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Integrated siphon-based metering and sedimentation of whole blood on a hydrophilic lab-on-a-disk

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Abstract In this paper, we present a novel and fully integrated centrifugal microfluidic "lab-on-a-disk" for rapid colorimetric assays in human whole blood. All essential steps comprising blood sampling, metering, plasma extraction and the final optical detection are conducted within t=150 s in passive, globally hydrophilized structures which obviate the need for intricate local hydrophobic surface patterning. Our technology features a plasma extraction structure (V=500 nL, CV<5%) where the purified plasma (c_{RBC}<0.11%) is centrifugally separated, metered by an overflow and subsequently extracted by a siphon-based principle through a hydrophilic extraction channel into the detection chamber.

Keywords Centrifugal microfluidics · Siphon · Sedimentation · Metering · Sample preparation · Whole blood

1 Introduction

The transfer of clinical diagnostic routines to compact point-of-care devices is subject of various academic (Oosterbroek and van den Berg 2003; Schulte et al. 2002; Thorsen et al. 2002; Sia et al. 2004) and commercial efforts (Ducrée and Zengerle 2004; Schembri et al. 1995; Examples: Careside Inc, Biosite Inc., Pelikan Technologies Inc., Chempaq A/S, i-STAT Corp., Gyros AB, Abaxis Inc). An paramount issue in medical diagnostic systems is the integration of the full process chain from the preparation of a patient's whole blood to an analytical result. To meet the stringent requirements of clinical diagnostics and the rapidly emerging field of point-of-care testing (POCT), so-called "lab-on-a-chip" systems which feature a full process integration, reduced consumption of sample and reagents as well as short times-to-result and ease of handling are the most prominent candidates (Vilkner et al. 2004; Reyes et al. 2002; Auroux et al. 2002; Figeys and Pinto 2000; Stone et al. 2004). So far, key laboratory unit operations such as sample injection, separation, metering, mixing, reaction, and detection have been successfully demonstrated (Gustafsson et al. 2004; Schembri 1993), but they have rarely been integrated to full-fledged applications.

Among the various types of lab-on-a-chip systems, centrifugal technologies have proven the capability to integrate complete assay protocols on whole blood (Madou and Kellogg 1998; Madou et al. 2000, 2001a,b; Ekstrand et al. 2000; Thorsén et al. 2003; Inganäs et al. 2001; Gustafsson et al. 2004; Duffy et al. 1999; Schembri et al. 1995). This is achieved by tailoring the interplay between the frequency-controlled centrifugal force for pumping and sedimentation and the capillary force which is statically controlled by the geometry and the contact angle. By capitalizing on the rotational symmetry, a high degree of parallelization of reaction channels can be obtained.

The work presented here focuses on the efficient blood sedimentation and metering to implement metabolic assays on whole blood on a centrifugal microfluidic platform. While previous approaches are based on hydrophobic microfluidics (Zeng et al. 2000; Mc Neely et al. 1999),

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this work presents a novel, globally hydrophilic siphon-based structure with a centrifugal overflow. While reducing the complexity of manufacturing, the structure can in principle be operated at arbitrarily high frequencies, thus excelling with fast sedimentation and meniscus flattening and to enhance to the precision of metering.

The paper structures as follows. First, the basic design concept of the microfluidic structures is described. The next section investigates the integrated sedimentation and plasma extraction structure. Finally, the high potential for process integration and automation is demonstrated by a fully integrated, colorimetric, absorption-based assay on alcohol on an untreated sample of whole blood.

2 Disk design

We use a hot embossed polymer substrate (*COC, Cyclic olefin copolymer (Topas®)*, www.ticona.com) of the size of a conventional compact disc (CD) featuring fluidic and optical elements to perform colorimetric assays. The fluidic elements provide ports for sample and reagent uptake as well as a sample preparation structure for an integrated blood sedimentation and plasma metering which is connected to a combined mixing and detection chamber (Fig. 1).

For guiding the optical probe beam, triangular V-grooves are embedded next to the measurement chamber on the reverse side of the chip. When the beam of a standard laser (λ=532 nm, *Flexpoint* 532 nm, *Laser Components IG*) enters the polymer chip, it is deflected by 90° via total internal reflection (TIR) at the side face of the V-grooves inclined by 45° against the chip surface (Grumann et al. 2006). After deflection into the chip-plane, the probe beam is attenuated in the measurement chamber and then reflected at another V-groove towards an external microspectrophotometer (*UV/VIS Microspectrometer, Boehringer Ingelheim MicroParts AG*). With this setup, an optical path length of l_{abs}=10 mm through the measurement chamber has been realized, even though the height of the chamber amounts to 1 mm, only. The liquid transport is controlled by the centrifugal force F_ν scaling in a well controllable fashion with the square of the spinning frequency ν.

