDMS are eventually assembled. Electrical and thermal characterization together with multiphysics modeling have been performed. The SiO_2 surfaces of the channels and silica beads used as carriers of the stationary phases have been end-grafted with PNIPAM and employed to study the reversible adsorption and release kinetics of albumin-fluorescein conjugates by fluorescence video microscopy. It is clearly shown albumin-fluorescein complexes adsorb on beads surfaces above the transition temperature of PNIPAM (hydrophobic state), and are released when the temperature decreases (hydrophilic state), yet not fully reversibly.

1 INTRODUCTION

The challenge of proteomics is to develop high-throughput and integrated approaches to identify and understand the structure, functions and interactions of proteins. Nano-liquid chromatography (nanoLC) in combination with electrospray ionization mass spectrometry (ESI-MS) detection has become a major experimental method owing to its high separation power and sensitivity (Ishihama, 2005). In general, proteins are fractionated, isolated and digested into peptides to be analysed and identified by nanoLC / ESI-MS. Miniaturization provides a number of advantages such as low limit of detection, small volumes of analyte required and reduced intermediate manipulation steps (Gauthier and Grimm, 2006). Thus, many efforts have been made to integrate on-chip separation devices providing the column, connection capillaries and MS coupling via a nanospray emitter (Hernández-Borges et al., 2007). Though, due to MS high sensitivity to salts, peptides need to be desalted and concentrated on C4 or C8 columns prior to their analysis (Wilm and Mann, 1996). During this step, certain hydrophobic peptides can be lost on the hydrocarbon surfaces because they show a greater affinity to the stationary phase as compared to the mobile one utilized for desorption of the purified peptides (Peterson et al., 2003).

We propose here a novel approach based on stationary phases prepared from poly(*N*-isopropyl acrylamide) (PNIPAM) that can reversibly adsorb and release peptides upon external activation in a purely aqueous environment. We present the development of an integrated device for sample desalting and preconcentration for nanoLC / ESI-MS analysis, combining PNIPAM grafted surfaces and the means to dynamically control their temperature by integrated microheaters.

2 THEORY

PNIPAM is a stimuli-responsive polymer which undergoes a reversible coil-to-globule transition at its

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lower-critical solution temperature (LCST) around 32°C. PNIPAM grafted surfaces can be switched from a swollen, hydrophilic and non-fouling state to a collapsed, hydrophobic and protein-adsorbing state using thermal actuation (Kanazawa et al., 1996; Huber et al., 2002). Such surfaces have been previously reported for spatio-temporal control of flows in fluidic microsystems by our group (Sudor et al., 2006).

The idea presented here is to use PNIPAM-decorated beads as stationary phases to trap peptides during desalting and pre-concentration steps prior to the nano-LC / ESI-MS analysis. The reversible transition of PNIPAM surfaces upon temperature allows controlled adsorption and release of peptides without the change of quality of a solvent. To increase specific surface of interaction between PNIPAM and peptides, PNIPAM is grafted on micrometric silica beads injected into the channel. The channel height is reduced at its center to block the beads, while its width is widened to preserve the constant surface area.

A resistive heating device is directly integrated on the pyrex subtrate to control the temperature inside the channel. Microfluidic pre-molded PDMS channels and reservoirs are eventually assembled to the substrate to form the final fluidic microsystems (Fig. 1).

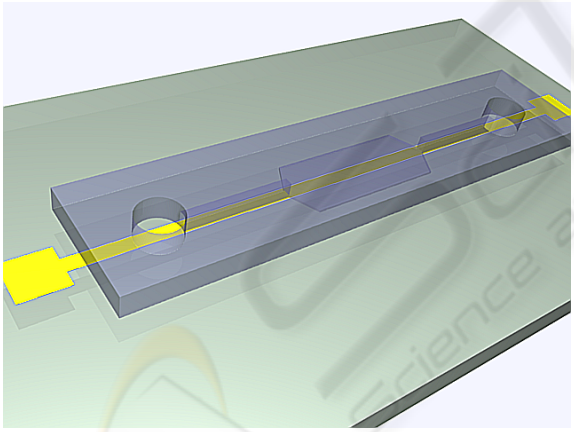

Figure 1: 3D view of the assembled prototype: pyrex substrate, heating line and PDMS channel.

