Mixing with bubbles: a practical technology for use with portable microfluidic devices

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Received 29th July 2005, Accepted 1st December 2005
First published as an Advance Article on the web 15th December 2005
DOI: 10.1039/b510843h

This paper demonstrates a methodology for micromixing that is sufficiently simple that it can be used in portable microfluidic devices. It illustrates the use of the micromixer by incorporating it into an elementary, portable microfluidic system that includes sample introduction, sample filtration, and valving. This system has the following characteristics: (i) it is powered with a single hand-operated source of vacuum, (ii) it allows samples to be loaded easily by depositing them into prefabricated wells, (iii) the samples are filtered *in situ* to prevent clogging of the microchannels, (iv) the structure of the channels ensure mixing of the laminar streams by interaction with bubbles of gas introduced into the channels, (v) the device is prepared in a single-step soft-lithographic process, and (vi) the device can be prepared to be resistant to the adsorption of proteins, and can be used with or without surface-active agents.

Introduction

This paper describes an approach to one of the outstanding problems in the design of microfluidic devices—making them portable. We illustrate this approach with a model microfluidic chip that has many of the features needed for a portable bioanalytical system, and performs the rudimentary tasks associated with handling fluids at the microscale.

The technology of microfluidics has been relatively slow to propagate into devices that are widely used, especially when the devices are portable or intended for use in resource-limited applications or environments. The reason for this slow propagation is that even relatively simple devices must include and integrate a substantial number of components. The creation and integration of these components represents a more substantial problem than it might seem, but the development of new microfluidic elements, and means to incorporate them into devices, are proceeding. Systems for bioanalysis and separation,[1–3] or high-throughput screening[4,5] are already available. We are especially interested in devices to be used in resource poor environments—*e.g.*, in healthcare in developing countries, by first responders and the military, and in analogous problems—where portability and ruggedness are key concerns.[6–8] Here we describe an example of an integrated microfluidic device that performs several of the basic functions required from a portable analytical system (introduction, filtering and mixing of the analytes) automatically and with power only from a hand-operated source of vacuum. The device can be fabricated using a single layer of micro-molding, and it operates efficiently without using electrical power.

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Mixing is an enduring problem in microfluidics.[9,10] Rapid homogenization of analytes at the microscale requires either active 'stirring'—aimed to achieve unsteady flow conditions[11,12]—or enhancement of the three dimensional character of the flow.[13,14] Neither of these approaches is—so far—compatible with the idea of inexpensive, in-field devices. Currently available passive mixers require multilayer lithography,[13,14] while active mixers demand external machinery[11,12] or involve moving parts[12] that also complicate fabrication. The availability of simple and inexpensive methods for mixing is thus a necessity in many types of analytical microsystems, especially those that are intended to be simple in use and disposable, and those that make use of homogeneous assays.[15] In the demonstration of the portable system that we report here, we incorporate strategies for micro-mixing that are based on the movement of immiscible slugs in microchannels.[5,16–18] Multiphase flows in capillaries have a long history of use for segmentation,[19] mixing[20] and homogenization of residence times[21] of reagents in flow-through reactors. Recent advances in the engineering of microscale flows have demonstrated the usefulness of these techniques for mixing *inside*[22–25] immiscible droplets suspended in a host liquid, and mixing in the *host* liquid using gaseous plugs.[17,18] We show that these techniques[17,18]—previously demonstrated for mixing in microfluidic chips operated in laboratory conditions—can be adapted for reliable operation in a rugged, portable system powered by application of negative pressure[6,7,26,27] and operating efficiently with analytes having viscosities corresponding to those of most physiological fluids.