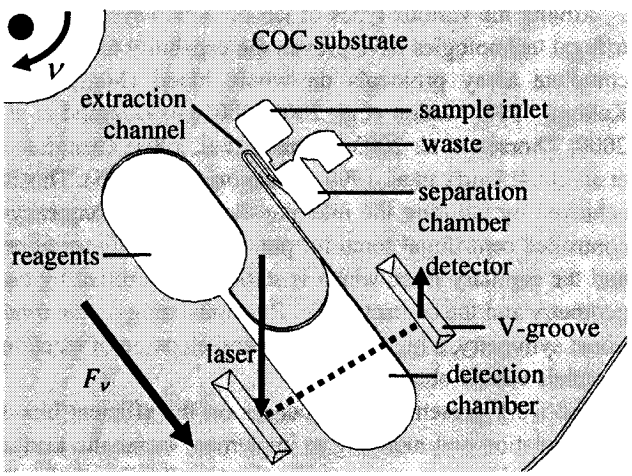

Fig. 1 Disk design with integrated microfluidic and optical structures to perform an enzymatic absorption assay for determining the alcohol concentration in human whole blood

3 Plasma extraction structure

The basic sedimentation and metering structure in Fig. 2 consists of a separation chamber which is connected to one inlet for the blood sample and two outlets. One of the outlets is an overflow to initially meter the raw blood sample at ν=50 Hz. Operating the structure at such elevated frequencies is made possible by using the siphon principle driving the liquid into a dead-end compartment rather than a capillary burst valve realized by hydrophobic microfluidics.

The second outlet represents a siphon-like extraction channel which bends at a crest point located radially farther inward than the maximum radial filling liquid level. The radial position of the outlet of the sedimentation chamber towards the siphon defines the volume of the extracted plasma as well as the "dead" volume collecting the sediment, e.g. the cellular pellet, to stay in the sedimentation chamber.

The liquid motion in the extraction channel depends on the radial spacing Δr between its downstream meniscus with respect to the liquid level in the sedimentation chamber. Above a certain frequency threshold ν_c, the centrifugal force F_ν prevails over the capillary force F_θ in the inbound section of the extraction channel, and the positions of the corresponding menisci are balanced by the principle of interconnected tubes.

Once the rotation ceases, the hydrophilic extraction channel is primed by capillary action. In our structure, time for the filling of the hydrophilic channel (contact angle θ=50°) amounts to less than 2 s. Instead of a complete stop (ν=0 Hz), the extraction channel can also be primed by keeping $\nu<\nu_c$ so the capillary pressure surpasses the (initially) counteracting centrifugally induced hydrostatic pressure.

The capillary priming of the siphon channel below ν_c is maintained until the advancing meniscus has passed the crest point of the siphon and settles below the position of the receding meniscus in the chamber, i.e. $\Delta r<0$. Now a slight centrifugal field is sufficient to extract the supernatant from the separation chamber towards the outer perimeter of the disk. The extraction process continues as long as the continuous liquid column remains intact and ceases when air is sucked into the extraction channel. A tight control of the break-off process which is governed by the geometry of

