DROPLET MIXER BASED ON SIPHON-INDUCED FLOW DISCRETIZATION AND PHASE SHIFTING

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ABSTRACT

We present a novel mixing principle for centrifugal microfluidic platforms. Siphon structures are designed to disrupt continuous flows in a controlled manner into a sequence of discrete droplets, displaying individual volumes as low as 60 nL. When discrete volumes of different liquids are alternately issued into a common reservoir, a striation pattern of alternating liquid layers is obtained. In this manner diffusion distances are drastically decreased and a fast and homogeneous mixing is achieved. Efficient mixing is demonstrated for a range of liquid combinations of varying fluid properties such as aqueous inks or saline solutions and human plasma. Volumes of 5 μL have been mixed in less than 20 s to a high mixing quality. One-step dilutions of plasma in a standard phosphate buffer solution up to 1:5 are also demonstrated.

INTRODUCTION

Mixing and diluting are essential steps in many assay procedures and constitute important unit operations for lab-on-a-chip platforms. In particular for point-of-care applications, mixing and dilution methods need to be fast. In contrast to macroscopic systems where liquid mixing can be easily achieved by stirring, shaking or other methods promoting turbulence in the liquid system, mixing in microfluidic systems is more challenging. Due to the small characteristic dimensions of microfluidic devices the flow is typically laminar and microfluidic mixers have to rely on diffusion and chaotic advection. Several microfluidic mixing principles have been introduced in the past [1] [2]. Among these are lamination mixers where liquids are laminated in a common channel to decrease diffusion distances. This can be further enhanced by placing obstacles in the channel or introducing curvatures and abrupt changes in cross-sectional area of the channels to promote chaotic advection or vortex mixing. Other mixers, especially suited for centrifugal microfluidics explore the Coriolis force present in rotating systems to induce secondary flows and promote mixing [3] or use periodically changing angular accelerations to perform batch mixing [4].

The novel mixing strategy presented here, performs mixing of two liquids by alternating the injection of discrete liquid volumes into a common reservoir under the impact of a centrifugal field. This approach enables mixing within short timescales (typically seconds) by generating an alternating pattern of thin lamellae which reduce the diffusion length, and also the kinetic impact of the discrete liquid volumes on the predeposited liquid volumes.

Given a liquid medium with a heterogeneous spatial distribution of species i.e. a concentration gradient ∇C, there will be a flux Φ of the species following a gradient in concentration. This flux is described by Fick's first law:

$$\Phi = -D \cdot \nabla C \qquad (1)$$

where D is the diffusion coefficient of the species in the particular medium. The time t_D required for a species to travel a distance l_D by diffusion is given by [5]:

$$t_D = \frac{l_D^2}{D} \qquad (2)$$

Equation 2 shows that diffusion times are proportional to the square of the diffusion distances and hence minimizing the diffusion length is a requirement for fast mixing. For discrete alternating layers of different liquids such as the approach being proposed, minimizing the thickness of the layers and maximizing the contact area has a significant impact in reducing the mixing time.

For continuous flow mixing this multilayer lamination approach has already been demonstrated. [1] [6]. The main advantages of the present approach is the simplicity for forming multiple thin layers from only two inlet channels plus the enhanced mixing effect of the kinetic impact of the alternate liquid volumes on a pre-deposited liquid. Moreover, the strategy is also highly suitable for mixing unequal liquid volumes such as required for dilutions.

MIXING MECHANISM

In order to create a fine striation pattern of liquids from two different inlets in a common outlet reservoir, two steps are necessary:
1. Liquid discretization in small volumes
2. Issuing the discrete volumes in alternating fashion into a common chamber to create a multi layer stack.

The first requirement is achieved using siphon-induced flow discretization which is illustrated in Figure 1.

