PURIFICATION OF miRNA FROM WHOLE BLOOD BY CHEMICAL LYSIS AND PHASE SEPARATION IN A CENTRIFUGO-PNEUMATIC MICRO-HOMOGENIZER

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ABSTRACT

In this paper a method for the efficient chemical extraction of miRNA from whole blood is first reported. Our novel, centrifugo-pneumatic homogenization scheme allows fast mixing of whole blood with the chemical TRI Reagent®(TRI) to provide a very efficient cellular lysis and later separation of the blood homogenate. Thus, at the end of the process, the sample is separated in two phases, an aqueous and an organic phase, with the total RNA fraction remaining in the aqueous phase separated from the DNA and proteins entrapped in the organic phase. To avoid chemical degradation of the chip, a solvent resistant cyclo olefin polymer (Zeonor®) disc was used in this case.

KEYWORDS: Microhomogenizer, centrifugal microfluidics, miRNA extraction, whole blood

INTRODUCTION

On-chip extraction and purification of nucleic acids from clinical samples such as whole blood, plasma, serum, tissue, or other body fluids is critical for research fields such as forensic sciences and molecular diagnostics, where purity of the sample and short analysis time are factors of outmost importance. A remarkable example of the importance of the extraction and analysis of nucleic acids came with the discovery in the early 1990s of the microRNAs (miRNAs). During the last decade, miRNAs have emerged as a new generation of biomarkers and target therapeutics for cancer. The aberrant expression of miRNAs has been observed in multiple pathologies and proven to be implicated in most human cancers [1].

Therefore, numerous attempts within microfluidics research have focused in the extraction, purification and downstream analysis of nucleic acids. However, to date, the most commonly reported approach is the use of solid phase extraction methods. This approach implies the integration of a solid phase capture mechanism (commonly silica based) within the channels of a chip [2,3]. Although widely used, this technique also presents some drawbacks like the integration of an additional material in the chip, adding steps to the fabrication process and sometimes conditioning the fabrication material, and finally, silica based capture phases do not discern amongst nucleic acids during the extraction process, capturing both RNAs and DNAs at the same time which for some applications might not be optimal.

Chemical liquid-liquid extraction remains an alternative for isolating RNA from biological samples and it is widely used in medical research. In this work, the phenol derivative TRI is employed for the concentration and purification of RNA from a whole blood sample [4]. After lysing the blood cells and thorough homogenization, three phases emerge: an aqueous phase where the RNA resides**,** an interphase containing the DNA, and an organic phase where the proteins are retained. However, this technique is difficult to implement on a miniaturized plastic chip, as most plastics used in microfabrication offer poor chemical resistance to organic solvents and mere diffusive mixing is insufficient at critical volumes for clinical analysis. To overcome this limitations, we report on a new, centrifugo-pneumatic microhomogenizer made of the chemically resistant polymer Zeonor®, which uses a pneumatically assisted pumping design to create turbulent conditions for the efficient lysis and chemical extraction of RNA from whole blood.

THEORY

The principle that drives this device combines the use of centrifugal forces and pneumatic compression/relaxation as previously described by Gorkin *et al*. [5]. The here presented novel design allows the generation of turbulence improving the homogenization of the blood sample. In more detail, fluids are periodically driven between a loading chamber and the compression compartments through a narrow, nozzle-like neck which generates turbulence (Fig. 1).

