

Reduction of the hydrophobicity of thiol-ene microfluidic devices using an adsorbed polymeric coating

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ABSTRACT

Thiol-ene formulations have gained a considerable interest in the field of micro and nano fluidics, because of their physico-chemical properties and their polymerization characteristics. However, the hydrophobic nature of this material represents a considerable drawback to its application in micro and nano fluidics. In fact, the introduction of aqueous solutions into narrow channels, is complicated by the low wettability of this material. In addition, hydrophobicity makes its use with biological samples unsuitable. Here, we present a simple and fast approach to reduce dramatically the hydrophobicity of thiol-ene surfaces and to avoid protein unspecific binding by photo-grafting to the surface a ter polymer of dimethylacrylamide (DMA), glycidyl methacrylate (GMA) and 3-(trimethoxysilyl) propyl methacrylate (MAPS) which is very stable to aging, solvents and high temperature treatments. Due to its versatility, the proposed polymeric coating is suitable to enhance the analytical performance of any thiol-ene based micro and nano fluidic device.

Keywords: thiol-ene, microfluidics, nanofluidics, polymeric coating, hydrophilicity

1 INTRODUCTION

Thiol-ene formulations have gained a considerable interest in the field of micro and nano fluidics, because of their physico-chemical properties and their polymerization characteristics. In fact, the thiol-ene reaction forms a cross-linked polymer by adding a monomer that contains several sulphhydryl moieties to a monomer containing multiple double bonds, also in absence of an initiator. Furthermore, the reaction shows no sensitivity to oxygen, low volume shrinkage and low polymerization shrinkage stress. This characteristics facilitate the use of this material for the prototyping and manufacturing of several microfluidic devices in the order of seconds with optical properties similar to glass and with low permeability to solvents (1). The main advantage offered by thiol-ene photopolymerization relies on the possibility of modulating the monomers stoichiometry so to provide an excess of double bonds or thiol groups, leading to a functional surface that can anchor biomolecules and polymer films (2).

Unfortunately, the hydrophobic nature of this material represents a considerable drawback to its application in micro and nano fluidics (3, 4). In fact, the introduction of aqueous solutions into narrow channels, for instance, is

complicated by the low wettability of this material. In addition, hydrophobicity makes its use with biological samples unsuitable: it is generally recognized, in fact, that proteins, being attracted by hydrophobic and electrostatic interactions, adsorb irreversibly on the surface of microdevices leading to poor analytical performance.

In this work, we present a simple approach to dramatically reduce the hydrophobicity of thiol-ene surfaces and to avoid protein unspecific binding by photo-grafting to the surface a ter polymer of dimethylacrylamide (DMA), glycidyl methacrylate (GMA) and 3-(trimethoxysilyl) propyl methacrylate (MAPS), poly(DMA-co-GMA-co-MAPS). This copolymer has been recently introduced to suppress electroosmotic flow and protein adsorption in fused silica capillary for electrophoresis (5). The main advantage in the use of this copolymer is the easiness of the coating procedure which simply consists in immersing the thiol-ene slides into a diluted aqueous solution of the polymer for few minutes and leads to a very stable film. Moreover, oxirane groups (deriving from GMA monomer) along the polymer chains, easily react with the excess of sulphhydryl groups on the thiol-ene surface, thus allowing the use of this polymer without any surface pre-treatment, which is very complicated and in some cases unfeasible, when considering nano-channels.

The presence and stability of the coating have been assessed by measuring water contact angle of microscope-like thiol-ene flat slides. Coating conditions and polymer composition have been deeply investigated as well, so to obtain a film stable to aging, organic solvents and high temperature treatments.

2 MATERIALS AND METHODS

1,3,5-Triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione, pentaerythritol-tetrakis(3-mercaptopropionate), ammonium sulfate, ethanol, were from Sigma-Aldrich (St. Louis, MO, USA), Lucirin TPO-L was from BASF. Water contact angle were measured with using a CAM200 instrument (KSV Ltd), which utilizes video capture and subsequent image analysis.

2.1 Manufacturing of thiol-ene slides

Microscope-like thiol-ene flat slides (2.5 x 7.5 cm) have been obtained by mixing the two monomers, 1,3,5-triallyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione and pentaerythritol-tetrakis(3-mercaptopropionate) in a weight-to-weight ratio of 2,058 in order to have 40 % excess of thiol groups exposed on the surface. The mixture have been properly

stirred and 10 μL of Lucirin TPO-L have been added to the mixture which has been poured into a PDMS mould. The mixture has been cured under UV light (365 nm) for 30 minutes.