The basic mechanism can be described as follows: a given liquid enters a reservoir featuring a siphon outlet at flow rate Q_i (Figure 1 a). Under the influence of a density dependent volume force such as centrifugal force and given that the rotational frequency is sufficiently high to suppress capillary priming of the siphon channel, liquid entering the chamber will accumulate in the reservoir (Figure 1 b). With time, the liquid levels in the reservoir and siphon outlet rise. Provided the liquid level is below the crest point, the siphon acts as a cloesed valve, retaining the liquid while the chamber is filling. Once the liquid level is above the crest point, the liquid flows outwards through the siphon channel at an outgoing flow rate Q_o (Figure 1 c). If the outgoing flow rate is higher than the incoming flow rate, the liquid level in the chamber will decrease until the liquid column breaks and gas enters the siphon (Figure 1 d). When the liquid column is disrupted, the same process resumes, thus inducing the periodic emission of droplets.
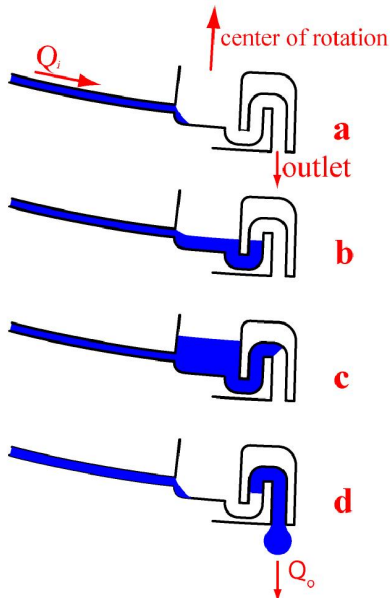
Fig 1: Working principle of the siphon-induced flow discretization. Liquid fills the discretization chamber and is held back by the outlet siphon until the crest point of the siphon is reached (a and b). When the crest point is passed (c) liquid starts flowing out at a flow rate Q_o, higher than the inlet flow rate Q_i, thus emptying the chamber (d). When the chamber is empty, the process is resumed.

Since a discrete volume / droplet is issued once the liquid in the discretization chamber reaches the crest point of the siphon, the volume of each droplet can easily be tuned varying the geometry and dimensions of the discretization chamber. The drop volume is given by $V_s + V_c$, where V_s is the volume of the siphon channel until the crest point and V_c is the volume of the chamber (see Figure 2 a). Discrete liquid volumes can be further reduced to the siphon volume alone (Figure 2 b) by eliminating the chamber provided that the relation between incoming and outgoing flow rates is maintained.
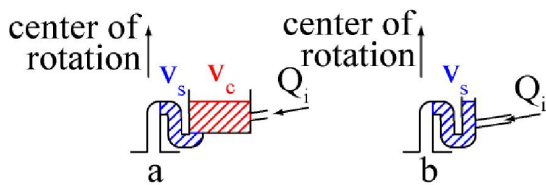

Fig 2: Definition of droplet volumes by geometrical properties of the discretization chambers.

To fulfil the second requirement (alternately issuing droplets into a common reservoir) the outlets of two of the above described discretization structures are brought together in a common reservoir, while the inlet channels are connected to different supply reservoirs (Figure 3).

The combined action of the centrifugally induced artificial gravity field as well as the kinetic impact make the droplets spread upon impact, thus forming thin lamellae (Figure 3). An alternating striation pattern evolves in the mixing chamber as a result of an arbitrary phase shift between the action of the two siphons. Non-

uniform mixing ratios can be enforced by an asymmetry between the fluidic structures defining the two discretizing structures.
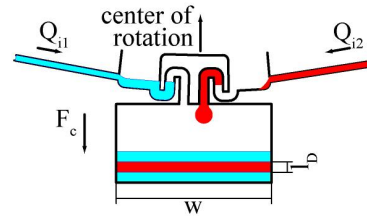

Fig 3: Schematic drawing of the siphon-induced flow discretization mixing structure.

EXPERIMENTAL

MATERIALS AND FABRICATION

Disk based microfluidic devices were fabricated by standard photolithography procedures using Dry Film Resists of different thickness (Ordyl P50000 and SY300 series, Elga Europe, Nerviano, Italy). Reservoirs and mixing structures were fabricated with depths of 100 or 120 µm (P50000 series) on top of 2 mm thick PMMA substrates (Repsol Glass, Repsol, Spain). Disk shapes and fluidic connections such as IO ports were machined by CO_2 laser ablation of the PMMA substrates. Channels were produced with depths of 30 or 55 µm (SY300series) on top of flat, 0.6 mm thick PC substrates consisting of blank DVD halves. After developing and etching the structures, disks containing reservoirs and channels were aligned and bonded by thermo-lamination. Micro fluidic devices for experimental characterisation of mixing performance contained either reservoir depths of 100 µm and channel depths of 30 µm (for aqueous ink mixtures) or reservoir depths of 120 µm and 55 µm deep channels (for human blood plasma and PBS mixtures or dilutions). PBS buffer was obtained from Invitrogen, Carlsbad, CA, USA. Blood plasma was obtained from Hytest, Turuk, Finland.

