OSTE MICROCHIPS BASED ON GOLD NANOPARTICLES FOR CHEMICAL AND BIOLOGICAL ANALYSIS

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Abstract: *Here we present the feasibility to graft, in one-step, gold nanoparticles (AuNPs) on thiol-ene polymers by using UV irradiation, so as to create localized preconcentration areas onto OSTE (Off-stoichiometry thiol-ene) microchips. These selectively modified areas on OSTE microchips will permit (i) to concentrate the targets present at trace levels in complex matrices (such as biological samples) through the interaction with affinity ligands such as aptamers or (ii) to develop an active surface area to SERS through the immobilization of AuNPs. We demonstrate that OSTE microchips can be used for electrokinetic separations thanks to the chemical resistance of thiol-ene polymers plates when immersed into usual solvents (phosphate buffer without or with CTAB, or SDS) used for electrodriven separations and, thanks to gated injection with fluorescein into OSTE microchips. Finally, we present Raman spectroscopy measurements to demonstrate the possibility to graft gold nanoparticles on thiol-ene plates with an UV irradiation treatment.*

Key-Words*: Microchip, thiol-ene polymers, gold nanoparticles, surface modifications*

Introduction: From all substrates used for microfabrication, PDMS (polydimethylsiloxane) is the most common one. This polymer has many advantages such as its elastomeric properties, optical transparency and biocompatibility [1]. However, some molecules can adsorb on its surface due to its hydrophobicity and the generation of an electroosmotic flow into microchannels is quite difficult [1]. Moreover, the grafting of ligands into PDMS microchannels needs a pretreatment step of the microchannel walls [2]. Therefore, several surface functionalization methods have been developed such as plasma treatment, vapor chemical deposition, layer-by-layer deposition, covalent bond modification and silanization [2]. These PDMS surface treatments need several steps and an increase in microfabrication time. Moreover, these surface modifications are rarely permanent.
New classes of polymers (thermoplastics) with interesting functional groups permit an electroosmotic flow and the grafting of ligands on their surface without any pretreatment. Among thermoplastics, THV Dyneon or OSTE polymers are particularly interesting. OSTE polymers are based on the versatile UV-curable thiol-ene chemistry and take advantages of off-stoichiometry ratios to enable important features for a prototyping system, such as one-step surface modification. The other advantages of thiol-ene polymers are their good optical properties, chemical resistance and low gas permeability [3].
In this poster, we present the possibility to use OSTE microchip for electrokinetic separations and the feasibility to graft in one-step, gold nanoparticles on OSTE plates due to UV treatment.

Experimental: For the preparation of OSTE substrates (plates or microchips) two precursors (allyl (1,3,5-trially-1,3-5-triazine-2,4,6(*1H,3H,5H)*-trione) and thiol (pentaerytriol tetrakis (mercaptocetate)) precursors) were mixed in disposable polystyrene cups using two different ratios: one with an allyl excess (for micro channels or allyl plates) and the other one with a thiol excess (for the top of the chip or thiol plates). The ratio of precursors (thiol:allyl) used for OSTE plates and chips are: 1:1.3 w/w (channels) and 2.5:1 w/w (microchip's top). The proper amount of each precursor was weighted and the mixture was mixed vigorously. Then, 0.01% w/w of photo initiator (ethyl phenyl(2,4,6-trimethylbenzoyl)phosphinate) was added for the polymerization reaction. The mixture was left in a dark room for 40 min to remove air bubbles. Finally, the polymer was poured over PMMA mold (for plates) or PDMS counter-molds (for microchips) and exposed to the natural light for 1 day. After exposure, all substrates were heated at 60°C during 10 min in oven to facilitate unmolding. Reservoirs were made using a 3 mm biopsy puncher. To seal the microchip, the two parts were heated at 120°C during 10 min and exposed to UV light (photolight-UV-MD2-A4, Carimbos Medeiros Ltda, Brazil) during 20 min. The exposure to UV light induces a click chemistry reaction between thiol and allyl moieties. PDMS molds for the preparation of OSTE microchips were made as follow: first, a counter-mold in PMMA was made using a laser engraving machine (Gravograph LS-