2.2 Coating of thiol-ene slides

A 2% w/v aqueous solution of poly(DMA-co-GMA-co-MAPS) (synthesized as reported elsewhere (5)) has been prepared. This solution has been diluted 1:1 with ammonium sulfate at 40% saturation level either at pH 5.5 and the same solution buffered at pH 8.5. The thiol-ene slides, freshly manufactured, have been immersed into the polymer solutions and have been exposed to UV light either for 20 or 60 minutes. The slides have been rinsed with DI water and cured at 80°C under vacuum for 15 minutes. The same procedure has been applied for uncoated thiol-ene slides and for slides coated with poly(DMA-co-GMA) (monomer molar fraction, 96%, 4%, respectively; synthesized as reported elsewhere (5) without adding MAPS).

2.3 Water contact angle measurements

The presence and stability of the coating has been assessed by water contact angle measurements which were collected via the sessile drop method. Deionized water was used, and its purity was confirmed by correlating the measured surface tension based on the pendant drop shape to the literature values for pure water (72 mN/m at 25°C).

Water contact angle has been measured immediately after the coating step and after 14 days of storage at room temperature. To assess stability in harsh conditions, coated slides have been either immersed in ethanol for 2 minutes or boiled in water for 10 minutes, and then contact angle has been measured.

Water contact angle of uncoated thiol-ene slides, treated as previously described, has been measured as a comparison.

3 RESULTS AND DISCUSSION

Reduction of hydrophobicity of microfluidic devices surface is fundamental for their use with biological samples. In fact, it is generally recognized that proteins unspecifically interact with surfaces through hydrophobic and electrostatic interactions, thus affecting the performance of analyses. In particular, the high hydrophobicity of thiol-ene cross-linked polymers hampers their use in manufacturing microfluidic devices, despite the several advantages of this material.

To overcome this issue, we have photo-graphpted to thiol-ene slides a ter polymer of *N*, *N*-dimethylacrylamide (DMA), glycidyl methacrylate (GMA) and 3-(trimethoxysilyl) propyl methacrylate (MAPS) (monomer molar fraction is, respectively, 95%, 4% and 1%), recently introduced to suppresses electroosmotic flow (EOF) and protein adsorption in fused silica capillary for electrophoresis (5). This polymer (**Figure 1**) contains the following functionalities: i) a DMA segment that binds to

the surface by weak, non-covalent interactions such as hydrogen bonds, Van der Waals or hydrophobic forces, ii) a pending silane hydrolyzable monomer (MAPS), which strengthen the coating stability on the surface and iii) a chemically reactive monomer (GMA) whose oxiranes easily react with the excess of thiols on the surface, when exposed to UV light (365 nm).

The single-step coating procedure simply consists in the UV light assisted grafting of the polymer dissolved in a diluted aqueous solution (1%w/v). The process is robust, and does not require any surface pre-treatment, as a consequence it is highly advantageous when working with micro-channels and nanostructures.

In this work, the coating conditions have been optimized and the hydrophobicity reduction of thiol-ene surfaces assessed by water contact angle measurements. As can be easily noticed from **Figure 2** (blue bars), water contact angle of uncoated thiol-ene is about 80°, thus showing a very high hydrophobicity. This characteristics is maintained after 14 days of storage at room temperature and after treatment at high temperature (100°C) or organic solvents (ethanol). On the contrary, the coating step assures a significant reduction of hydrophobicity (as shown in **Figure 3**): after twenty minutes of exposure to UV light the contact angle of the substrate decreases from 70-80° to 40°. However, a better stability of the coating in harsh conditions such as organic solvents (ethanol) or high temperature (boiling water) is achieved only by extending the UV exposure time to 1 hour and by buffering the polymer solution at alkaline pH. This is due to the higher reactivity of the epoxy rings in alkaline conditions.

In a control experiment, we have checked that the thiol-ene substrate is not damaged or modified by long UV light exposure as shown in **Figure 2** (green and pink bars). This experiment demonstrates that the decrease of contact angle values results from the presence of the stable hydrophilic film and not from surface oxidation.

The role of monomers along the polymer chains has been deeply investigated as well. The presence of MAPS is fundamental to assure a high stability of the coating in harsh treatments. In fact, the coating performed in the same conditions as reported below, but with a polymer which does not contain pending silane monomers (poly(DMA-co-GMA)) is not stable when the slides are treated with solvents or with boiling water. As shown in **Figure 4**, in fact, after treatment with ethanol and boiling water, contact angle value increases up to 70°, which is similar to the value of an uncoated thiol-ene slide (see **Figure 2**, blue bars). It is very likeley that silanol groups promote the condensation of contiguous polymer chains, increasing the stability of the coating.

Moreover, oxirane moieties are essential to assure the covalent bonding to thiol groups without any surface pretreatment. In fact, substitution of GMA with another monomer, *N*-acryloyloxysuccinimide, leads to a polymer with the same performance in terms of unspecific protein adsorption suppression (5), and hydrophobicity reduction (data not shown), but requires pre-treatment of the surface with oxygen plasma which is unfeasible in case of micro and nano-channels.