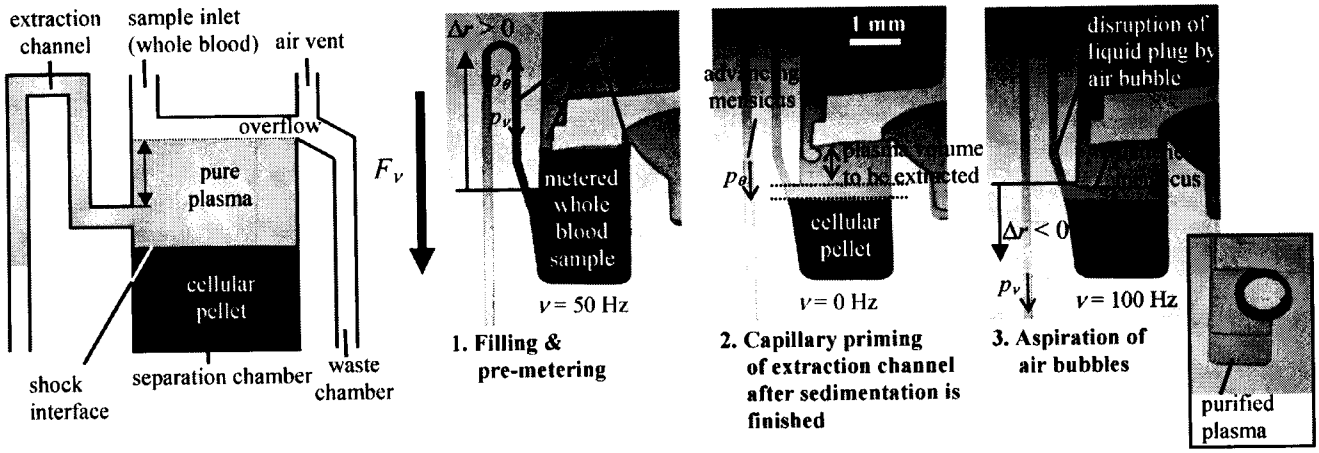

Fig. 2 Sketch of the plasma extraction structure. (1) After injection through the inlet, a droplet of raw blood is pre-metered. (2) The shock interface separating pure plasma and cellular blood proceeds and stops at a position radially outwards compared to the extraction channel. Then, the disk is stopped and the extraction channel is filled by the capillary pressure p_θ. (3) If the net radial length Δr between the downstream meniscus and the liquid level in the separation chamber is negative, a centrifugal pressure p_ν exists to pump the plasma through the extraction channel until air is sucked into the extraction channel. The extracted plasma is collected in a reservoir attached to the extraction channel for further use

the edges at the point of disruption is key to the metering precision of the extracted plasma.

The well defined, reproducible disruption of the liquid column by a sharp edge at the entrance of the outlet channel also clearly distinguishes this principle from the siphon mechanism in a similar, previously introduced structure by Schembri et al. (1995). In their structure which was designed for the extraction of a much larger plasma volume exceeding 18 μL, the flow through the siphon-like outlet channel is stopped when the radial height of upstream meniscus in the separation chamber matches the radial height of the exit of the outlet channel. As a detailed geometrical layout and a thorough experimental characterization are lacking in the paper by Schembri et al. (1995), it is virtually impossible to soundly compare the performance in terms of the accuracy of the metered plasma.

Figure 3 sketches the frequency protocol for the centrifugally integrated metering, sedimentation and plasma extraction with the structure displayed in Fig. 2. By spinning for 2 s with a frequency ν=50 Hz, the separation chamber is filled and the undefined inlet volume is premetered by the overflow. Raising ν to 100 Hz for 10 s leads to a rapid sedimentation of the whole blood as illustrated by the formation of a moving shock interface between the pure plasma in the supernatant and the cellular pellet at the bottom.

An excellent volumetric precision of the extracted plasma in the detection chamber is verified by a series of 23 measurements (V=500 nL) with a CV better than 1%. The volume measurements were carried out by processing the series of digital pixel images taken by the stroboscopic camera. The pixels within the parts filled with liquids are counted by an image processing software. Pixel counts are converted into volumes by means of a substrate-based reference scale. A 30 μm wide frame was assumed as error along the outline of the pixel area, leading to a volume deviation smaller than 1%.

The required time for a complete separation of the blood sample in plasma and cellular constituents is proportional to $1/\nu^2$ (Fig. 4). A residual cell concentration c_{RBC}<0.11% in the plasma is determined by optical comparison to calibrated cell suspensions.

This concept of valving a flow in a siphon-like extraction channel is an attractive alternative to capillary burst valves based on hydrophilic and hydrophobic microfluidics. This is because the siphon only requires a plane and sufficiently hydrophilic coating of the extraction channel exhibiting a constant cross section. Such a structure, is much easier to manufacture, e.g. by standard polymer replication schemes and subsequent dip-coating of the entire substrate and lid, than the capillary burst valves based on well defined hydrophobization of geometrical constrictions. Furthermore, there

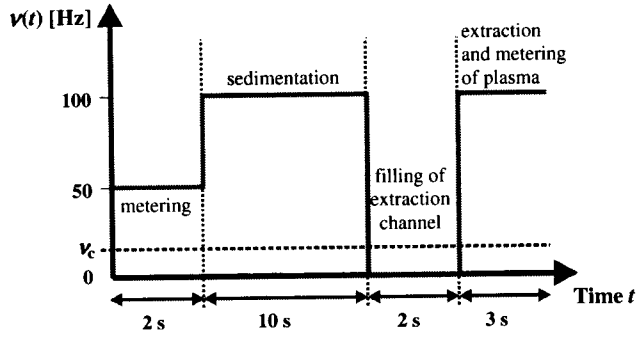

Fig. 3 Frequency protocol $\nu(t)$ for the sample preparation including an integrated blood sedimentation and the subsequent extraction of highly purified plasma via the siphon channel