100); second, Sylgard 184 silicone kit was mixed in proportion of 10:1 (w/w) pre-polymer/catalyst and poured over the PMMA counter-mold; third, bubbles were removed under a low pressure in a desiccator and left in oven at 70 ºC for 3 hours. All OSTE substrates were washed using ethanol, nanopure water and dried under nitrogen before experiments.
The surface of OSTE plates immersed into different solutions (Milli-Q water, phosphate buffer without or with CTAB or SDS, ethanol and acetone) was characterized using an inverted optical microscopy (10x objective, Axio Observer, Zeiss). The images were acquired and treated with ZEN software. To show the feasibility of electrokinetic injection (gated injection) in OSTE microchip, fluorescein was used as fluorescent probe and was detected with an inverted fluorescent microscopy (GFP filter, Zeiss). Movies were recorded with Zen software. The gold nanoparticles synthesized according to the Lee´s process [4], were characterized with SEM (Scanning Electronics Microscopy, JEOL JSM-6360LV, Thermo Electron Corporation, USA) and UV-vis spectrophotometry (Hewlett Packard 89090A, ChemStation software for acquisition of spectra). The grafting of gold nanoparticles on OSTE plates was evaluated using Raman spectrometry and Raman image spectroscopy, performed with a Raman Station 400F spectrometer (Perkin Elmer) equipped with a 785 nm and 250 mW excitation laser. Data was acquired using Spectrum software (Perkin Elmer).

Results and discussions: The stability of OSTE plates was tested in different solvents: deionized water, 10 mM phosphate buffer (pH 7.4), without or with CTAB (0.3 mM) or SDS (0.3 mM) (two commonly used surfactants for electrokinetic separations) and ethanol (used for cleaning of OSTE microchips and to remove air bubbles trapped in microchannels). OSTE plates were immersed in these solvents for one week and pictures were taken with an inverted optical microscopy to characterize the surface of these treated OSTE substrates. We observed that OSTE polymer resists to the exposure to deionized water, phosphate buffer without or with CTAB or SDS and ethanol. Fluorescein prepared into 10 mM phosphate buffer (pH 7.4) was then introduced in an OSTE microchip with a gated injection mode under positive voltage. As fluorescein and thiol-ene polymers are negatively charged at pH 7.4, the detection of fluorescein in these conditions proves a high electroosmotic flow compared to the electrophoretic mobility of the fluorescent dye. Therefore, OSTE microchips are suitable for further electrokinetic separations. Two grafting methods for gold nanoparticles on OSTE plates were evaluated: incubation for 24 h (OSTE plates immersed into gold nanoparticles solution) and UV treatments (a 10 µL drop of gold nanoparticles solution followed by UV exposition during 30 min or 1 h). After these treatments, plates were washed with deionized water during 1 min. Once dried, a drop of crystal violet (10 µL, 100 ppm) was deposited on the modified plates to perform SERS (Surface-enhanced Raman spectroscopy). Raman spectrometry spectra of crystal violet adsorbed on gold nanoparticles when exposed to UV treatment, present new peaks (1620, 1176, 420, 334 cm^{1}) compared to the blank spectra of allyl (1 thiol: 1.3 allyl) and thiol (2.5 thiol: 1 allyl) plates. Therefore the grafting of gold nanoparticles on the surface of OSTE plates with this treatment is demonstrated. Moreover, Raman spectra show that maximum nanoparticles are grafted on allyl plates for a 1h UV treatment. Incubation of plates allows to confirm that gold nanoparticles are grafted only on thiol plates. So, the grafting process with incubation and UV treatment are different.

Conclusion: The OSTE microchip was proved to be resistant to the main solvents used for electrokinetic separations. An injection (gated mode) of model molecule (fluorescein) indicated a high electroosmotic flow. We then proved the efficient grafting of gold nanoparticles on thiol-ene microchannel walls by a 1h UV treatment. Therefore, UV exposition through a mask will allow the creation of a defined area in the microchannel with gold nanoparticles functionalization, so as to generate a concentration zone for chemical or biological targets present at low concentration into complex samples.

References and acknowledgements:
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