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Electroosmotic properties of microfluidic channels composed of poly(dimethylsiloxane)

Xueqin Ren[a], Mark Bachman[b], Christopher Sims[c], G.P. Li[a,b,*], Nancy Allbritton[a,c,*]

[a]*Center for Biomedical Engineering, University of California, Irvine, CA 92697, USA*
[b]*Department of Electrical and Computer Engineering, University of California, Irvine, CA 92697, USA*
[c]*Department of Physiology and Biophysics, University of California, Irvine, CA 92697, USA*

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Abstract

Microfluidic devices fabricated from polymers exhibit great potential in biological analyses. Poly(dimethylsiloxane) (PDMS) has shown promise as a substrate for rapid prototyping of devices. Despite this, disagreement exists in the literature as to the ability of PDMS to support electroosmotic (EO) flow and the stability of that flow over time. We demonstrate that in low ionic strength solutions near neutral in pH, oxidized PDMS had a four-fold greater EO mobility (μ_{eo}) compared to native PDMS. The greater μ_{eo} was maintained irrespective of whether glass or PDMS was used as a support forming one side of the channel. This enhanced μ_{eo} was preserved as long as the channels were filled with an aqueous solution. Upon exposure of the channels to air, the mobility decreased by a factor of two with a half-life of 9 h. The EO properties of the air-exposed, oxidized PDMS were regenerated by exposure to strong base. High ionic strength, neutral in pH buffers compatible with living eukaryotic cells diminished the EO flow in the oxidized PDMS devices to a much greater extent than in the native PDMS devices. For analyses utilizing intact and living cells, oxidation of PDMS may not be an effective strategy to substantially increase the μ_{eo}.

Keywords: Electroosmotic mobility; Microfluidic channels; Poly(dimethylsiloxane)

1. Introduction

Traditionally, microfluidic devices have been fabricated by chemical etching in glass, quartz or silicon. This fabrication process is expensive, time consuming and labor intensive. In addition, the lithography-based process requires access to a clean room facility. Therefore, there is a trend to find cheaper materials and alternative methods of microfabrication to meet the requirements for low cost, disposable chips. Many polymers such as acrylic, polystyrene, and copolyester have been used for this purpose. Techniques such as hot embossing, injection molding, casting, and laser ablation have been adopted [1–9].

Polydimethylsiloxane (PDMS) is a bulk polymer consisting of repeated units of $-OSi(CH_3)_2O-$. Its high use and acceptance by the biomedical com-

*Corresponding author. Tel.: +1-949-824-4194; fax: +1-949-824-3732.
E-mail address: gpli@uci.edu (G.P. Li).
*Corresponding author. Tel.: +1-949-824-6493; fax: +1-949-824-8540.
E-mail address: nlallbri@uci.edu (N. Allbritton).

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munity make it an attractive material for constructing microfluidic chips. The use of PDMS for microfluidic channels was first introduced by Whitesides et al. in 1993 [10]. Since then PDMS has become a popular material for building microfluidic devices [11]. Among its desirable properties are: (1) the monomer can be polymerized at low temperatures; (2) it is transparent down to ~280 nm allowing optical detection at many wavelengths, and visible alignment during lamination; (3) it is self releasing and leaves little residue on a micromold, allowing the mold to be used indefinitely; (4) it is elastomeric allowing it to be peeled from micromolds without damaging the microchannels or master; (5) it seals readily with glass, PDMS, and other polymers; and (6) it is non-toxic and biologically inert, so it will not damage living cells.

Despite the growing interest in microfluidics devices fabricated from PDMS, disagreement exists in the literature regarding some of the properties of the channels. Work by Duffy et al. demonstrated that channels composed of native PDMS were extremely hydrophobic, difficult to fill with aqueous buffers, and consequently difficult to use for electrophoretic separations [12]. All of these results are entirely consistent with the repeating, hydrophobic structure of PDMS. In contrast Ocvirk et al. and other groups reported that microfluidics channels fabricated from native PDMS were readily filled with aqueous buffers and supported an EO flow similar to that of glass or quartz devices [13–15]. This data suggested that the surface of native PDMS in the microchannels is negatively charged and may be much more hydrophilic than was previously thought. The source of the surface charge which could generate this EO flow is not known. Additionally the rationale for the discrepant results of the various investigators has yet to be resolved.

