

A one-step protocol for the chemical derivatisation of glass microfluidic devices

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A simple and robust derivatisation system for glass and silica microdevices is described. The device surface is coated in a one-step treatment with a highly cross-linked polystyrene/divinylbenzene/allylsiloxane copolymer. The surface derivatisation is highly resistant to solvents, acids, bases and oxidising or reducing agents.

Introduction

Microfluidic devices can be defined as analytical systems that have typical dimensions most readily expressed in microns. Many materials are used in the manufacture of microfluidic systems, but chief among these are silicon,¹ metals,² polymers,³ fused silica⁴ and glass.⁵ The choice of substrate material is often defined by the chemical or biological system being studied, and will involve considerations including use at elevated temperatures and pressures, chemical- and bio-compatibility and use with oxidising agents or organic solvents. Moreover, when using microfluidic systems as environments in which to perform molecular synthesis, the chemical nature of microchannel surfaces is often key (due to elevated surface area-to-volume ratios) in defining success and applicability.

Such considerations have led to a number of derivatisation protocols being developed for microfluidic surfaces. Unfortunately, many of these systems require multiple synthetic steps or the use of high temperatures to deposit even a simple, non-functionalised wall coating.⁶ The most common systems for glass microfluidic systems are based upon the formation of self-assembled monolayers of alkyl siloxanes covalently attached to the silanol surface groups of the glass channel wall.⁷ Although this approach is normally simple in its implementation it suffers from the fact that surface-associated siloxane groups are labile and have limited lifetimes, particularly in aqueous base.

Polystyrene/divinylbenzene copolymers have long been utilised as solid supports in combinatorial chemistry.⁸ The polymer is robust, and when sufficiently crosslinked is inert and insoluble in most organic solvents. The basic polymer scaffold can also be derivatised by the inclusion of functionalised monomer units, to vary the surface chemistry. The radical polymerisation of this system can be accomplished by thermal or photoinitiated systems⁹ and is tolerant of a variety of solvent systems. Virtually any alkene

can be incorporated into this system as a functionalised monomer, which makes it facile to engineer a system that will adhere strongly to glass. The key factor in producing a robust, covalently attached derivative on a glass or silica surface is suppressing the lability of the siloxane self-assembled monolayer. We have chosen to address this problem through the incorporation of the siloxane R groups into a heavily crosslinked styrene/divinylbenzene copolymer (Fig. 1). This has the dual effect of limiting reagent access to the glass surface, so inhibiting chemical attack, and of providing a firm attachment to the glass surface for the styrene/divinylbenzene polymer system.

In this communication we describe a system for a robust one-step derivatisation of glass or fused-silica microchannel surfaces using a polystyrene/divinylbenzene/allylsiloxane copolymer that takes place at only moderately elevated temperatures and leads to functionalised and tunable surfaces.

Experimental

Microfluidic device fabrication

Glass microfluidic devices were constructed according to methods previously described.⁵ Briefly, soda lime glass substrates precoated with a positive photoresist (AZ 1518) and a low reflective chromium layer (Nanofilm, Westlake Village, California) were exposed using a direct-write laser lithography system (DWL2.0, Heidelberg Instruments, Heidelberg, Germany) to transfer the channel pattern. After the photoresist was developed (Microposit

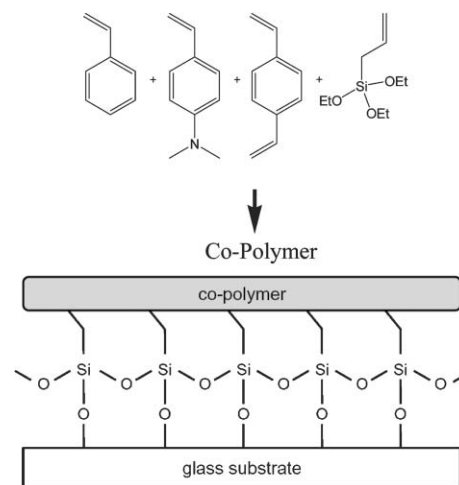


Fig. 1 Dual layer surface derivatisation of glass. The allylsiloxane attaches to glass as a SAM then copolymerises with the styrene/DVB system to give a protective second layer.