3 EXPERIMENTAL

3.1 Heating Device

Our heating device was made with lines fabricated on a silicon, glass or pyrex substrate with deposited Ti/Au (1000 / 8000 Å) electrodes serving as built-in resistive heating sources. Lines of 100 μm and 500 μm width (respectively 32.5 Ω and 6.75 Ω on pyrex) were realized and characterized. Infrared imaging showed the heated zone was localized around the heater (Fig. 2). Suitable temperatures were obtained for acceptable voltages: 51°C for 4 V (500 μm wide) and 7 V (100 μm wide), given that LCST of PNIPAM is around 32°C. For 500 μm-wide lines around 4 V, we obtained a homogeneous heated zone more than 1.5 mm wide (microfluidic colons are 50 μm wide). Response time in heating is very short (< 1s); cooling happens in seconds.

Multiphysics modeling using Comsol was also performed. First modeling results fit relatively well with experimental data (Fig. 3), however, a slight refinement of the model is still necessary. Work is also underway to develop more complex heating devices allowing more precise control of heated zones (Paumier et al., 2007).

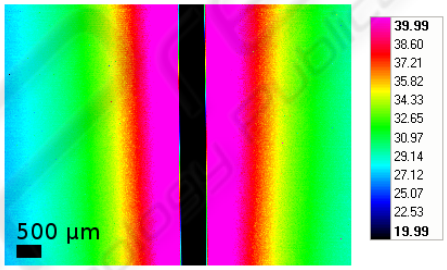

Figure 2: Infrared thermal imaging of the Ti/Au electrode (°C). The resistor appears black because of the infrared reflection on gold.

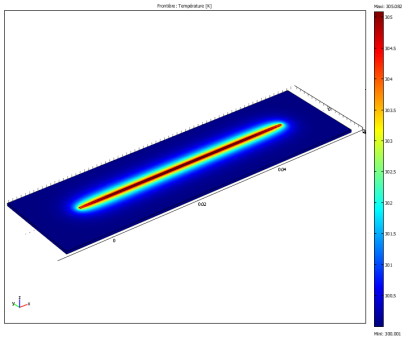

Figure 3: Multiphysics modeling of the heating process using Comsol.

3.2 Surface Chemistry

A 500 nm SiO_2 layer was deposited by plasma-enhanced chemical vapor deposition (PECVD) on the substrate and the electrodes to provide an electric insulator, and to allow homogeneous PNIPAM grafting. The SiO_2 surfaces of the channels and silica beads (used as carriers of the stationary phases)

were end-grafted with PNIPAM according to literature (Hjertén, 1985), through an intermediate silane layer (3-trimethoxysilyl propylmethacrylate). Surfaces were characterized with dynamic contact angle measurements and multireflection infra-red spectroscopy. The latter showed specific peaks identifying chemical groups from PNIPAM (Fig. 4).

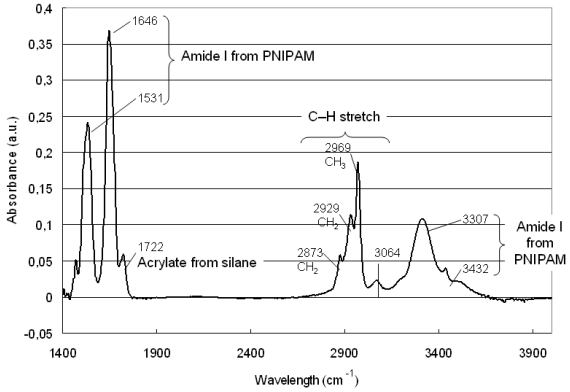

Figure 4: Multiple internal reflection infrared spectrum of surfaces grafted with PNIPAM.

4 RESULTS AND DISCUSSIONS

4.1 Controlled Adsorption/Release of Proteins

To prove feasibility of using beads decorated with PNIPAM to adsorb/desorb proteins, we made our first experiments in capillaries. We used silica beads (5 μm in diameter) end-tethered with PNIPAM chains in fused silica capillaries (100/385 μm inner/outer diameter). The inner surface of the capillary was end-grafted with polyacrylamide (PAM), which is not sensitive to temperature changes in the studied range. Bovine serum albumine (BSA) - fluoresceine conjugate (1 mg/ml) dissolved in sodium phosphate buffer (pH 7) was injected into the capillary. When temperature was increased to about 40°C (above LCST), PNIPAM chains on beads became hydrophobic; they trapped and concentrated BSA-fluorescein conjugates on beads, making them fluorescent, as shown in Fig. 5. By decreasing the temperature below the LCST, BSA-fluorescein conjugates were released into the solution.