To insure that the microchannels were charged and hydrophilic, Duffy et al. treated native PDMS channels with an oxygen plasma [12]. Treatment with an oxygen plasma modifies the polymer surface by two mechanisms: (i) transferring energy to the polymer which catalyzes reactions that otherwise would not occur, and (ii) incorporating O^- from the plasma into the polymer. Either of these mechanisms can substantially alter the hydrophobicity of the PDMS surface. Oxygen plasma treatment of PDMS is known to generate silanol groups which are ionizable [11,16]. This negative surface charge was thought to be the basis of the measured EO flow which was near that of glass or quartz channels. These channels were also easily loaded with aqueous buffers. While oxygen plasma treatment appeared to greatly increase the wetability of the PDMS channels, the effects of the plasma on the channels were reported to be short-lived. Thus the utility of the oxygen plasma-treated PDMS as a substrate for electrophoretic separation systems remains to be proven.

The purpose of this study was to determine the surface properties of PDMS microchannels and the stability of those properties under various conditions. The EO flow and the infrared absorption spectra of both native PDMS and oxygen plasma-treated PDMS were measured. The stability of the oxidized PDMS was studied over a span of 14 days as was the effect of exposure of the devices to air, aqueous buffer, and base. μ_{eo} was also measured when glass was used as a substrate for the PDMS channels since glass has optical properties superior to that of PDMS. In addition since microfluidics devices are highly attractive for the analysis of biological cells, the impact of cell-compatible buffers on μ_{eo} was assessed.

2. Experimental

2.1. Chemical reagents and solutions

Sylgard 184 was purchased from Dow Corning (Midland, MI). Silicon nitride-coated silicon wafers were acquired from Wafernet Inc. (San Jose, CA). Shipley 1827 positive photoresist and developer was obtained from Mircro Chem. Corp (Newton, MA). All other reagents and materials were purchased from Fisher Scientific (Pittsburgh, PA). Phosphate buffers (10 and 20 m*M* phosphate, pH 7.0) were made from potassium dihydrogenphosphate and adjusted to pH 7.0 with NaOH. The cell-compatible buffer was 135 m*M* NaCl, 5 m*M* KCl, 10 m*M* HEPES, 1 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, pH 7.4. All solutions were degassed by sonication for 15 min immediately prior to use.

2.2. Fabrication of PDMS devices

Microfluidic channel patterns were designed by a CAD program (Freehand 8.0, Macromedia, San Francisco, CA) and printed out at 5080 dpi on high resolution transparencies to form masks. Silicon wafers coated with 0.3 μm of silicon nitride (Wafer-Net) were cleaned and dehydrated. The wafers were then spin-coated (Karl Suss Spin Coater, Saint Jeoire, France) at 3500 rpm with positive photoresist (Shipley 1827, Shipley-Ronal, Newton, MA). The coated wafer was exposed to UV light through the mask using a contact aligner (Karl Suss). After development in a sodium hydroxide-based developer (Microposit MF351, Marlborough, MA), the patterned wafer was hard baked at 120°C. The silicon nitride coating was then etched using a reactive ion etcher (Plasmatherm) with CF_4-based chemistry to form a hard mask. The remaining photoresist was removed using an oxygen plasma. Wet etching was performed on the silicon using a mixture of HF, HCHOOH, HNO_3 (2:3:8). The isotropic etch produced surfaces 20–23 μm deep with an average roughness of 0.3 μm. After etching, the wafer was rinsed and dried. The remaining nitride was removed using a reactive ion etch (Plasmatherm). The etched silicon wafer formed the negative relief pattern for the channels which were molded in PDMS.

