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Flame Aerosol Deposition of TiO₂ Nanoparticle Films on Polymers and Polymeric Microfluidic Devices for On-Chip Phosphopeptide Enrichment

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Abstract

Direct and fast (10s of seconds) deposition of flame-made, high surface-area aerosol films on polymers and polymeric microfluidic devices is demonstrated. Uniform TiO₂ nanoparticle films were deposited on cooled Poly(methyl methacrylate) (PMMA) substrates by combustion of titanium(IV) isopropoxide (TTIP) – xylene solution sprays. Films were mechanically stabilized by in-situ annealing with a xylene spray flame. Plasma-etched microfluidic chromatography columns, comprising parallel microchannels were also coated with such nanoparticle films without any microchannel deformation. These microcolumns were successfully used in metal-oxide affinity chromatography (MOAC) to selectively trap phosphopeptides on these high surface-area nanostructured films. The chips had a high capacity retaining 1.2 µg of standard phosphopeptide. A new extremely fast method is developed for MOAC microchip stationary phase fabrication with applications in proteomics.

1. Introduction

Flame-deposited [1] and annealed [2] metal oxide nanoparticle films possess a large surface area that facilitates interaction with analyte molecules in gas sensors, offering high sensitivity and fast response/recovery [3]. Deposition of such films has been demonstrated on various substrates, even on thermosensitive ones ($T < 400^{\circ}\text{C}$) such as CMOS integrated devices for gas sensing applications [4].

Large interaction surface is also useful in liquid chromatography. More specifically nanoparticle films are attractive in metal-oxide affinity chromatography (MOAC), where analytes are separated by interacting with the metal-oxide surface. There, a high retention capacity with small mass of stationary phase is necessary. The MOAC is used in proteomics for enrichment and separation of phosphopeptides, which are typically present in small quantities in biological fluids. MOAC is usually performed off-chip using titania packed in pipette tips [5-8]. Commercial tips are available, for example, by GL Sciences (MonoTip®) and by Glygen Corp. (NuTips™).

However, on-chip-based MOAC using microfluidic chips is more attractive for phosphopeptide enrichment, as it can be automated and uses tiny amounts of sample with less handling steps. In addition, polymeric microfluidic chips may be disposable and less costly [9]. For on-chip approaches metal oxides are commonly integrated into LC-MS systems in the form of precolumns filled with TiO₂ spheres [10]. However, the TiO₂ nanoparticle packing of microcolumns or capillaries may be a complex and time consuming step, and requires frits to prevent loss of the nanoparticles with liquid flow and will create

high pressure drop across the column due to their nanoporosity. For this reason deposited nano-TiO₂ films would be preferable as stationary phases [11]. We have recently demonstrated liquid-deposited TiO₂-ZrO₂ microcolumns [12] on PMMA. Nevertheless, liquid deposition is a time consuming method (several minutes to hours), which may therefore be costly for production of disposable polymeric microfluidic chips.

Gas-phase (aerosol) synthesis and direct deposition on the other hand is an attractive alternative as it reduces the steps and time needed to produce the nanoparticle films [13], thus potentially reducing cost of the final product [14]. With the flame spray pyrolysis (FSP) process used here, the particle size is easily controlled in a wide range from 5 to 100 nm offering high specific surface area, and a wide range of single- and mixed oxide nanoparticles are usually crystalline [13]. While particle formation takes place at the high temperature of the flame, the deposition can take place at much lower temperature downstream [14].

Here, we show, for the first time to our knowledge, the successful direct flame aerosol deposition of TiO₂ nanoparticle films on polymers and on polymeric microfluidic devices without substrate damage or microchannel deformation. In addition it is shown that in-situ flame annealing can be used to mechanically stabilize the films [2] in order to withstand liquid flow. Films in microchannels were used as stationary phase for MOAC. It is also shown that phosphopeptides are trapped in acidic environment in such chips having a high capacity, and are eluted-recovered in alkaline environment.

2. Experimental procedures

Flame aerosol direct deposition of titanium dioxide nanoparticles films on polymer substrates was performed with a laboratory scale FSP unit [15] described elsewhere [16] in combination with a water cooled substrate holder [1]. A titanium-tetraisopropoxide solution in xylene was used as a precursor. A schematic of the setup is shown in Fig. 1 (click to see video of setup). The films were mechanically stabilized in a second step by flame annealing with a particle-free xylene flame with the same FSP unit [2]. The nanoparticle titania layers were deposited on PMMA substrates (IRPEN, Spain) 18 × 52 × 2 mm in size either flat or with open microfluidic channels, which become the affinity microcolumns after deposition and sealing. Details of the deposition conditions can be found in supporting information available on line for this paper.

