TOWARDS A COMPREHENSIVE CENTRIFUGAL PROCESS INTEGRATION BY ROTATIONALLY INDUCED LYOPHILISATE DISSOLUTION AND CELL LYSIS

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ABSTRACT

This manuscript shows how the rotational „shake mode“ which has previously been used for mixing of liquids [1] can be adopted for implementing two key steps in typical point-of-care applications, the efficient dissolution of lyophilized beads and cell lysis. The process time is reduced by optimizing the aspect-ratio of an on-disk chamber containing an overall volume of 50 µl. The two upstream processes supplement our existing portfolio of unit operations [2].

Keywords: Centrifugal Microfluidics, Dissolution, Lyophilisate, Blood Lysis

1. INTRODUCTION

One of the key success factors for lab-on-a-chip technologies is linked to the ability to fully integrate and automate complex lab protocols on a single substrate which is loaded by a sample and then inserted into a reusable device. To this end, the protocols of protein or nucleic acid assays have to be translated into a sequence of fluidic unit operations. One of the most key unit operations for assay development is mixing which influences assay steps like incubation, dissolution or sample preparation as shown in Figure 1A. In this work we focus on the optimization of microfluidic mixing processes in centrifugal platforms to enhance the efficiency of typical assay steps: the dissolution of lyophilizate and cell lysis.

2. FUNCTIONAL PRINCIPLE

Our modular setup consists of a microstructured disk (Fig. 1B) which is spun by a frequency programmable rotary motor. During our shake-mode protocol (frequency amplitude: 32 Hz, rotational acceleration: 8 Hz/s, hold time: 0.4 s), steep acceleration ramps imposed by the frequent reversal of the sense of rotation induce inertial mixing in a disk-based chamber [1]. Due to the interplay of viscous shear-stress meditated by the surface and the inertial volume force, the mixing efficiency is tightly linked to the aspect ratio and overall volume of the mixing chamber.

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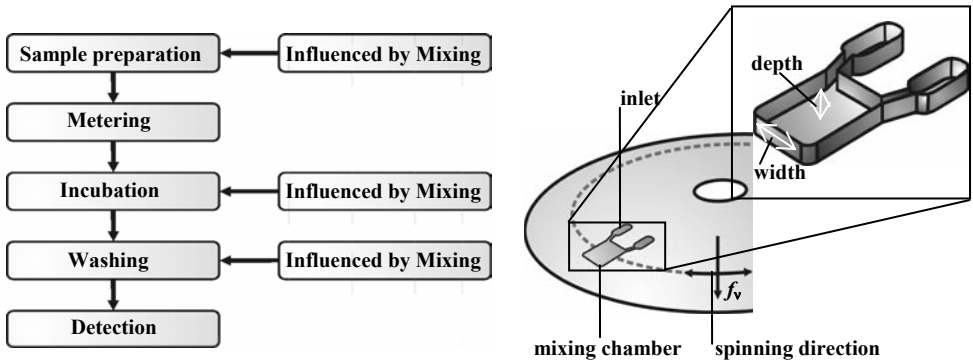

Fig. 1A) Typical unit operations for diagnostic assays and their dependence on mixing. ***B)*** A microstructured COC-disk containing the mixing chamber is used for our experiments. The chamber features a reagent volume of 50 µL at varying aspect ratios.

3. EXPERIMENTS

In Figure 2, the time to dissolve dry reagents by shake mode is experimentally characterized at aspect ratios between 0.04 and 0.5. In all experiments, the chamber volume is kept constant at 50 µl, an amount commonly used in many biological assays. The process time is evaluated by observing the dissolution of 5 mg of the red colored salt potassium ferricyanide in DI-water.

The best performance is achieved at aspect ratios between 0.1 and 0.29 where the shake-mode enhanced dissolution time is cut down by more than 70% with respect to mere diffusion at rest. The presence of silica beads in the mixing chamber does not further enhance the dissolution process (Fig. 3). We assume that their high density (3 g mL^{-1}) pins the beads to an „inefficient domain“ on the outer perimeter of the mixing chamber.