Sylgard 184 PDMS prepolymer was mixed thoroughly with its crosslinking catalyst at 10:1 (weight:weight) and degassed by application of a vacuum. The polymer was cast against the silicon mold and polymerized at 70°C for 1 h. After curing, the PDMS was peeled from the mold and cut to size. The physical dimensions of the channels were measured with a Tencor Instruments profiler (Alpha-Step 200). Holes (3.5 mm diameter) were punched into the polymer to create access ports and reservoirs. A flat piece of PDMS substrate was obtained by casting the polymer mixture on a clean flat surface. Final polymerization was performed by placing the PDMS in a 65°C oven overnight.

The micromolded PDMS was sealed against a PDMS or glass substrate. Both halves were successively rinsed with water, methanol, and water and then dried. A reversible seal was formed by mating the two pieces. Additional PDMS was sometimes applied and cured near the outer edges of the parts for additional strength as needed. In some instances the unmated PDMS (or glass) halves were placed in an oxygen plasma for 55 s (50 W at 60 mTorr). When joined together the oxygen plasma-treated parts sealed irreversibly.

Table 1
Channel dimensions (μm)[a]

Bottom width (a)	35	55	75
Middle width (b)	60	80	105
Top width (c)	70	100	120
Depth (d)	20	22	23

[a] Refer to Fig. 1B for the location of the measurement sites along the channel cross-section.

Straight channels 3 cm in length with varying widths and depths were created for experiments (Table 1). Due to the isotropic nature of the chemical etching, the channels had curved sidewalls (Fig. 1). For each experiment, five different batches of devices were molded with at least five devices per batch. Considerable care was taken during the fabrication process to produce consistent devices with clean, uniform, well trimmed reservoirs.

2.3. Measurement of μ_{eo}

A modification of the current monitoring method was used to measure the EO flow in the microfabricated channels [14,17,18]. Measurements were performed as described previously with the following exceptions. Just prior to use the channels were cleaned with methanol, water, and buffer successively. Buffers were loaded into the channels by application of a vacuum to the reservoirs. For the EO flow measurements with the phosphate buffer, the higher ionic strength buffer was 20 m*M* phosphate, pH 7.0 while the lower ionic strength solution was 10 m*M* phosphate, pH 7.0. As described previously the dilution factor was selected to ease end point detection [14,18]. For the EO flow measurements with the cell-compatible buffer, the higher ionic strength buffer was undiluted buffer and the lower ionic strength was a 19/20 dilution of the cell-compatible buffer. Each measurement of μ_{eo} was performed a minimum of three times. A high voltage power supply (Ultravolt, Long Island, NY) was used to

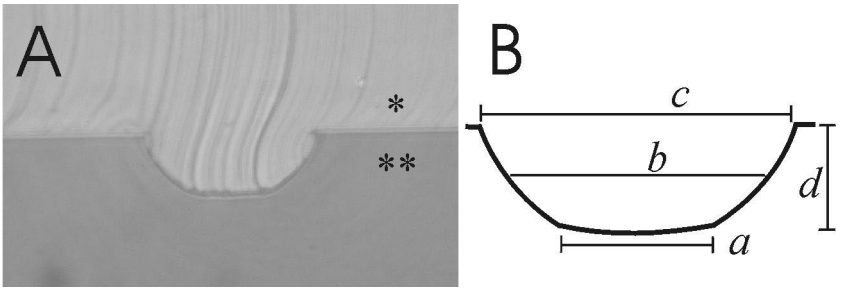

Fig. 1. Channels fabricated from PDMS. (A) Photomicrograph of a negative cast of a channel cut in cross-section (*) and mounted on a solid surface (**). The negative cast was created in PDMS. The vertical curved lines in the upper half of the photomicrograph were produced by the razor blade's movement through the PDMS. (B) Schematic of a channel cut in cross-section. The location of the dimensions given in Table 1 are marked as a, b, c, or d. The mid-width (b) of the channel shown in (A) was 60 μm.

produce a voltage potential (100 to 1000 V) across the microchannels.