The process for fabricating polymeric microfluidic devices with standard MEMS technology namely lithography on PMMA substrate and plasma etching was described in detail in a previous publication [12, 17]. A thin Silicon containing negative-tone photoresist (ORMOCER; Microresist, Germany) was directly spin-coated and lithographed on the polymer followed by deep oxygen plasma etching of the polymer. The resist solvent did not attack PMMA, probably due to the short duration of the resist dispensing and spinning process. The resist developer (1:1 MIBK:IPA methylisobutylketone:isopropanol mixture) also did not attack PMMA. For sensitive polymers (e.g. PET or PS) dilution of the developer with IPA helped avoid solvent attack [12, 17]. The chip fabrication process was repeatable and lasted approximately 15 min, of which 12 min was the etching process at a rate of greater than 1 μm/min. The microfluidic channels had a depth of 12 μm.

The on chip chromatographic setup is shown in Fig. 2 (click to see video of the setup). Lamination sealed (with Mega Photopolymer Laminator) microfluidic chips were placed in a “Lab-on-a-Chip Kit 4515” from Micronit Microfluidics BV and connected with fused silica capillary tubes (internal diameter 150 μm, external diameter 360 μm). Chromatographic eluents were introduced into a 150 μm optical path quartz tube, and were

detected by UV absorbance using a UV spectrophotometer setup from θ -metrisis (www.θ-metrisis.gr, Athens, Greece) employing a UV-Sensitive Peltier cooled CCD detector with signal to noise ratio 1000:1. The experimental setup used micropumps (Labsmith Company) and short tubing such that the flow-through time was less than 6 min. The set-up used three micropumps: The first for the acidic mobile phase, the second for the sample injection, and the third for the alkaline mobile phase. The infusion rate for all micropumps was held constant at 2 $\mu\text{L}/\text{min}$. A mono or tetra phosphopeptide solution was injected in the column in acidic environment and eluted in basic environment. Details of the materials and separation procedure can be found in supporting information available on line with this paper.

3. Results & Discussion

3.1 Nanoparticle film deposition and characterization

First, nanoparticles were collected as a powder downstream and analyzed for specific surface area (SSA). A high SSA was measured of 148 m^2/g , corresponding to an equivalent primary particle diameter of 10 nm. The collected powder has the same primary particle and crystallite sizes as the deposited nanoparticle film [1,2], since the particle formation is finished and only agglomeration and formation of the film takes place at this height (40 cm) as has been determined by thermophoretic sampling and microscopy [18]. XRD reveals that the TiO_2 nanoparticles have an average crystallite size of 9.5 nm for the anatase (86 wt. %) phase and 8 nm for the rutile phase. The measured XRD crystal sizes correspond well with the primary particle size which indicates monocrystalline particles as has been observed for flame synthesis and direct deposition of SnO_2 [1] and TiO_2 particles [18].

Fig. 3 (a) shows an example of a titania film on a flat polymer substrate as deposited for 30 s without flame annealing. The fractal morphology of the as deposited films is visible, similar to SnO_2 and TiO_2 films on glass substrates [1,18]. The particles (bright) are very loosely packed. This highly porous, lace-like structure is typical for flame made aerosol deposited films [1]. Porosities of such films are typically about 98% [1] ensuring excellent accessibility of the particle surface. The problem with such a structure of the films is their fragility, which is improved by in-situ direct flame annealing [2]. In-situ flame annealing was done for 3 s with a larger (compared to the flame used for deposition) particle-free xylene flame. Annealing parameters were chosen with caution so as to restructure and compact the film without damaging the polymeric substrate. Temperature measurements during deposition and annealing (see supplementary material) show that the polymer is heated below its glass transition temperature. Fig. 3 (b) shows an in-situ flame annealed titania film, restructured to more densely-packed agglomerates of TiO_2 particles (white) exposing the underlying polymer surface (dark) quite similar to antifogging TiO_2 films deposited on glass [18]. Uniform films are also observed over the whole substrate after the annealing step, indicating that the film was not damaged by the flame. AFM thickness measurements of the annealed films with 30 s deposition time reveal an average thickness of about 70 nm with maximum up to 120 nm. Films were stable against washing, but could be removed by rubbing with ethanol impregnated tissue paper. However, this is not a problem for application in microfluidics.

Fig. 3 (c, d) shows microfluidic channels coated by titania. The TiO_2 particles (white) uniformly cover both the channel bottom and the top-walls in between. Measurements of the channels in SEM before and after the deposition / annealing process showed no change in dimensions or damage of the microfluidic device. Microfluidic channels had a rough bottom surface due to plasma nanotexturing [17], which helped increase the surface area even more. The rough microchannels can be coated as-prepared or after stabilization by washing with de-ionized (DI) water [19] (see supporting information).