Materials

We fabricated the device (Figs. 1a) in a polydimethylsiloxane (PDMS) slab sealed to a PDMS substrate. We chose to use PDMS because it is the most useful material for testing concepts in microfluidics, and because it is easily coupled with

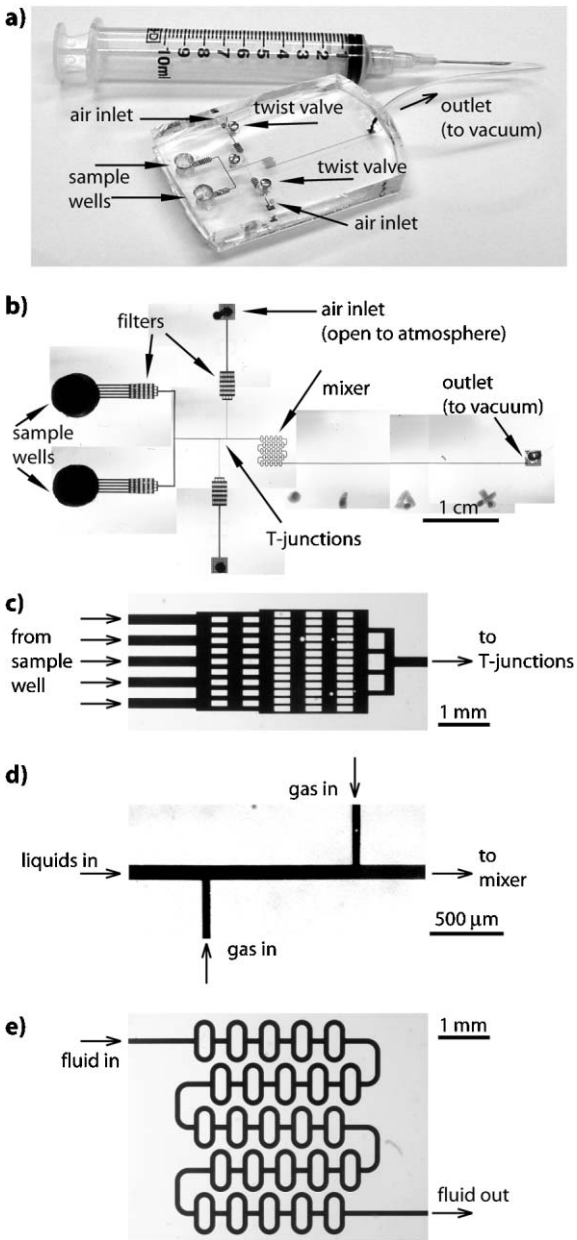

Fig. 1 Pictures and micrographs of the portable microfluidic device. The arrows guide the eye to the labeled components of the system and mark the direction of flow of the fluids, as described on the figure. (a) A picture of the device used in our study. The two liquids to be mixed are deposited as drops in the sample wells. When vacuum is applied to the exit, air and liquids flow through the device; the air breaks into separated bubbles in the T junctions. The movement of the bubbles in the mixer system facilitates mixing. The only external component required for the device to operate is an evacuated container (here a syringe barrel). We regulate the rate of flow of the fluids using TWIST valves. In this picture we have filled the channels with black dye in order to make them visible. (b) Optical micrograph (assembled from eight individual images) of the network of microchannels. The analytes deposited in wells are sucked into the device *via* a set of filters. Gaseous plugs are formed at the T-junctions. The liquids mix in the mixer and exit into the outlet channel. (c) Enlarged micrograph of the filter that is part of each inlet channel. (d) The two T-junctions. (e) The micromixer with sections of branched channels.

soft lithography[28,29] for fabrication. Formation of slugs of an immiscible phase requires that the continuous phase preferentially wets the walls of the channels. Plasma oxidation, which is a part of the sealing procedure[28,29] used to assemble devices in PDMS, renders the surface of PDMS hydrophilic and allows for reliable formation of bubbles of gas in an aqueous solution. The surface of dry, plasma-oxidized, hydrophilic PDMS restructures in time (hours to days[30,31]) and regains the hydrophobic character of untreated PDMS. In fabricating devices intended for practical use—where maintaining appropriate surface properties during prolonged shelf-storage before use will be critical, and the cost of the polymer may be important—materials other than PDMS may prove superior for specific applications.[31,32] The model system we have designed easily fits on the palm of a hand (Fig. 1a), and is thus compatible with applications requiring portability.