Prior to measurement of μ_{eo}, the current at different voltages (100 to 1000 V) was measured to determine whether the relationship between the current and voltage was linear (to identify significant Joule heating). For the phosphate-buffered solutions (10 and 20 m*M* phosphate), the current was proportional to the voltage at the highest voltage tested, 1000 V (333 V/cm), for all channels. For the cell-compatible buffer, the current was proportional to the voltage for voltages less than 250 V (83 V/cm) for the channels with a mid-width of 60 μm (see Table 1). Therefore, all the experiments were performed at 200 V (66 V/cm) or lower.

2.4. Measurement of infrared absorption by total attenuated reflection (ATR-IR)

ATR-IR spectra of PDMS films on a wedged germanium crystal were recorded using a single beam spectrometer (Nicolet Magnet 860, Madison, WI) equipped with a helium neon laser and a TGS (triglycine sulfate) detector. Spectra were recorded at 4 cm^{-1} resolution, and 4096 scans were collected per trace. A single-beam reference spectrum of a freshly cleaned germanium crystal was recorded before the measurements and used as the background spectrum. A water spectrum was also recorded, scaled empirically, and subtracted from the PDMS spectra to remove the water peaks in the region of 3500–4000 cm^{-1}.

3. Results and discussion

3.1. Characteristics of native PDMS channels

Hemispherical channels with a variety of depths and widths were fabricated from PDMS (Fig. 1A and B, Table 1). Channels composed of unmodified PDMS were difficult to load with aqueous solutions presumably due to the hydrophobic nature of PDMS. Preloading the channels with methanol and then water as reported by Ocvirk and coworkers did increase the ease with which aqueous solutions were introduced into the channels [13]. However air bubbles rapidly appeared in the channels of most of the devices within a short time after introduction of the aqueous solution into the channel when applying a negative pressure to one reservoir. Air bubbles formed at such a rapid rate in the channels that most unmodified devices (~90%, $n>75$) were not usable. Bubbles formed predominantly on the low pressure end of the channel, i.e. the side to which the vacuum was applied. Use of a stronger vacuum increased the amount of bubble formation. Application of a positive pressure to a reservoir to load the channels with buffer significantly diminished the rate of bubble formation in the devices. Since the buffer solutions were degassed and no electrophoresis was performed, a potential source of the air bubbles was the polymeric matrix of the device itself. The permeability of PDMS to gases is very high supporting this hypothesis [19]. An alternative explanation for the bubble formation was that the reversible bonding of the two PDMS surfaces was disrupted by the

application of the vacuum to load the channel with buffer. While this cannot be completely ruled out, we did not observe fluid leakage from the channels into the interface when pressure was used to load the channels with buffer. These results contrast sharply with those of Ocvirk and others in which untreated PDMS channels filled with aqueous solutions did not exhibit bubble formation [13–15]. It is possible that the different polymerization conditions utilized in this report compared to Ocvirk and colleagues resulted in PDMS channels with differing surface and bulk properties, i.e. hydrophobicity and gas permeability.

Experiments to measure μ_{eo} were performed on the devices in which the channels did not rapidly fill with air. The measured μ_{eo} in the devices composed of native PDMS was approximately 1×10^{-4} cm^2/Vs irrespective of the channel dimensions (Fig. 2A). The magnitude of the EO flow was stable over 14 days (Fig. 2B). The measured value of μ_{eo} was similar to that reported by Ocvirk et al. [13]. Conditioning the channels for 1 h in 1 *M* NaOH prior to use did not alter the EO flow. For a given device, the relative standard deviation (RSD) of μ_{eo} was typically less than 10% ($n=5$ measurements per device). For devices fabricated at the same time from the same mold, the RSD was also less than 10% ($n=15$ devices). For devices fabricated on different days from the same mold, the RSD of μ_{eo} was as high as 33% ($n=5$). The large RSD suggests that some variables influencing μ_{eo} are not well controlled during device fabrication on different days. This is perhaps not surprising since the origin of the surface charge which creates the EO flow is not known. PDMS is composed of repeating $-OSi(CH_3)_2O-$ groups and was therefore originally not expected to have a net surface charge or EO flow. The charge present on the PDMS surface of the channels may be due to impurities in the PDMS such as the cross-linking agent or silica fillers [13]. Alternatively, the surface properties of organic polymers may be quite different from those of the bulk polymer [20]. The surface may undergo chemical transformations (particularly oxidative reactions) or adsorb molecules from the surrounding environment. All of these mechanisms could alter the surface charge of the polymer and consequently the EO flow. To investigate whether –OH groups were present on the surface of the PDMS, the absorbance in the infrared was measured by ATR-IR. No peaks were present near 3200–3400 cm^{-1} suggesting that any –OH groups present were at concentrations below the limits of detection.