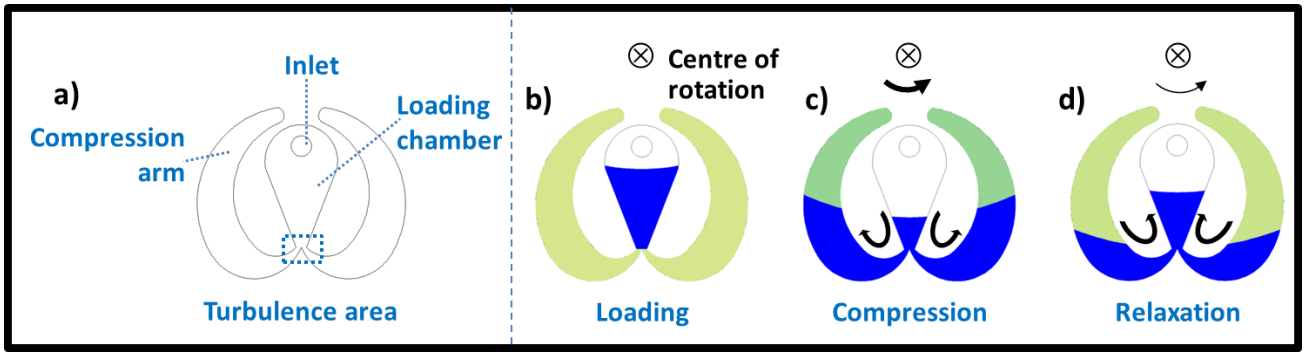

Figure 1: ***a*** *Main parts of the micro-homogenizer.* ***b*** *Loading of the chamber with liquid (blue) entrapping the air (green) in the compression arms.* ***c*** *Spinning the disc at high rpms leads in the compression of the air (dark green) towards the top of the compression arms while the liquid moves forward.* ***d*** *Backing down of the liquid by the air expansion in after reduction on the rpms.*

Initially, the disc is spun at high rotational velocity and acceleration; this drives the loaded towards the dead-end side arms where it pressurizes the entrapped air. Decreasing the (mean) rotational speed (even while maintaining high acceleration) lets the air in the side arm expand to drive the liquid back towards the central container. Reciprocation of this centrifugo-pneumatic actuation enhances the quality of mixing.

EXPERIMENTAL

The microfluidic disc was assembled using a three layers system, two Zeonor® blank discs bonded by a pressure sensitive adhesive layer (Fig.2a). The full structure was milled on a blank disc using a Modela™ MDX-40A milling machine (Roland DG, UK). The pressure sensitive adhesive layer featured the empty silhouette of the micro-homogenizer using a Craft ROBO-Pro vinyl cutter (Graphtec America Inc., USA) and a second blank disc was used as a lid to close the system. The layers were aligned and pressed together using a roller to ensure good adhesion of the system.

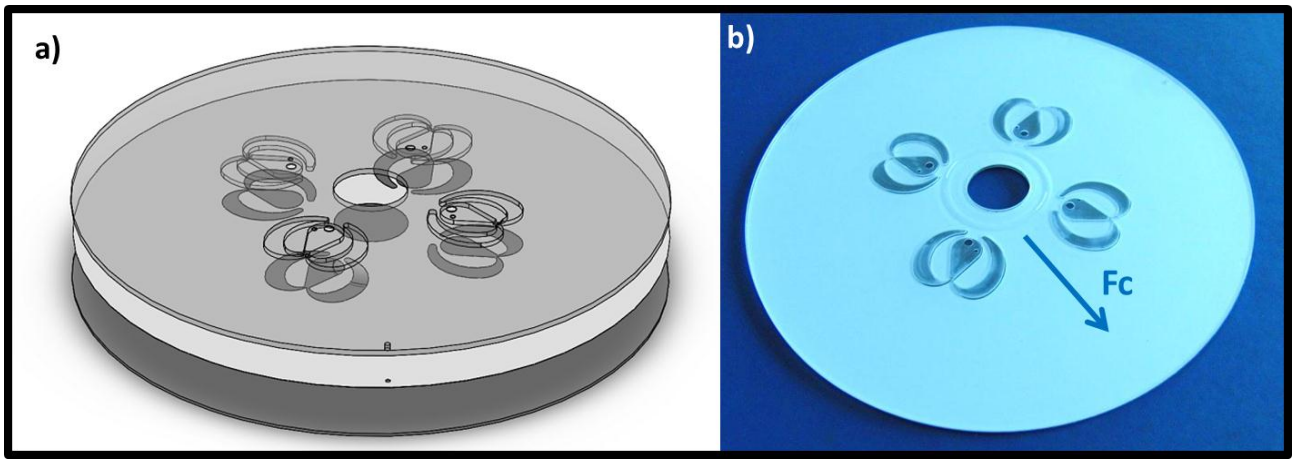

*Figure 2:****a*** *Schematic showing of the assembly composed of two Zeonor® discs and the intermediate adhesive layer.* ***b*** *Photograph of the system after assembly.*

Initial microfluidic testing by visual inspection of colored dyes demonstrated effective mixing and biological tests were subsequently performed. For miRNA extraction, fixed amounts of rabbit whole blood and TRI, scaled down from the standard protocol [4], were mixed using multiple cycles of compression and relaxation at 6000 rpm and 600 rpm, respectively (Fig. 3).