Inlets and flow regulators

As usual in devices made of soft polymers, we accessed the microchannels by boring holes through the PDMS slab.[28] Large holes served as inlets (wells of volume of approximately 100 µL) for the two analytes. The user deposits these liquids as drops onto the chip (or—alternatively—inserts capillary 'sippers' containing the liquids into smaller holes). There are two inlets for the gas (air) that forms the bubbles; these inlets remain open to atmosphere during use. We connected the end of the outlet channel to a container (a syringe barrel and plunger or a hand-operated pump) maintained at a pressure $p < 10$ kPa (*i.e.*, <1/10 of atmospheric pressure; $p_0 \approx 100$ kPa). The operation of the device is not critically dependant on the exact pressure differential between inlet and outlet, provided it is on the order of one atmosphere.

We approximate the rate of flow Q of the liquid through the network of channels (Fig. 1b) by the Hagen Poiseuille equation $Q \approx (p_0 - p)A^2/\mu L$, where A is the cross section area of the channel ($A \approx 1 \times 10^3$ µm^2), μ is the viscosity of the liquid (we approximate the rates of flow for a range of viscosities between 1 and 6 mPa s) and L is the length of the channel ($L \approx 10$ cm). This combination of parameters yields values of Q between 0.15 µL s^{-1} (μ = 10 mPa s) and 1 µL s^{-1} (μ = 1 mPa s). The corresponding Reynolds numbers are given by $Re = \rho Q/\mu w$, where ρ is the density of the fluid ($\rho \approx 10^3$ kg m^{-3}) and w is the width of the channel (w = 100 µm). The value of Re ranges from 0.1 (μ = 10 mPa s) to 10 (μ = 1 mPa s), thus the flow of liquid through the channels is laminar.

In practice, in the presence of bubbles, we obtained smaller rates of flow ($Q \approx 0.3$ µL s^{-1} for μ = 1 mPa s and $Q \approx 0.04$ µL s^{-1} for μ = 5.8 mPa s) than those estimated above. We used TWIST valves[8] to tune the rates of flow continuously by adjusting the cross-section of the inlet channels. We found the following minimum rates at which the device still functioned properly: $Q \approx 0.06$ µL s^{-1} for μ = 1 mPa s and $Q \approx 0.015$ µL s^{-1} for μ = 5.8 mPa s. Using only 25 µL of each analyte, TWIST valves allowed us to tune the time it took this volume of analyte to flow through the device from ~3 to ~12 minutes for μ = 1 mPa s and ~10 to ~30 minutes for μ = 5.8 mPa s.

Filters

The macroscopic wells—where analytes are deposited—are connected to the micro-mixer *via* a set of inlet channels (multiple channels minimize the risk of clogging at the entrance to the micro-network) and filters that are prefabricated into the device. The filters have a graded design (Fig. 1c) that prevented particulates present in the drops of reagents, or in the air, from entering and blocking the network of channels.

Formation of bubbles—T-junctions

We introduced bubbles into the main channel and the mixing section using T-junctions.[33] This geometry has been successfully applied to form droplets[33] and bubbles[34] in devices operated either with positive pressures applied to the inlets,[33] or with controlled rate of inflow of the fluids into the device.[34] Formation of bubbles by application of negative pressure is equivalent to emulsification in the pressure-controlled devices with the additional constraint that the pressure applied to all the inlets is the same. We find that the T-junction reliably forms bubbles in a vacuum-operated device. With the use of the TWIST valves we could tune the T-junctions to steadily produce bubbles at a volume fraction ϕ required for efficient mixing ($\phi > 0.2$), and the bubbles were usually uniform in size. We did not measure the standard deviation of the size of the bubbles because we observed that the distribution of size of the bubbles does not affect the efficiency of mixing appreciably. In the experiments in which we did not use surfactant, the bubbles often coalesced in the channels or broke at the branch points in the mixer—both effects lead to a significant variation of sizes of bubbles in the mixing unit. These effects, however, did not decrease the efficiency of mixing.