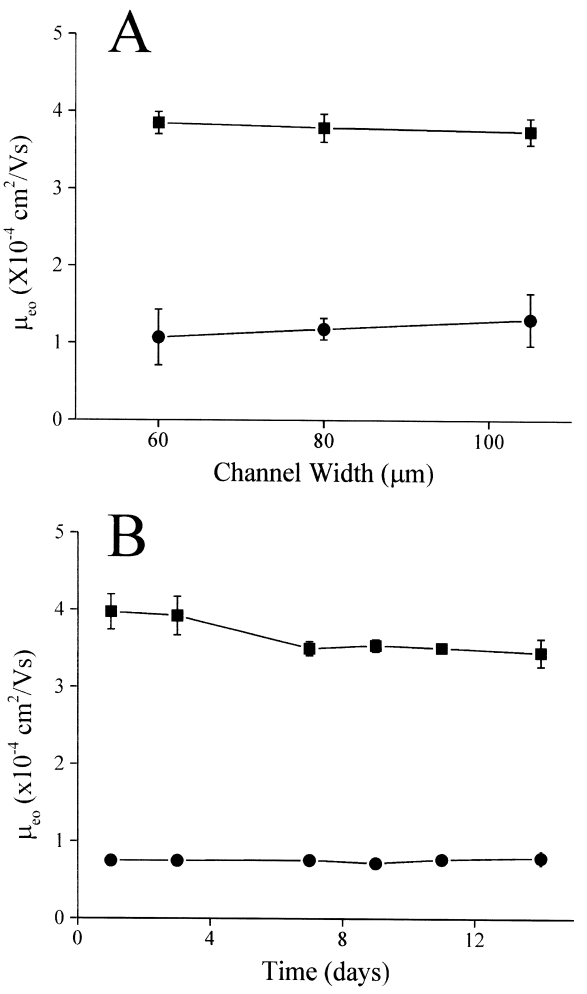

Fig. 2. Electroosmotic properties of native (●) and oxygen plasma-treated (■) PDMS channels and substrates. (A) μ_{eo} of channels with different mid-widths (*x*-axis). (B) Stability of μ_{eo} for channels stored in water. Native PDMS channels and substrates or oxygen plasma-treated channels and substrates were constructed and immediately filled with water. At the times indicated on the *x*-axis, the water was replaced with the appropriate buffer solutions and μ_{eo} was measured. Between measurements the channels were maintained in water. The mid-width of the channels was 60 μm. The error bars represent a single standard deviation of the data points and were frequently smaller than the symbol representing the data in this set of experiments.

3.2. Characteristics of oxygen plasma-treated PDMS channels

The properties of PDMS devices in which the channel and substrate were both treated with an oxygen plasma were compared to that of untreated PDMS channels. The devices treated with an oxygen plasma were substantially easier to fill with aqueous buffers than untreated devices. In addition only a minority of the channels (<5%) exhibited the formation of air bubbles after filling with an aqueous buffer. It is possible that the oxygen plasma treatment diminished the gas permeability of the PDMS or alternatively that the treatment masked nucleation sites or reduced the number of nucleation sites for bubble formation.