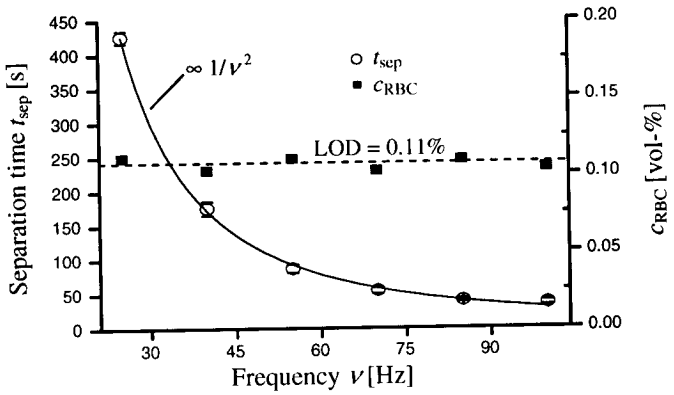

Fig. 4 The measured separation time t_{sep} of the metered 500-nL blood volume (CV<1%) decreases with the inverse square of the spinning frequency ν. The quality of the extracted plasma represented by the residual red blood cell concentration c_{RBC} remains below 0.11% (limit of detection) for all ν

is no (capillary) burst frequency to limit the maximum frequency and thus the separation time.

The availability of high rotational frequencies also implies that the centrifugal force can be set to prevail over surface tension to form extraordinary flat menisci in the sedimentation chamber as can be seen, for instance, in Fig. 2. Such flat menisci are of particular benefit for the metering of the extracted plasma volume defined between the centrifugal overflow and the tear-off point when air is sucked into the siphon channel.

4 Integrated absorption assay for alcohol

An integrated colorimetric absorption assay is conducted by a fully automated frequency protocol to eliminate error-prone handling steps (Steigert et al. 2006a,b,c; Grumann et al. 2006). The colorimetric reaction is based on a standard two-step enzymatic reaction using the well-established alcohol oxidase (AOX) method.

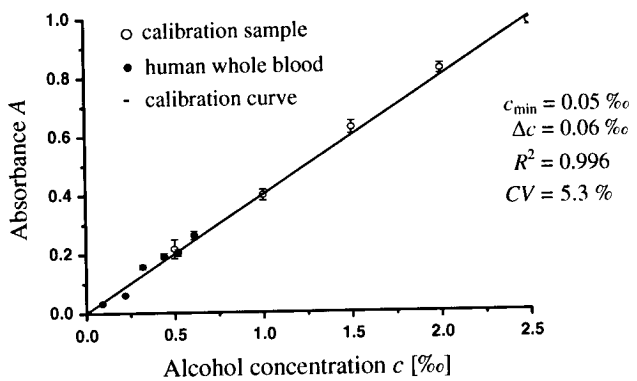

Fig. 5 Calibration curve of the alcohol assay. After sedimentation and mixing has completed (t=50 s), the increase of the absorbance A is recorded for t=100 s. Our measurements well reproduce the concentrations of calibrated ethanol solutions as well as reference measurements on human whole blood with a CV of 5.3%, a lower limit of detection c_{min}=0.05‰, a resolution of Δc=0.06‰, and a linearity of R^2=0.996

To carry out the absorption assay, the extracted plasma in the detection chamber is efficiently mixed with enzymatic reagents (*ALK 121, Diaglobal GmbH*) by using "shake-mode" mixing, i.e. a frequent reversal of the sense of rotation (Grumann et al. 2005). Finally, the absorbance which quantifies the alcohol concentration is monitored in real-time during constant (slow) spinning (Steigert et al. 2006a,b,c) with our previously introduced optical total internal reflection (TIR) concept (Grumann et al. 2006). By benchmarking (*Vario-Photometer, Diaglobal GmbH*) with calibrated samples (*ALK QS, Diaglobal GmbH*), our measurements (Fig. 5) display a CV of 5.3%, a lower limit of detection c_{min}=0.05‰ (five times standard deviation of background signal), an excellent resolution (Δc=0.06‰), and a high linearity between the alcohol concentration and the optical absorption signal (R^2=0.996). The presented data is derived from five measurements, each conducted on at least three different samples. Additionally, the real time monitoring under rotation (Steigert et al. 2006a,b,c) allows to drastically reduce the time-to-result with respect to standard kits from 8 min down to 150 s. This performance is comparable to common breath analyzers while avoiding the ambiguous correlation between the alcohol concentration in breath and whole blood.

5 Conclusion

We introduced a novel, centrifugal microfluidic sample preparation scheme for whole blood, displaying an integrated metering and plasma extraction that is based on the use of overall hydrophilized substrates. Its performance was successfully demonstrated in a fully process integrated enzymatic absorbance assay on alcohol. The assay is conducted by an automated frequency protocol to eliminate error-prone handling steps. The underlying unit operations of our integrated sedimentation and metering structures, such as stopping, metering, and/or valving a flow, are enabled by a siphon-like hydrophilic extraction channel. This implies an

appreciable simplification of the fabrication process compared to structures based on capillary burst valves which require a locally well defined hydrophobization of tiny channel constrictions. In addition, higher frequencies of rotation can be applied to accelerate sedimentation and shape flat menisci for high-accuracy sample metering prior to the quantitative assay.

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