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351, Shipley Europe Ltd, Coventry, UK), channels were etched into the glass substrate using a buffered oxide etching solution (20% 7 : 1 HF/NH₄F) and external access holes were drilled. To form enclosed channels, a glass coverplate cleaned with concentrated H₂SO₄ was thermally bonded to the structured glass substrate in a furnace (Heraeus Instruments GmbH, Hanau, Germany) at 858 K.

Reagents and materials

All chemicals and solvents were purchased from Aldrich (Sigma-Aldrich Ltd., Gillingham, UK) and used as received. Copolymer solutions for simple double layer derivatisation were made up as follows: to a solution of dicyclohexylazo-bis-carbonitrile (0.12 g) in styrene (1.45 ml) was added decamethylpentasiloxane (4 ml). To the resulting mixture was added divinylbenzene (mixed isomers, 98%, 0.35 ml) and allyltriethoxysilane (0.1 ml). The resulting mixture was kept refrigerated until use, with a maximum storage lifetime of 4 h. Copolymer solutions for *active surface double layer derivatisation* were made up as follows: to a solution of dicyclohexylazo-bis-carbonitrile (0.12 g) in styrene (1.19 ml) was added decamethylpentasiloxane (4 ml). To the resulting mixture was added divinylbenzene (mixed isomers, 98%, 0.35 ml), *N,N*-dimethylaminostyrene (0.26 ml) and allyltriethoxysilane (0.1 ml). The mixture was kept refrigerated until use, with a storage lifetime of 4 h.

Procedure for chip derivatisation

Glass microfluidic devices were initially washed with a flow of isopropanol and dried by heating to 60 °C whilst purging with a flow of dry nitrogen. After drying microchannels were filled with copolymer solution and allowed to stand for 10 minutes. The entire microfluidic device was then lowered into a thermostatically controlled oil bath held at 120 °C and maintained at this temperature for 2 min. On removal the device was allowed to cool to ambient temperature and microchannels were purged with a flow of dry nitrogen. After the removal of excess polymerisation mixture microchannels were washed with cyclohexane, then isopropanol, and finally dried using a flow of dry nitrogen.

Procedure for surface activation

Derivatised microchannels were filled with methyl iodide and left to react at ambient temperature for 24 h, evaporative losses were replaced as necessary.

Raman spectroscopy

Raman spectra were measured using a Jobin–Yvon LabRam Infinity 1010 Microscope. The instrument consisted of an inverted microscope (Olympus System Microscope Model BX40) with a 10×/0.25 infinity corrected objective for illumination and scattered light collection. A HeNe laser (17 mW at 633 nm) was used as an excitation source. Detection was performed using a CCD array held under vacuum. The CCD detector consisted of an array of 1024 × 256 pixels and 1000 cm⁻¹ is dispersed by the spectrometer over the CCD detector to afford a resolution of 1 cm⁻¹ per pixel.

Results and discussion

Glass microchannel surfaces derivatised by this method were found to be coated with a robust layer of polymer over the interior surface, Fig. 2. Optical microscopic imaging indicated layer thicknesses of approximately 5 μm.

Although in places the polymer layer exhibited surface features, on close examination these were found to be due to outgrowths of polymer, possibly from surface roughness on the glass, and do not represent holes in the polymer layer. Significantly, the thickness of polymer layer could be controlled (between 5 and 50 μm) by altering the concentration of the initial polymerisation mix, but above a threshold concentration microchannels became susceptible to blockage during the polymerisation process. Using the stated techniques under normal conditions we achieved a blockage rate of less than 1% on a batch process.

The unusual choice of solvent is necessitated by the relatively high temperatures involved in the process: standard non-polar solvents boil at these temperatures, invariably leading to poor coverage and blocking of the microchannel. Highly polar solvents lead to interference in polymerisation or problems in solubility of the monomers. Decamethylpentasiloxane was found to have the ideal combination of solvation profile and boiling point for this process, and moreover is inexpensive and non-toxic.

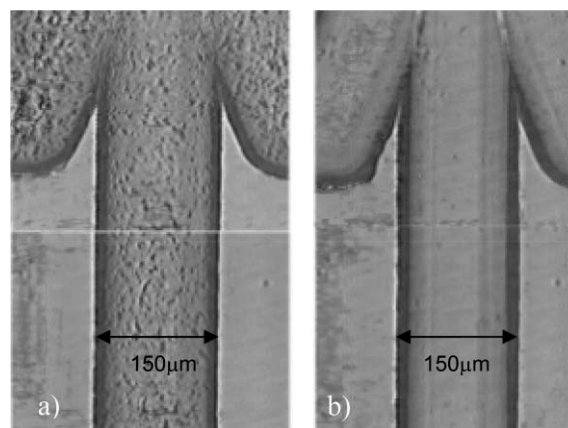


Fig. 2 A glass microfluidic device (a) after and (b) before derivatisation with styrene/DVB/allylsiloxane copolymer.