μ_{eo} in the oxygen-plasma treated devices was 4×10^{-4} cm^2/Vs, approximately four-fold greater than that of the untreated channels and comparable to that of fused-silica under similar conditions [21,22]. As with the untreated devices, μ_{eo} was not dependent on the channel dimensions (Fig. 2A). Analysis of the surface of oxygen-plasma treated devices by ATR-IR revealed the presence of a large peak centered at 3200 cm^{-1} suggesting that –OH groups were now present at much higher concentrations compared to untreated PDMS (Fig. 3A). These results are in agreement with the hypothesis of Duffy and coworkers that oxygen-plasma treatment increases the EO flow by converting $Si–CH_3$ to $Si–O^-$ [12]. These same investigators also reported that the oxidized layer of PDMS was stable for a period of only 3 h after which time the migration times of analytes began to drift. For this reason we tested the stability of the channels by measuring the ATR-IR spectra and μ_{eo} of devices treated with an oxygen plasma and then exposed to air or water. Within 3 h after exposure to air, the peak at 3200 cm^{-1} in the ATR-IR spectra of the oxygen plasma-treated PDMS was nearly absent suggesting that the stability of the oxidized PDMS in the presence of air was considerably less than 3 h (Fig. 3B). In contrast when water was maintained in a channel, the EO flow was nearly constant over 14 days, the longest time tested (Fig. 2B). When the aqueous buffer was removed and the channel exposed to air for varying periods of time (3, 6, 9, 24, or 48 h) prior to refilling with buffer, the EO flow decreased with a half-life of ~9 h to ~60% of its original value (μ_{eo} of 2.7×10^{-4} cm^2/Vs) (Fig. 4). The EO flow was stable at this lower value for periods of air-exposure up to 24 h (the longest time tested). When these air-exposed channels were treated with 1 *M* NaOH, μ_{eo} increased to the value obtained prior to air exposure (Fig. 5). However, the incubation time with base was quite long (~3 h) making this an unsuitable strategy to regenerate the PDMS surface between electrophoretic runs. Taken together these results suggest that some of the increased charge on the surface of PDMS treated with an oxygen plasma is due to the formation of surface Si(OH) which are unstable in the presence of air. The instability of the surface Si(OH) on oxidized PDMS after exposure to air has been noted by others but the mechanism is not well understood [12,13,23,24]. Current evidence suggests that the ''hydrophobicity recovery'' is due to the migration of low molar mass PDMS to the surface and/or due to reorientation of hydroxyl groups from the surface into the bulk by rotation about sigma bonds [25–27]. The regeneration of the surface Si(OH) upon prolonged exposure to base may be due to the removal of the low molar mass PDMS or reorientation of the hydroxyl groups onto the surface. However, a portion of the surface charge on the

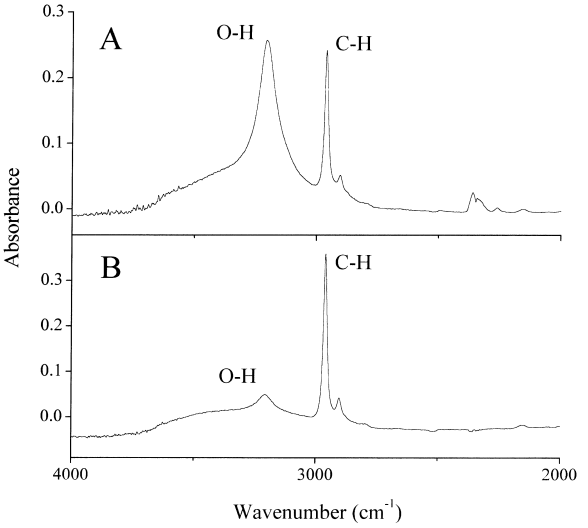

Fig. 3. Measurement of the infrared absorbance of PDMS by ATR. (A) A PDMS substrate immediately after treatment with an oxygen plasma. (B) A PDMS substrate treated with an oxygen plasma and then incubated in air for 3 h.