The kinetics of reversible adsorption and release was also studied. The graph on Fig. 6 shows the adsorption and release of albumin-fluorescein conjugates on beads. We observed the release of proteins was not fully reversible. A proposed explanation is the low grafting density of PNIPAM chains and consequent protein adsorption on the non-modified surfaces of silica beads.

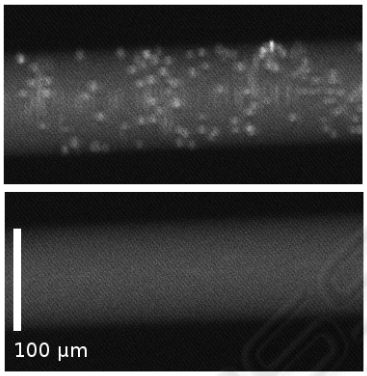

Figure 5: Albumine-fluorescein conjugates adsorbed on 5-μm beads (top, $T > LCST$) and then released (bottom, $T < LCST$). Background fluorescence is due to complexes in solution.

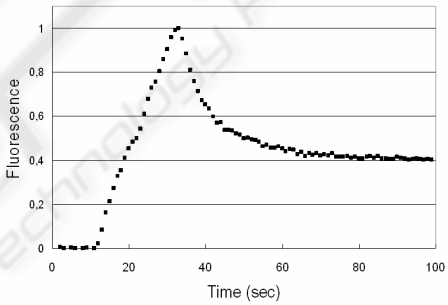

Figure 6: Adsorption/release kinetics of albumin-fluorescein conjugates from glass beads functionalized with PNIPAM (normalized units).

4.2 Beads in Microchannels

Then, we went a step further and injected silica beads inside our PDMS microchannel. Silica beads were not functionalized with PNIPAM at this point. The microchannel was made of three parts. At the center, the channel was 1250 μm wide, 1500 μm long and 4 μm high. Side channels were 50 μm wide, 3000 μm long and 100 μm high each. This geometry allows to trap beads where section changes.

Several sizes of beads and central height of the middle section of the channel were tested and characterized through fluorescence microscopy. When these sizes were too close, beads managed to slip inside the central part, due to PDMS ductility: Young's modulus of PDMS depends on the mixing ratio of elastomer and curing agent but it remains about 10^5 Pa (Armani et al., 1999). We observed this phenomenon with

5 μm beads and 4 μm central height. To prevent this, we injected a limited amount of 10 μm beads prior to the 5 μm beads, that were prevented from entering the central part because of the bigger beads. Experiments with PDMS devices and beads modified with PNIPAM are currently underway.

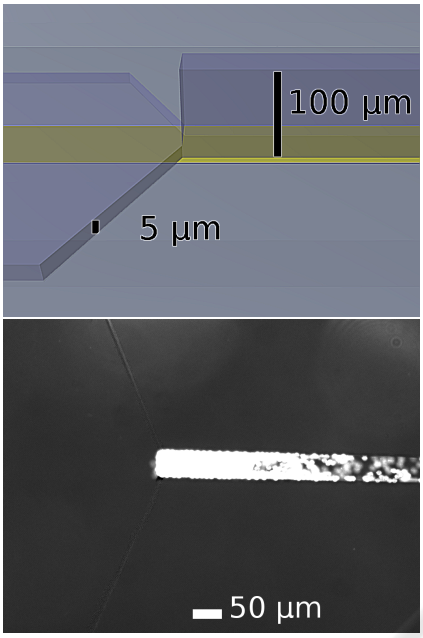

Figure 7: Top: 3D close-up on the channel zone where beads are blocked. Bottom: Top-view of fluorescent 10 μm and 5 μm beads blocked at the entry of the central part.

5 CONCLUSIONS

Thermoresponsive properties of PNIPAM upon temperature are well known and have been demonstrated as switchable surfaces for protein adsorption. We demonstrated in this work the possibility to integrate such switchable surfaces into fluidic microsystems dedicated to sample preparation for nanoLC / ESI-MS. We have developed essential components and know-how about heating sources, reversible protein adsorption and release, and injection of beads in PDMS microchannels. We are now demonstrating the feasibility of the microsystems for desalting and preconcentration of various peptide samples.

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