METHODS

The experimental setup for testing the fluidic structures consisted of a PC controlled servo-motor for spinning the disks (cool muscle 2, muscle corp., Osaka, Japan), a flood illumination system and a fire wire camera (Unibrain 501b, Unibrain Inc, San Ramon, Ca, USA). The camera was externally triggered by a signal from the motor and acquiring one image per rotation. This allowed for recording images of a specific position of the disk while spinning.

Mixing quality was assessed by two different methods. When mixing aqueous inks, a colorimetric method was used. After the liquids have been mixed, an image of the resulting mixture was acquired, and converted to a 8 bit grey scale image. Using an image processing software (ImageJ, NIH, USA), the area which contains the mixture was selected and the standard deviation of the histogram calculated. To compensate systematic errors due to the experimental setup, the standard deviation of a reference mixture was measured, and subsequently used to normalize the other standard deviations. The reference

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mixture was prepared by mixing the aqueous inks in the same ratios using a vortex mixer. After the mixing was performed, the same volume of liquid as obtained during the experiments was pipetted to an identical disk device and a reference image was acquired. The experimental setup consisted of an illumination table, a surrounding box to keep the illumination conditions constant and a digital camera (D 80, Nikon, Japan).

The assessment of mixing quality using plasma and PBS was done using a spectrophotometer (Nanodrop 1000, Thermo Scientific, Waltham, MA, USA). After mixing was performed, the mixture was split in 1,5 µL aliquots. The concentration of plasma proteins in these aliquots was subsequently determined using absorbance measurements at 280 nm wavelength. A perfect mixture would have the same concentration of proteins in all aliquots.

In order to collect the aliquots an additional microfluidic structure was employed. (Figure 4).
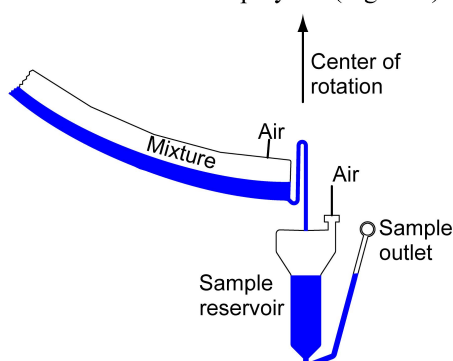

Fig 4: sample collection structure used in experiments where plasma and PBS was mixed. Under rotation liquid from the mixing reservoir is transferred to to the sample reservoir. Whenever a liquid level corresponding to 1,5 µl is reached, the disk is stopped and the liquid is collected. This process is repeated until all liquid in the mixing reservoir is collected.

Briefly, the aliquot collection procedure is done as follows: after the mixing step is performed, the disk is stopped and the siphon at the outlet of the mixing reservoir primes. When the disk resumes spinning, liquid from the reservoir is transferred into the sample reservoir. The rise of the liquid level in the sample reservoir is observed using a magnification lens coupled to the camera. The disk is stopped when the liquid level reaches a point which is known to correspond to a volume of 1,5 µL. This volume is then collected via the sample outlet using a standard micro-pipette. The process is repeated until all liquid from the sample reservoir is collected. Typically 7 to 9 aliquots were collected per mixing trial.

RESULTS

All results reported here concern mixing experiments which were carried out at a rotation frequency of 40 Hz. When mixing unequal liquid volumes (dilutions), the flow rates were adjusted via changes to the hydraulic resistance of the inlet channels in order to achieve a simultaneous emptying of both supply reservoirs. The mixing quality was evaluated immediately after performing the experiments.

Experiments using equal volumes of aqueous inks employed three different structure designs to enable discretization of volumes with 60, 180 and 360 nL. Additionally the discretization structures emptied directly either at the centre or at the side of the receiving reservoir. In all experiments 5 µl of each ink were used and mixing has been performed in less than 20 s. As expected reducing the discretization volumes leads to an increased homogeneity of mixing (Figure 5).
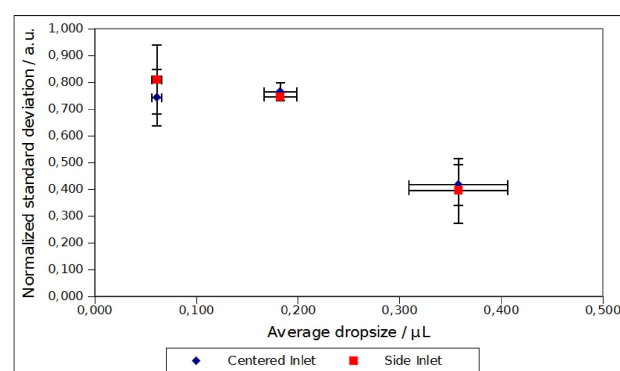

Fig 5: Normalized standard deviation (1 = perfect mixing) of mixed solutions as a function of the discrete volume and type of mixing structure. The measured standard deviation of the mixtures were normalized using the standard deviation of a sample mixed using a vortexer.