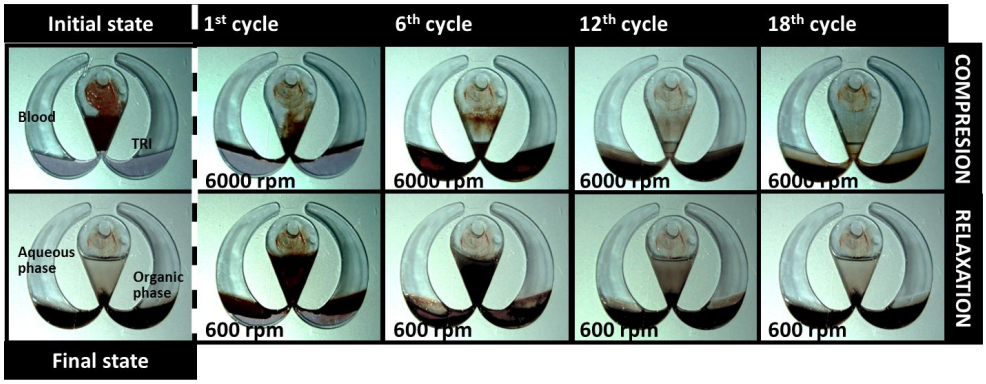

Figure 3: Sequence of images representing the mixing and phase separation process. Left-top image represents the initial state with 13 µL of blood and 38 µL of TRI and the bottom one the final result. Each cycle lasts 8 s.

RESULTS AND DISCUSSION

After a total number of eighteen cycles, the aqueous phase where the total RNA is isolated and the organic phase containing the lysed red blood cells are fully separated. The aqueous fraction was retrieved from the disc, purified and analyzed using a Small RNA Kit and a Bioanalyzer (Agilent Technologies) (Fig. 4). The collected fractions indicated 3X higher miRNA concentration using our microhomogenizer than the ones analyzed following the standard procedure off-chip.

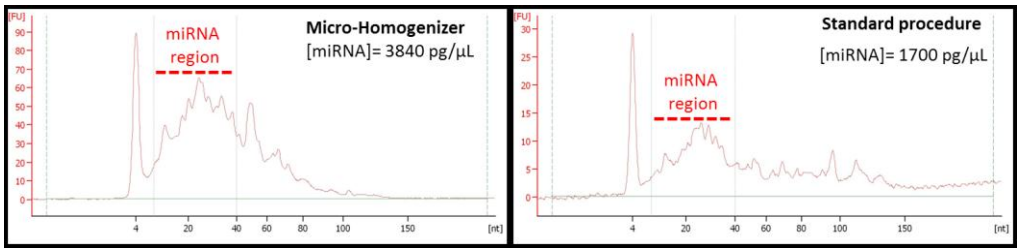

Figure 4. Electropherograms obtained from the collected fractions on the CD (left chart) and with the standard procedure (right chart).

CONCLUSION

Our novel, centrifugo-pneumatic liquid-liquid homogenization scheme has proven to enable an efficient and fast lysis and chemical RNA extraction from whole blood on a solvent resistant Zeonor® chip. Moreover, our system was able to improve the extraction efficiency when compared to the standard procedure off-chip. Continued work is focused on further applications of the technology (Fig. 5 shows its use for creating emulsions) as well as its integration with other processing units towards a fully integrated and automated molecular diagnostics.

*Figure 5. **a** Loading of oil (transparent phase) and water (dark phase). **b** Compression of the liquids while spinning. **c** Formation of water droplets with the backing down of the liquids at low rpm.*

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