While testing formation of bubbles by application of negative pressure, we found an interesting behavior that we do not observe in devices operated with positive pressure applied to the inlets: in the absence and at very low (<0.1% (w/w)) or very high (>10% (w/w)) concentrations of a neutral surfactant (Tween 20, Aldrich), the device generated a stream of regularly-spaced bubbles. At intermediate concentrations of surfactant, the device generated irregular bursts of bubbles (with a frequency of approximately 10^{-1} to 10^{-2} Hz, and with tens to hundreds of bubbles generated in a single burst), interrupted by pauses with typical intervals on the order of tens of seconds. We associate this behavior with dynamic surface tension effects:[35–37] as bubbles break off from the tip of the stream of gas, the concentration of surfactant on the tip is diminished and the value of interfacial tension increases, pulling the tip upstream—into the gas inlet channel—and reducing the rate of formation of bubbles. As liquid flows by the tip, surfactant molecules adsorb onto the interface, lower the capillary pressure and formation of bubbles starts again. We found that we can direct the system towards stable operation by using the TWIST valves to reduce the flow rate of the liquid and gas.

We used two inlets for gas to improve the robustness of the device. When each of the two T-junctions generated steady series of bubbles, they worked out-of-phase. When the bubble generators were sending long series of bubbles interrupted by long intervals of inactivity, they also worked out-of-phase—that is—when one of the generators was inactive, the other one produced bubbles, and *vice-versa*. This behavior ensured that bubbles are always present in the mixing unit.

Branching channel mixer

Jensen *et al.* have previously shown a microfluidic mixer based on flow patterns introduced into the continuous liquid by bubbles flowing through winding channels.[17,38] In this work we use a micro-mixer that comprises a series of branching sections: the main channel splits into two, and these branches subsequently recombine. We have shown previously [18] that bubbles flowing through branched sections stretch and fold the continuous liquid, and reduce the typical size of unmixed volumes of the liquid exponentially in the length traveled downstream. Briefly, in the absence of bubbles, the liquid splits at equal rates of inflow into the two arms of the branched section. When the bubble enters one of the arms, it increases the resistance to flow of fluid through the branch in which it travels;[39] it thus decreases the inflow of the liquid to this arm, and increases the flow of fluid into the opposite branch. As a result, the next bubble enters the opposite arm and reverses this pattern of flow. The periodic oscillation of inflow into left and right arms crosses the laminar streamlines of the liquid and results in mixing. All of the channels in the mixing section of the device are designed to form arcs, since the curvature of the channels also enhances mixing[17] and the lack of sharp corners eliminates stagnation points and residual eddies.

Efficiency of mixing

We tested the efficiency of mixing and applicability of the portable device to liquids of different viscosities, both with and without surfactant. To visualize mixing we used two streams of aqueous solutions of glycerol, one of which was dyed with a black ink (Waterman). In the absence of bubbles, the two liquids flowed laminarly with only small diffusive broadening of the black–clear interface (Fig. 2a). When air was allowed to flow into the channel and break into bubbles, the gaseous plugs mixed the liquids. We quantified mixing by taking intensity profiles $I(x)$ from optical microscopes across the main channel before the mixer and after each of branching sections (Fig. 2c) (here x is the spatial coordinate in the direction perpendicular to the length of the channel, and $I(x)$ is the average intensity of pixels positioned a distance x from the wall of the channel).We normalize this intensity so that $I(x)$ can acquire values between 0 (corresponding to the original, unmixed black liquid) and 1 (clear liquid). We calculated the normalized standard deviation $\sigma^* = \sigma(I(x))/\langle I(x)\rangle$, where $\sigma(I(x))$ is the standard deviation of $I(x)$. The value of $\sigma^* = 0$ corresponds to ideally homogeneous distribution of ink, while $\sigma^* = 0.5$ signifies two separate streams of original solutions. In Fig. 2d we show the evolution of σ^* along the channel in experiments on mixing of (i) clear water and water–dye solution ($\mu \approx 1$ mPa s), (ii) two aqueous solutions of surfactant (Tween 20, 1% (w/w), and (iii) two aqueous solutions of glycerol (50% (w/w), $\mu \approx 6$ mPa s). The rapid decay of σ from ~ 0.5 before the first branching section to ~ 0 after