Moreover, the mixture performed with 360 nL discrete volumes took more than 40 minutes in order to reach the mixing quality obtained with 60 nL discrete volumes directly after mixing. It is also noteworthy that mixing quality of the lower discretization volumes is already close to that of the reference mixture. The position at which the discretization chamber enters the mixing reservoir was observed to be of minor importance for the mixing quality.

Figure 6 shows images collected during the mixing process with aqueous inks. There is clear evidence of liquid lamellae which are concentric around the point where the discrete volumes impinge the liquid pre-deposited in the mixing reservoir. With increasing distance from the center the boundaries between lamellae become fuzzier, indicating an ongoing mixing process.
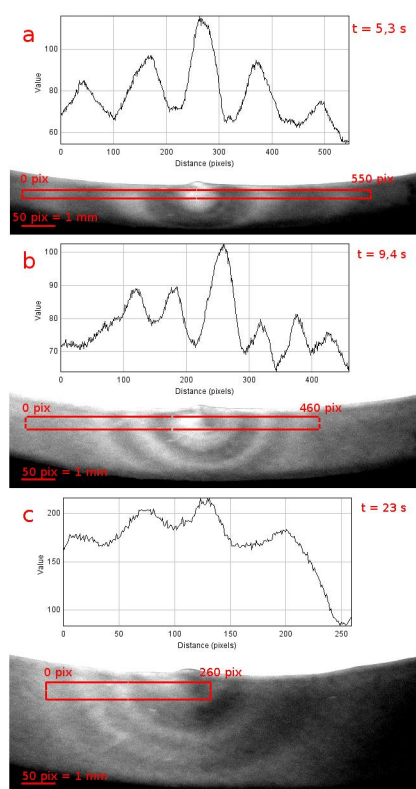
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Fig 6: Image sequence of the mixing of two differently colored aqueous inks. Displayed is the center of the mixing chamber, where the droplets are issued and the histograms of the areas enclosed by the rectangles. The black part on the right side of image c is due to deficient illumination.

Since the mixing structures designed to obtain 60 nL discrete volumes showed superior mixing quality, the same discretization level was chosen for diluting plasma in PBS in ratios of 1:2.6 and 1:5. Figure 7 shows the results obtained for several samples of the two dilutions, taking into account all aliquot determinations per sample as shown in the standard deviation error bars. These results show that the approach proposed is suitable for diluting human plasma in PBS.
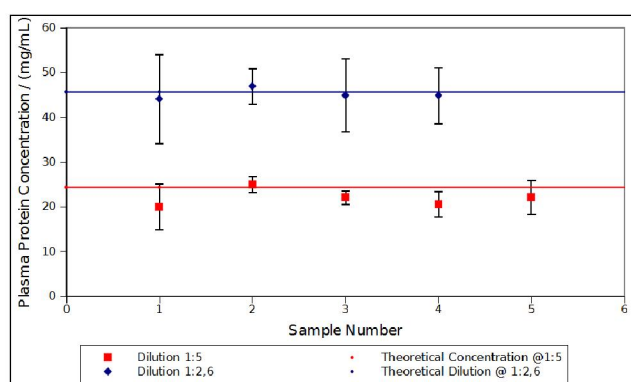

Fig 7: Results of the plasma dilution experiments. Shown is the concentration of plasma proteins in different samples. A homogeneous mixture would have the concentration which is indicated by the horizontal lines in all samples.

CONCLUSIONS

A novel mixing structure based on siphon-induced flow discretization has been developed and implemented on a centrifugal microfluidic platform. The structure is suitable for mixing liquids at volume ratios between unity and 1:5. Additionally, our mixing technology is also suitable for mixing liquids of significantly different hydrodynamic characteristics such as human blood plasma and standard buffer solutions. Furthermore, the mixer is simple to integrate and run in centrifugal microfluidic platforms, being also amenable to large scale manufacturing materials and processes such as plastic injection molding. Moreover, it does not require any surface modification processes and mixing is achieved in a fast, one-step centrifugation procedure.

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