CENTRIFUGO-PNEUMATIC HANDLING OF MICROPARTICLES WITHOUT EXTERNAL ACTUATION AS A NEW UNIT OPERATION FOR CENTRIFUGAL MICROFLUIDICS

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ABSTRACT

For the first time we present a microfluidic method for handling of microparticles that requires neither surface treatment of chambers and channels nor external actuators such as magnets. Thus it is not limited to handling of magnetic particles. Using centrifugal forces and temporary storage of pneumatic energy, only, we demonstrate 1) liquid mediated microparticle loading, 2) re-suspension of microparticles by shake mode at low centrifugation; sedimentation of microparticles and afterwards exchange of liquids with particle loss below 2% and supernatant removal efficiency of more than 99.5%, 3) re-suspension and subsequent transport of microparticles together with liquid reagent with particle loss of 6% or less.

KEYWORDS: Microparticle, Centrifugal Microfluidics, Centrifugo-Pneumatic Flow Control

INTRODUCTION

Microparticles play an essential role in solid phase assays e.g. nucleic acid extraction and immunoassays. In such assays, functionalized microparticles have to get in contact with sample liquid to specifically bind analytes where sufficient suspension of the microparticles is required. Afterwards, washing steps remove all unbound species where a complete removal of sample liquid and low particle loss are essential to achieve high purity and sensitivity. Finally, an elution step (DNA-extraction) or direct detection of analytes attached to the microparticles (Immunoassay) may be performed. Hence, an efficient microfluidic particle handling method is required. Existing approaches in centrifugal microfluidics successfully introduce external magnets to assist handling of microparticles[1,2]. These methods are limited to the use of magnetic microparticles. Instead of employing external magnets, physical structures for microparticle aggregation[3,4] and particle trapping[5] have been employed. These methods require sufficient large particles with reduced surface to volume ratio which results in long incubation times to achieve binding of analytes at low concentration[6].

In this paper, we present a microfluidic method for handling of microparticles controlled by centrifugation only, without any other means. The method does not require coating of microfluidic chambers or channels and is not limited to using magnetic microparticles.

FUNCTIONAL PRINCIPLE

The microfluidic structure consists of two compression chambers connected via a capillary with high fluidic resistance (timing channel) and two siphons with different fluidic resistances (Fig. 1-A). The handling of microparticles is achieved by three microfluidics process chains: 1) liquid mediated microparticle loading, 2) microparticle washing or analyte binding and 3) liquid mediated microparticle transport. Each process chain is comprised of several unit operations.

Liquid mediated microparticle loading: A suspension with microparticles is supplied from the inlet (Fig. 1-A). Upon centrifugation, the suspension is transported into the first compression chamber compressing the air and microparticles sediment (Fig. 1-B). At low deceleration to a medium spin frequency, the pneumatic energy of the compressed air is released slowly displacing liquid out of the first compression chamber. The liquid levels in the inlet chamber and the siphons are equally elevated until

the lower volume triggered siphon with high fluidic resistance is primed (Fig. 1-C). In this way, almost all the supernatant is transported into the waste chamber (Fig. 1-D).

Particle washing or analytes binding: After the microparticle loading, another liquid is transported into the first compression chamber compressing the air and microparticles sediment. At critical centrifugation, the first compression chamber is overfilled priming the timing channel and liquid fills the second compression chamber (Fig. 1-F timer setting). At deceleration, the release of pneumatic energy from the compressed air and thus the siphon priming is delayed due to the high fluidic resistance in the timing channel. As illustrated in Fig. 1-G, re-suspension of microparticles is achieved by shake mode[7] during the delay period at low frequency. Before the second compression chamber is empty, the microparticles are again sedimented at high centrifugation (Fig. 1-H). Subsequently, a slow deceleration to a medium frequency avoids re-suspension of microparticles and the liquid primes the volume siphon (Fig. 1-I). Consequently, the liquid is removed to the waste chamber (Fig. 1-J).