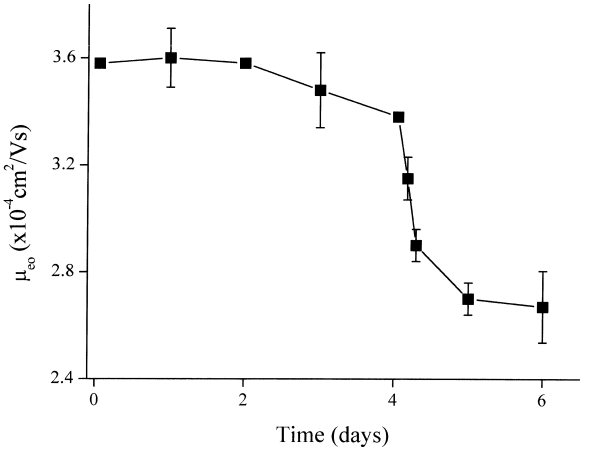

Fig. 4. Stability of μ_{eo} for channels exposed to air. Devices were fabricated from PDMS treated with an oxygen plasma and then immediately filled with water. At the times indicated on the *x*-axis between day 0 and 4, the water was replaced with the appropriate buffer solutions and μ_{eo} was measured. On day 4, water was removed from the channels and the lumens were filled with air. To measure μ_{eo} at the times indicated on the *x*-axis between days 4 and 6, the appropriate buffers were loaded into the channel. After the measurement, air was reintroduced into the channel. The mid-width of all channels was 60 μm.

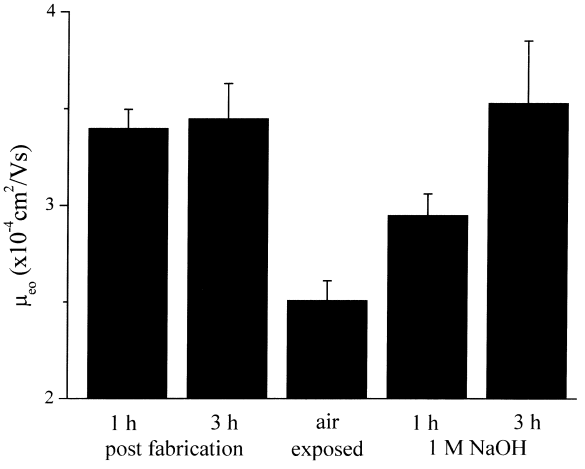

Fig. 5. Regeneration of air-exposed devices with 1 *M* NaOH. Devices were fabricated from PDMS treated with an oxygen plasma and then immediately filled with water. At 1 and 3 h post fabrication, μ_{eo} was measured as displayed on the bar graph. The devices were then filled with air for 24 h and μ_{eo} was measured (labeled as ''air exposed''). The lumens were then filled with 1 *M* NaOH and incubated for 1 h after which μ_{eo} was measured (labeled as ''1 h 1 *M* NaOH''). The devices were incubated for an additional 2 h in 1 *M* NaOH and μ_{eo} measured (labeled as ''3 h 1 *M* NaOH''). The mid-width of all channels was 60 μm.

oxygen plasma-treated PDMS must be due to either stable Si(OH) or other moieties since the air-treated PDMS had twice the μ_{eo} of native PDMS.