3.2 Enrichment of phosphopeptides

Titania deposited microcolumns were then used for phosphopeptide enrichment as described in supplementary information. Different titania film thicknesses were tested in order to determine the capacity of the microcolumns to trap phosphopeptides. Fig. 4 shows the chromatograms (elution and separation) of monophosphopeptide and of a mixture of mono- and tetra- phosphopeptides using PMMA microcolumns with TiO₂ film deposited for (a) 80 s, 3 s annealing (b) 2 × 40 s, 2 × 3 s annealing and (c) 30 s, 3 s annealing as stationary phases. Samples are introduced at time t=0 in trifluoroacetic acid and reach the detector at t = 5 min. No peak is observed at t = 5 min confirming complete retention of injected quantities. At t = 5 min NH₄OH is injected and reaches the detector at t = 10 min. Elution of the monophosphopeptide is observed at RT = 10 min after the basic (NH₄OH) mobile phase reached the detector and at RT = 17 min for the tetra-phosphopeptide.

The capacities of the microcolumns with different film thicknesses are at least (a) 0.8 , (b) 1.2 and (c) 0.4 µg of mono-phosphopeptide plus 0.6 µg of tetraphosphopeptide respectively. These are high values comparable to the capacity of a wet deposited TiO₂-ZrO₂ stationary phase on similar microfluidic columns [12], as well as the capacity of commercial tips.

However, we have observed that our chips are not good for multiple-use since the capacity decreased after repeated sample injections and elutions (see supporting information). This was caused by the continuous basic flow of NH₄OH, rather than by the flow of a small plug of NH₄OH, resulting in slow etching and removal of the nanoparticle film: We used a total of 40 µl of NH₄OH pH = 10 flowing at 2 µl/min for 20 min, rather than 10 µl plug of 10 µl/min for 1 min typically used in the literature). In addition the continuous flow of the mobile phase under pressure caused slow removal of the nanoparticles. We have observed with SEM microscopy that microfluidic channels after 30 min of water or NH₄OH flow showed reduced amount of particles on their internal surface. A further optimization of the deposition and annealing step will be necessary in order to produce chips capable of multiple use. Nevertheless the process of chip fabrication, and phosphopeptide enrichment was very reproducible for different batches and chips (see supporting information).

Conclusions

Proof of concept was shown for flame aerosol direct deposition on polymeric microfabricated substrates. The polymers and the microfluidic structures were not damaged by the high temperature flame when carefully selecting the process conditions. Thin films of titania nanoparticles were successfully deposited and in-situ flame annealed on PMMA polymer with sufficient stability to withstand liquid flow. Metal oxide affinity chromatography (MOAC) separation of model phosphopeptides was possible on the microcolumn and has shown a capacity of at least 0.4 µg for the thin film (30 s deposition) and 1.2 µg for the thicker films (80 s total deposition) which is comparable to commercially available MOAC tips and wet-deposited titania stationary phase. We note that the dry TiO₂ flame aerosol deposition process including annealing lasted only a few seconds compared to hours for wet deposited coatings. This represents a significant improvement for metal affinity microcolumn fabrication. Furthermore, the flame aerosol tool has the capability to produce a large variety of different metal oxide nanomaterials and their mixtures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

A schematic of the Flame Spray Pyrolysis apparatus. Click to see on-line the experimental procedure of deposition on polymeric substrates.

Fig. 2.

The microchip connected to detection setup. Click to see on-line the experimental procedure of the enrichment of standard phosphopeptides using the PMMA microchip deposited with the TiO_2 stationary phase.

Fig. 3.

SEM images of flame deposited titania films (a) as deposited on a flat polymer substrate for 30 s; The bright spots are titania nanoparticles. The film shows a fractal-like morphology of loosely agglomerated particles with a high porosity. (b) The in-situ flame-annealed film. The fractal morphology changed to a more densely agglomerated one exposing the polymer substrate surface underneath (dark regions). (c, d) The PMMA micro-column after the flame-deposition of TiO_2 , consisting of 7 μm wide, 12 μm deep microchannels. Notice that the microchannel bottom surface is rough (due to plasma roughening, [17]) and the walls in between are flat. The particles (white) cover both the walls and the microchannel bottom. The deposition is uniform over large areas.

Fig. 4.

Affinity chromatography on chip, with retention, elution and separation of mono-phosphopeptide (FQ-pS-EEQQTEDELQDK) and of a mixture of mono and tetra phosphopeptide (RELEELNVPGEIVEpSLpSpSpSEESITR) using a PMMA micro-column containing a TiO_2 stationary phase deposited for (a) 80 s, 3 s annealing (b) 2×40 s, 2×3 s annealing and (c) 30 s, 3 s annealing as stationary phases. The acidic mobile phase (pH 3) was trifluoroacetic acid 0.05 %, while the basic mobile phase was aqueous NH_4OH (pH 10). The sample quantity was (a) 8 μl (50 μM), i.e. 0.8 μg (b) 12 μL (50 μM), i.e. 1.2 μg , (c) 4+4 μl (50 μM), i.e. 0.4 μg +0.6 μg for mono and tetra phosphopeptide, and the flow rate of the mobile phase was 2 $\mu\text{L}/\text{min}$. Some baseline shifts are due to shifting of the intensity of the Deuterium lamp.