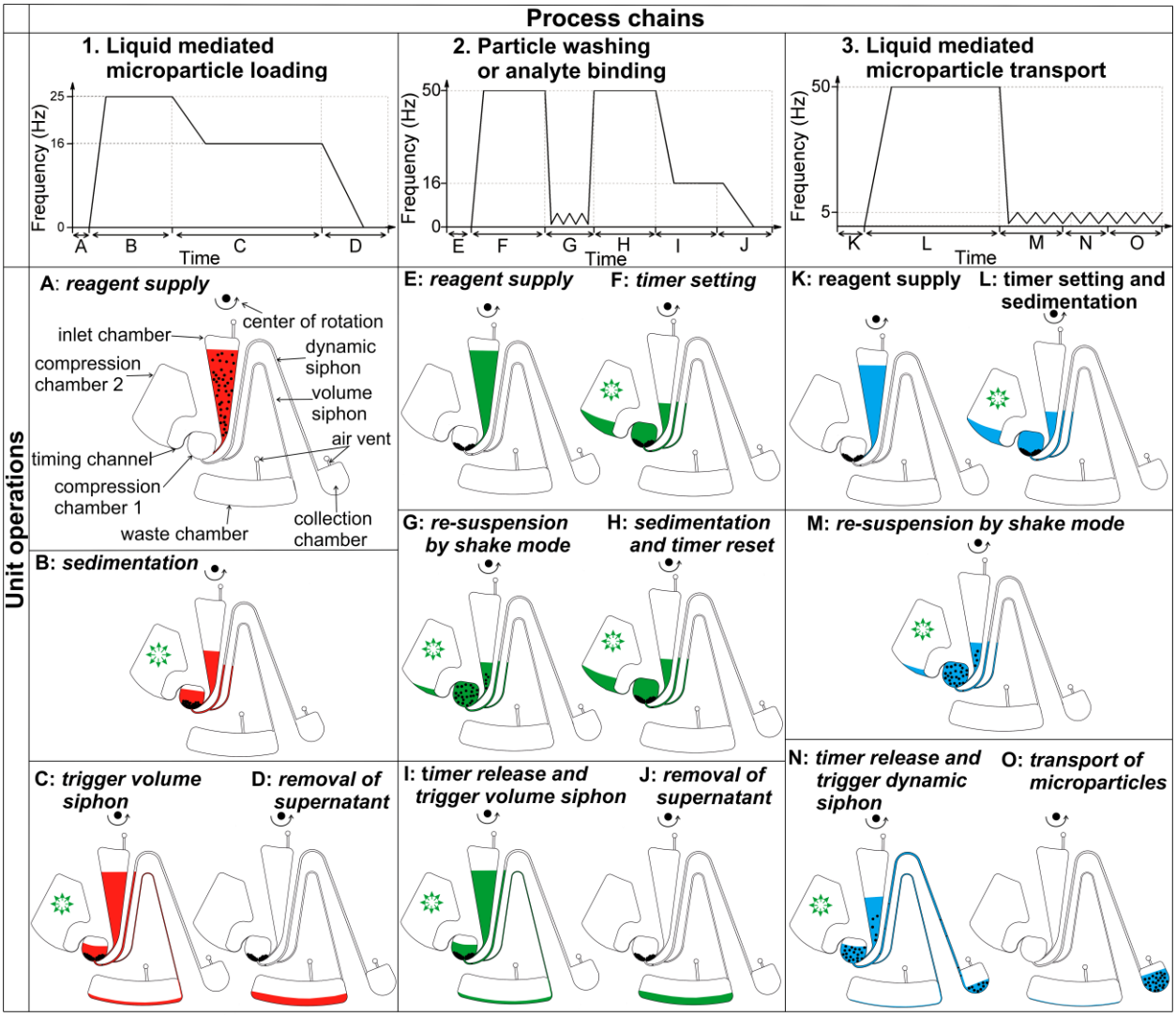

Fig. 1: Centrifugo-pneumatic Microfluidic process chains and unit operations for handling of microparticles

Liquid mediated microparticle transport: As illustrated from Fig. 1-K to Fig. 1-O, transport of microparticles is achieved by re-suspension and subsequent transport of microparticles together with a liquid buffer. After loading the timer, the shake mode is continued until the timer is released. In that case,

pneumatic energy is released abruptly. Thus, the dynamic siphon with low fluidic resistance is primed (Fig. 1-N). Consequently, almost all re-suspended microparticles are transported together with the liquid through the dynamic siphon to the collection chamber (Fig. 1-O).

EXPERIMENTS AND METHODS

All experiments are carried out on a 6 mm thick PMMA disk with milled microfluidic structure sealed with pressure sensitive polyolefin adhesive foil (900360, HJ-BIOANALYTIK GmbH). 150 µl of Dynabeads-M-280 suspension (Life Technologies AS, Norway) is pipetted into the disk. The disk is processed in a stroboscopic setup according to the frequency protocol in Fig. 1. The stroboscopic setup (BioFluidix GmbH) is based on a LabDisk player (Qiagen Lake Constance GmbH). It enables recording of real-time images during disk rotation. The recorded real-time images were used to determine the residual liquid after removal of supernatant. The particle loss was determined by measuring the particle concentration using their autofluorescence property. Concentrations down to 5 µg/ml can be detected.

RESULTS AND DISCUSSION

As shown in Tab. 1, the particle loss is 2% or less for four different initial particle concentrations during washing of microparticles. The particle loss is 6% or less during transport of microparticles. As determined by optical analysis of residual liquid after supernatant removal, the supernatant removal efficiency is independent form the particle concentration and is in all cases more than 99.5%.

Table 1: the particle loss (PL) and supernatant removal efficiency at four different initial particle concentrations

Particle concentration (µg/ml)	PL during removal of supernatant (%)	PL during transport (%)	Supernatant removal efficiency (%)
163 ± 1	2 ± 1	3 ± 1	99.5
339 ± 8	2 ± 2	4 ± 1	99.5
433 ± 6	2 ± 2	6 ± 1	99.5
921 ± 13	1 ± 1	2 ± 2	99.5

CONCLUSION

The centrifugo-pneumatic microparticle handling makes surface treatment and external actuators such as magnets obsolete. Thus, it is not limited to the use of magnetic microparticles. This broadens the choice of microparticles for centrifugal microfluidics. The new method features all fluidic functionalities for implementing microparticle based assays e.g. DNA extractions, Immunoassays and or Immuno-capture. The proposed method could be also used for direct processing of cells, liposomes or other disperse materials. In combination with sequential reagent release, the proposed microparticle handling method can be used for automation of microparticle based assays in centrifugal microfluidics.

REFERENCES

[1] O. Strohmeier et al., *LAB ON A CHIP*, 2013, **13**, 146–155.
[2] Y.-K. Cho et al., *LAB ON A CHIP*, 2007, **7**, 565–573.
[3] L. Riegger et al., *SENSORS AND ACTUATORS A-PHYSICAL*, 2006, **126**, 455–462.
[4] J. Park et al., *ANALYTICAL CHEMISTRY*, 2012, **84**, 2133–2140
[5] R. Burger et al., *LAB ON A CHIP*, 2012, **12**, 1289–1295.
[6] S.-H. Chuag et al., *SCIENCE AND TECHNOLOGY OF ADVANCED MATERIALS*, 2013, **14**.
[7] M. Grumann et al., *LAB ON A CHIP*, 2005, **5**, 560–565.

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