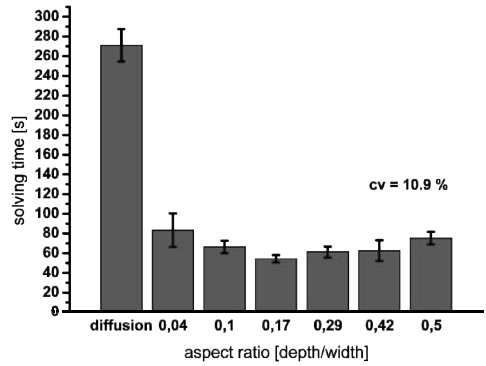

Fig. 2 Correlation between the dissolution time of potassium ferricyanide in 50µL of DI water in mixing chambers with varying aspect ratios.

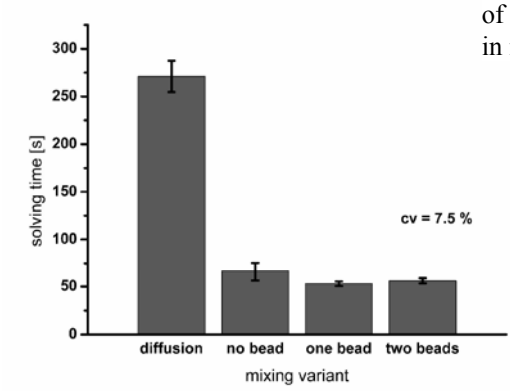

Fig. 3 The influence of silica beads in the mixing chamber on the dissolution time for potassium ferricyanide

We next investigated the dissolution of a single 10.6-mg bead containing the lyophilizate of a PCR master mix (with-

out primers, manufacturer: GE Healthcare) in a chamber displaying an aspect ratio of 0.29. A minimum dissolution time of 250 s, representing a 4-fold acceleration with respect to mere diffusive dissolution, is obtained without silica beads or obstacles for pinning the bead in the center of the chamber (Fig. 4).

Lysis of blood cells in a mixing chamber is demonstrated in Figure 3C. Mixing of lysis buffer and 50 µl of human whole blood is performed with a shake-mode protocol. Nearly 100 % of the blood cells are lysed within 20 s, in a chamber displaying a depth of 1 mm. Cell lysis without shake mode takes about 90 s. The centrifugal force transfers the lysate via a siphon directly into an Eppendorf tube inserted into a „flying bucket“ rotor (Fig. 5).

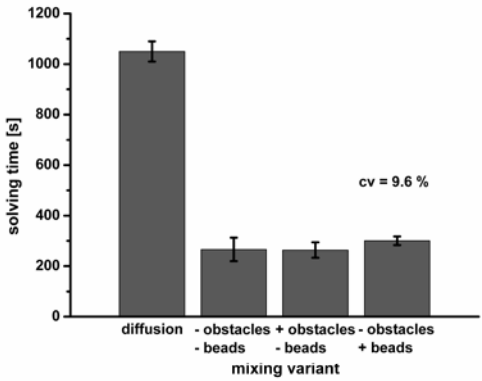

Fig. 4 Dissolution time for 10.6 mg “ready-to-go” PCR lyophilizate beads in 50 µL DI-water. The dissolution time is reduced by a factor of four compared to mere diffusion by using the shake-mode. Obstacles or beads in the mixing chamber show no significant influence on the speed of the dissolution process.

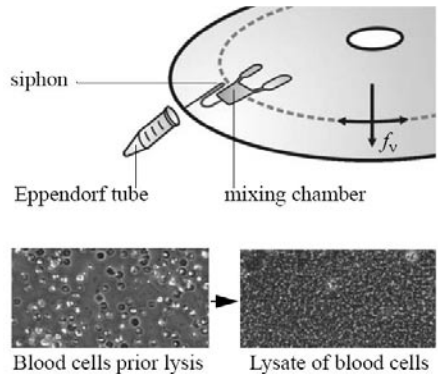

Fig. 5 Lysis of blood cells by shake-mode mixing of lysis buffer with human blood. The siphon keeps blood and buffer in the chamber during spinning of the disk. Adding 20 µL of buffer and spinning forwards the buffer containing the lysed cells into an Eppendorf tube mounted on a flying-bucket holder.

5. CONCLUSION

We identified the aspect ratio of the mixing chamber as the key impact parameters for using the rotational shake-mode to decisively enhance the efficiency of important unit operations on our centrifugal microfluidic platform: the dissolution of dry, lyophilized reagents and cell lysis. Adding these capabilities to our existing portfolio of unit operations [2] will allow to implement more complex protocols while cutting down the time-to-results and minimizing the costs of the disposable.

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