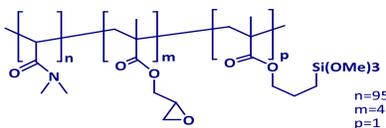


Figure 1 Structure of poly(DMA-co-GMA-co-MAPS) a ter-copolymer constituted of N,N-dimethylacrylamide (DMA), Glycidyl methacrylate (GMA) and 3-(trimethoxysilyl)propyl-methacrylate (MAPS). The monomer molar fraction is, respectively, 95%, 4% and 1%.

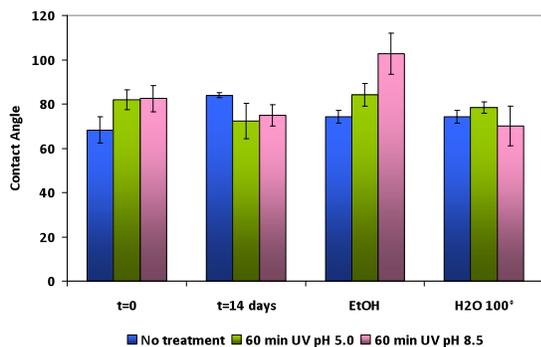


Figure 2 Water contact angle measurements of uncoated thiol-ene surfaces with thiol excess. Measures have been performed before and after the treatment with UV light of the substrate immersed in an ammonium sulphate solution buffered at pH 5.0 and 8.5. Thiol-ene slides have been stored at room temperature up to 14 days, immersed in ethanol (EtOH) for 2 minutes and boiled in water (H₂O=100°C) for 5 minutes to simulate harsh storage conditions

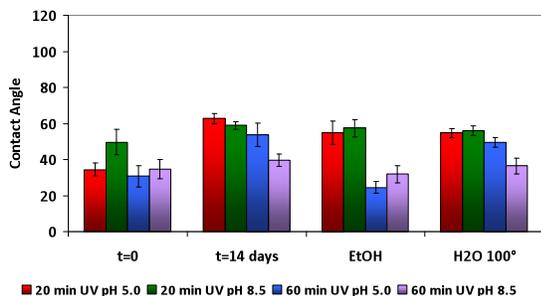


Figure 3 Water contact angle measurements of thiol-ene surfaces with thiol excess coated with poly(DMA-co-GMA-co-MAPS). Coating has been performed under UV light for 20 and 60 minutes in ammonium sulphate buffer at pH 5.0 and 8.5. Thiol-ene slides have been stored at room temperature up to 14 days, immersed in ethanol (EtOH) for 2 minutes and boiled in water (H₂O=100°C) for 5 minutes to simulate harsh storage conditions.

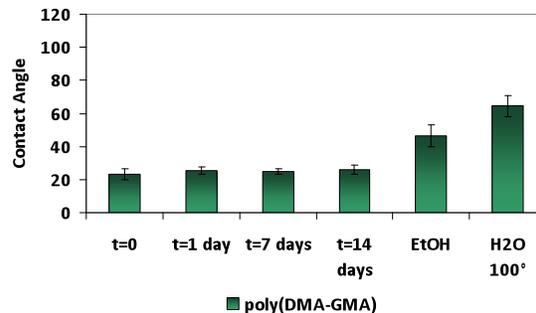


Figure 4 Water contact angle measurements of thiol-ene surfaces with thiol excess coated with poly(DMA-co-GMA). Coating has been performed under UV light for 1 hour in ammonium sulphate buffer at pH 8.5. Thiol-ene slides have been stored at room temperature up to 14 days, immersed in ethanol (EtOH) for 2 minutes and boiled in water (H₂O=100°C) for 5 minutes to simulate harsh storage conditions. In this case, the coating, missing the silane monomer, is less stable to harsh conditions: in fact after treatments with ethanol or boiling water, contact angle values increase up to about 70°, which is similar to the value of an uncoated thiol-ene slide.

4 CONCLUSIONS

An adsorbed polymeric coating has been used to dramatically reduce the hydrophobicity of thiol-ene slides showing an excess of sulphhydryl groups, without the need of any surface pre-treatment. The coating procedure is fast, easy and leads to a film stable to aging and in several harsh conditions. The coating conditions have been optimized by investigating the pH value of the polymeric coating solution and UV time exposure.

Monomer composition of the polymer has been investigated as well, demonstrating the fundamental role of both epoxy rings, which allow a fast UV reaction with thiol groups on the surface at alkaline pH, and silane moieties which strengthen the stability of the coating when subjected to harsh conditions.

Due to its versatility, the proposed photo-grafted polymeric coating is suitable to enhance the analytical performance of any thiol-ene based micro and nano fluidic device.

Acknowledgments

This work was partially financed by the EU through FP7 NADINE (NANosystem for early Diagnosis of NEurodegenerative diseases), project contract n° 246513.

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