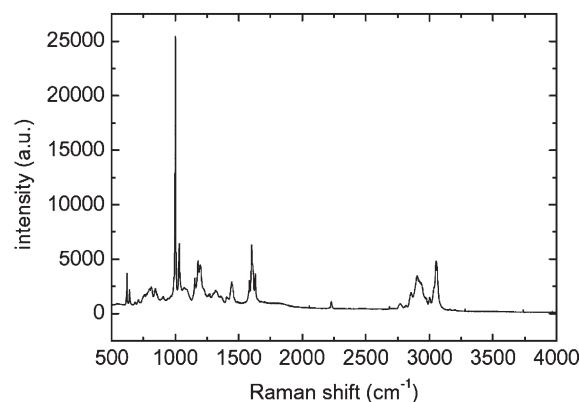


Fig. 3 Raman spectrum of polymer layer deposited during dual layer surface derivatisation of glass.

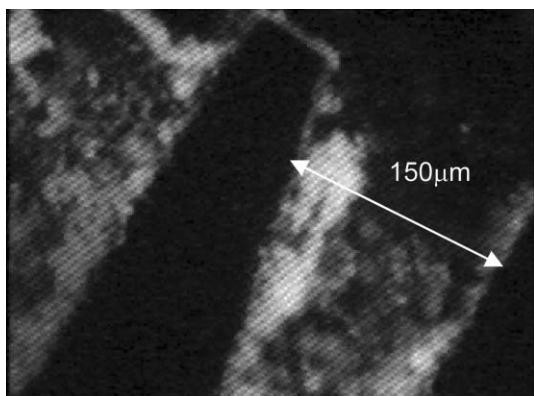


Fig. 4 Activated surface of a derivatised microchannel shown retaining fluorescein ions from sodium fluorescein solution. Surface irregularity induced outgrowths show highest activity.

The polymer layers deposited on the microchannel surfaces was analysed by *in-situ* Raman spectroscopy and found to exhibit a vibrational spectrum identical in all respects to that of a commercial styrene/DVB copolymer, Fig. 3. Notable peaks include 1603 cm^{-1} (C=C stretch), 1332 cm^{-1} (CH_2 bend), 1002 cm^{-1} (aromatic ring breathe) and 225 cm^{-1} (C–C–C bend). Furthermore, polymer layers proved to be resistant to treatment with diethyl ether, tetrahydrofuran, *N,N*-dimethylformamide, dichloromethane, dimethyl sulfoxide, acetone and cyclohexane at ambient and elevated temperatures. Resistance was assessed by microscopic examination.

Having established a protocol for the simple double layer derivatisation of glass microchannel surfaces we extended the protocol by introducing an active agent into the monomer mix. In these studies *N,N*-dimethylaminostyrene was used as an active agent, since by simple methylation this can be transformed into a rudimentary ion exchange function. Polymerisation proceeded as before and treatment with methyl iodide produced an activated surface which functioned as an anion exchange resin. This was demonstrated by flowing a solution of fluorescein disodium salt through the functionalised microchannel. As can be seen in Fig. 4, fluorescence emission can be observed from the polymer layer

demonstrating efficient anion exchange. Further, fluorescence could be removed by treatment with carbonate or hydroxide ions in solution. Finally, it should also be noted that the above procedure was used in all respects on fused silica capillaries (Composite Metals Ltd, OD $350\text{ }\mu\text{m}$, ID $150\text{ }\mu\text{m}$) with identical results.

Conclusions

A simple, robust and repeatable method for the introduction of an iterable, active surface derivatisation into a glass or fused silica microchannel was demonstrated. We believe this method has wide potential application, for example in the immobilisation of catalysts and enzymes on the chip surface through encapsulation, ion exchange attachment or covalent attachment, or for positioning scavenging moieties for sample pre-treatment. The general method will allow incorporation of multiple reagents within a single microchannel flow system, as well as tunable hydrophilicity of channel walls. Moreover the repeatability and simplicity of the method should facilitate automated chemical modification and allow for mass production of robust functionalised chips in the near future.

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