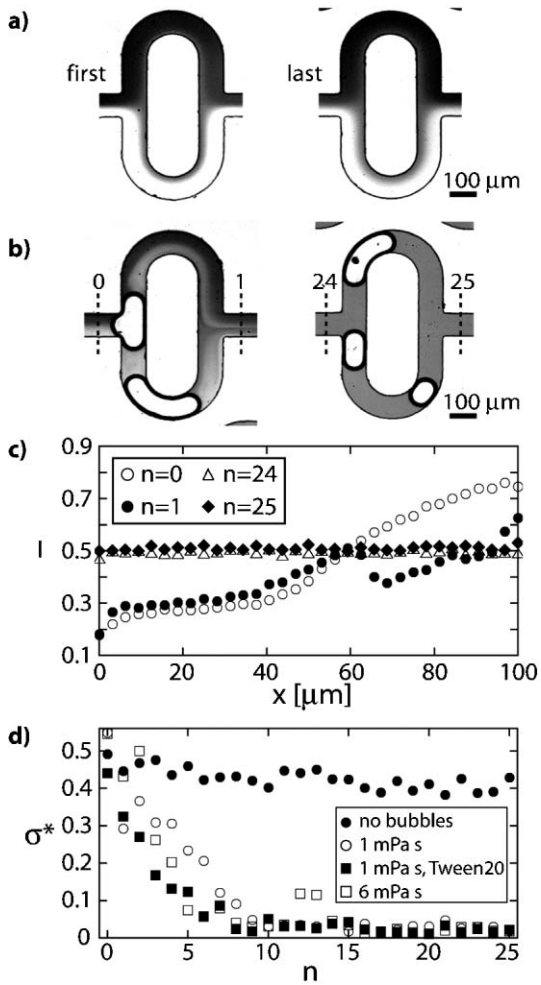

Fig. 2 Optical micrographs of the first and the last branching section of the mixer (experiment with 1 mPa s aqueous solutions of surfactant), (a) without bubbles and (b) with bubbles of air. The dashed lines in (b) show the positions at which we acquired the profiles of the intensity of light across the channel (from top to bottom on the picture). The numbers indicate the number of the branching sections after which the profile was taken. We show these profiles in inset (c). When bubbles are present in the system the two liquids mix partially even before the mixer. This mixing is due to the periodic variation of flow conditions around the T-junctions, while the bubbles are formed. When surfactant is not present in the liquid, the bubbles often coalesce at the points where channels split. Coalescence leads to a variation of sizes of the bubbles, but we found that this variation does not affect the efficiency of mixing. In (b) the liquids do not contain surfactant and bubbles coalesce in the branching sections resulting in a wide distribution of sizes of the gaseous slugs. We quantified homogenization of the two liquid streams by the normalized standard deviation of the intensity profiles taken along the positions marked with dashed lines in inset (b). Inset (d) shows the evolution of the normalized standard deviation σ^* of the intensity profiles as a function of the position in the mixer (n: number of branching sections passed) for four different conditions—no bubbles (●), the aqueous solutions without (○) and with (■) surfactant (Tween 20, 1% w/w), and the 50% w/w solutions of glycerol (□).

approximately 10 branching sections indicates efficient mixing of the all of the liquids tested in our experiments. The lack of dependence of the efficiency of mixing on the presence of surfactants is important for two reasons: (i) most physiological samples contain surface-active ingredients that might, in principle, influence the operation of the mixer, and (ii) specific diagnostic applications might require the use of surfactants to prevent adsorption of proteins to the gas/liquid interface,[15] or require their absence to prevent denaturation of proteins.[40] The mixer is compatible with viscosities corresponding to those of biological fluids that are of potential interest: the viscosity of human blood serum (~2 mPa s),[41] whole blood, which has a viscosity of approximately 5–6 mPa s at low rates of shear,[41] urine (~1 mPa s),[42] tears (2 to 9 mPa s),[43] and saliva (2 to 9 mPa s).[44]

Compatibility with solutions containing proteins

In order to check the compatibility of our system with proteins, we performed an enzymatic assay. We deposited aliquots of two solutions into the sample wells; one solution contained Amplex red reagent (200 μM H_2O_2, 100 μM Amplex red reagent, in buffer (50 mM sodium phosphate pH 7.4), Molecular Probes, A22188), and the other contained horseradish peroxidase (HRP, EC 1.11.1.7.) in the same buffer. When mixed together, HRP converts Amplex red into a fluorescent product with an emission maximum at $\lambda \approx 590$ nm.[45] The intensity of fluorescence that we observed was limited by the rate of reaction rather than by mixing. Using TWIST valves, we adjusted the average rate of flow of liquid through the channels to <1 cm s^{-1}; this value allowed us to see saturation of the fluorescent signal within the mixer (Fig. 3).