3.3. Characteristics of hybrid PDMS/glass channels

Glass is frequently used as a substrate to which the material (polymer or glass) with the imprinted channels is sealed. Glass possesses a number of attractive properties as a substrate: (i) electrodes and other features are easily fabricated on glass, (ii) glass has excellent optical properties with minimal fluorescence, (iii) glass is rigid and inelastic, and (iv) glass can be manufactured in very thin sheets making it compatible with the use of high numerical aperture lenses with short working distances. Despite these benefits the use of glass as a substrate results in a microchannel in which the walls are composed of different materials with potentially distinct zeta potentials. Previous investigators have reported that μ_{eo} is nearly identical in native and oxidized PDMS/glass devices [14]. Their reported μ_{eo} at pH 7 (4×10^{-4}cm^2/Vs) was similar to the measured μ_{eo} for glass devices under like conditions. This suggests that the surface properties of the glass substrates may dominate the flow properties irrespective of whether the PDMS is oxidized. To determine how the EO flow was altered when glass was used as a substrate for the PDMS channels, μ_{eo} was measured in PDMS/glass channels with a mid-width of 60 μm (Table 2). Many of these devices (~45%, $n=6$) exhibited problematic bubble formation although the percentage was approximately half that for the PDMS/PDMS devices. μ_{eo} was increased two-fold in native PDMS/glass devices compared to native PDMS/PDMS devices. The μ_{eo} of the PDMS/glass devices is in agreement with the estimated μ_{eo} (2×10^{-4} cm^2/Vs) calculated from the relative surface area contributions of the glass (40%) and PDMS (60%) in the channels. However, μ_{eo} of the native PDMS/glass channels was still one half of that of oxidized PDMS/glass channels (and all-glass devices) suggesting that the glass substrate substantially altered but did not dominate the flow properties. Oxidized PDMS/PDMS and oxidized PDMS/glass channels possessed identical μ_{eo}. The extent to which the different surface properties of the PDMS

Table 2
μ_{eo} of PDMS/glass hybrid channels

	Channel/substrate composition		
	PDMS/PDMS[a] ($\times 10^{-4}$ cm^2/Vs)	PDMS/Glass ($\times 10^{-4}$ cm^2/Vs)	PDMS/Glass[a] ($\times 10^{-4}$ cm^2/Vs)
10–20 m*M* PO_4, pH 7.0	3.8±0.5	1.8±0.2	3.6±0.2
Extracellular buffer	2.0±0.1	1.4±0.1	1.8±0.2

[a] The PDMS channel and the substrate (glass or PDMS) were treated with an oxygen plasma just prior to the measurements.

channel and glass substrate influence the separation characteristics of the devices is currently under investigation.

3.4. Properties of PDMS/PDMS and PDMS/glass channels in the presence of biologic buffers

Microfabricated devices are expected to have a major impact in biomedical research particularly in the analysis of single cells [28–31]. Miniaturized flow cytometers have been used to sort and manipulate cells [28]. In other devices, cells were loaded into the microchannels and lysed, followed by analysis of the cellular contents [29,30]. In most of these and future applications with cells, cell-compatible buffers will occupy at least a portion of the microdevice. These buffers are neutral in pH, possess a high ionic strength, and frequently contain divalent cations (Mg^{2+} and Ca^{2+}). To determine the extent to which these buffers altered the EO flow, μ_{eo} was measured in untreated and oxygen plasma-treated channels with glass or PDMS substrates (Table 2). As expected EO flow was diminished compared to that of the low salt phosphate buffers. The extent to which μ_{eo} was diminished was much greater in the oxygen plasma-treated devices than in the untreated devices so that all of the devices possessed a similar μ_{eo}. Thus in the presence of biologic, high-salt buffers, treatment of PDMS surfaces with an oxygen plasma may not be a useful tool to increase the EO flow-rate.

4. Conclusion

We have demonstrated that air bubbles rapidly nucleate in microlumens formed exclusively from native PDMS substantially limiting the utility of these devices. Oxidation of the PDMS dramatically decreased gas nucleation and increased μ_{eo} by a factor of four. While the oxidized surface was unstable in air, it could be maintained for long periods of time when immersed in an aqueous solution. In addition the surface could be regenerated by exposure to base. Combining the aqueous immersion with periodic regeneration in base may improve the long term stability of the oxidized PDMS surface. Channels composed of oxidized PDMS and glass had identical μ_{eo} compared to those fabricated entirely from oxidized PDMS. When the oxidized PDMS/PDMS or oxidized PDMS/glass was utilized in the presence of a cell-compatible buffer, the increase in μ_{eo} over that of native PDMS/glass devices was minimal. From these results it is clear that new strategies to create hydrophilic and stable surface properties are needed to improve the utility of PDMS-based devices.

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