Conclusions

We have demonstrated a portable microfluidic platform that integrates (i) easily accessible entry ports for the analytes, (ii) filters, (iii) a robust micro-mixer, and (iv) a hand-operated air pump as a source of reduced pressure to pump both liquid and gas. The system is easy to fabricate—it requires only a single step of lithography and replication—and it is simple in use—it requires only a single source of low-quality vacuum. The device operates over a range of viscosities that includes those of blood serum, whole blood at low rates of shear, urine,

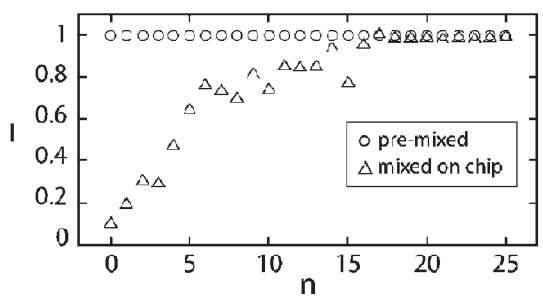

Fig. 3 Intensity of the fluorescent signal collected from a 100 by 100 μm area after each branching unit (n denotes the number of branching units passed by the fluid) measured in two experiments: (i) (control experiment) with pre-mixed solution delivered to the device, and (ii) with two separate streams of Amplex red and horseradish peroxidase delivered to the sample wells and mixed within the mixer. The intensity is normalized by the intensity obtained for the control.

saliva, and tears—and is relatively insensitive to the interfacial free energy of the gas/liquid interface. The T-junction geometry is suitable for formation of bubbles by application of negative pressure to the outlet of the device. The bubbles mix the continuous fluid and homogenize the residence times[17,19] of the analytes in the device.

We believe it will be possible to integrate additional components (*e.g.* for analytic purposes) into the device. In order to avoid both potential problems in processing the mixed analytes and in detection associated with the presence of bubbles in the stream of mixed liquids, it is possible to separate the gaseous slugs from the continuous fluid with the use of capillary pressure. Such separation has been demonstrated independently by Gunther *et al.*,[17,46] and by Hibara *et al*.[47] The residence times of the analytes—a parameter important in many biological assays—can be tuned easily (either in the fabrication process, or directly in the field by adjusting an imbedded TWIST valve). The device functions efficiently both in the presence or absence of surface-active agents, should be applicable for diagnostic assays involving proteins, and is resistant to many variations in the properties of biological fluids.

The necessity to maintain the hydrophilic character of the walls of the microchannels may require the development of additional technology before these devices have sufficiently long shelf-life to allow them to be used practically. This problem is one that is general to microfluidic devices fabricated with hydrophobic polymers. For PDMS devices a possible solution is to store them in hermetic, humidified containers. A choice of more hydrophilic polymer or use of passivated surfaces[48] (*e.g.* with proteins[49]), or addition of surfactants to the solutions containing the analytes, may also solve this problem. The presence of gas/liquid interfaces might be problematic in some specific diagnostic applications, as many proteins adsorb to these interfaces and denature. Use of high-concentration samples might help circumvent this problem, as the ratio of the area of the interface to the volume of the liquid is quite small.

To date, microfluidics has remained predominantly a research and laboratory technique. We intend the device described in this report to be a step toward an integrated, portable device for in-field diagnostics. The processes that we used in this system—that is: introduction of fluids, filtering, valving and mixing—all seem to function efficiently. Better control over the surface chemistry and wetting, improved strategies for detection,[7,15] and solutions for handling of multiple series of reagents[6] are probably the next elements to address in working toward practical devices.

Acknowledgements

P.G. acknowledges support of the Foundation for Polish Science. M.A.F. acknowledges a fellowship from the Hertz Foundation. S.K.S. acknowledges a fellowship from the Canadian Institutes of Health Research. This work was supported by the U.S. Department of Energy under award DE-FG02-OOER45852. Additional salary support was provided by the National Institutes of Health (NIGMS) under award GM065364. We thank Harvard MRSEC for the use of micro-fabrication facilities and fast cameras.

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