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25th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2021), pp. 213-214, 2022-08

FLOW THROUGH TARGET BACTERIA CAPTURE, LYSIS AND DNA EXTRACTION USING MAGNETIC CLOUDS

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ABSTRACT

A novel sample preparation method is presented which uses suspended magnetic nanoparticle (MNP) assemblies (termed *M-cloud*) formed within a micropillar array magnetized using an external magnetic field. Composed of aggregated MNPs, the M-cloud serves as a porous capture matrix for target analyte flowing through the array. The concept is applied for *in-situ* magnetic capture of target bacteria, lysis and DNA extraction performed on a polymer microfluidic chip in a flow-through manner. Quantitative assessment M-cloud performance using river water samples and downstream qPCR revealed that DNA output correlates linearly with the input bacteria concentration, making it possible to confirm *E. coli* O157:H7 at 10^3 cells/mL.

KEYWORDS: Magnetic separation, Flow-through sample preparation, Bacteria capture, Nucleic acid extraction.

INTRODUCTION

Sample preparation based on magnetic separation (MS) has gained increasing attention for broad spectrum of analytical applications, including pathogen detection using qPCR analysis. Conventional MS is typically performed in test tubes, however MS in combination with microfluidic devices has recently emerged as a viable, high-throughput and low-cost alternative. Most microfluidic MS systems rely on the separation of target analyte only after a pre-incubation step in which the targets are linked to the magnetic particles [1,2]. *In-flow* MS is an emerging sample preparation method that does not require pre-incubation [3]. However this approach suffers the displacement of beads under fluid flow, allowing only relatively low flow rates (e.g., 1 to 5 µL/min) for operation. To overcome this limitation, we developed *in-flow* MS using MNP cloud assemblies to produce PCR-grade gDNA from bacteria samples in a simple, flow-through microchip format. We validate the M-cloud concept by enriching *E. coli* O157:H7 from river water, followed by bacterial lysis and DNA extraction *in-situ* within a single microfluidic device.

EXPERIMENTAL

The M-clouds are generated using a microfluidic chip comprising embedded micropillar array that can be magnetized using permanent magnets. The device is fabricated from thermoplastic polymers (Zeonor 1060R and Mediprene OF 400M) using hot embossing. The capture region comprises an array of high-aspect ratio micropillars (16.5 µm in diameter and 85 µm in height) coated with a soft ferromagnetic (NiP) layer and thin gold film using electroless plating (Figure 1A). The assembled chip is placed inside a uniform magnetic field generated by external magnets so that the magnetic field lines are oriented perpendicular to the direction of flow through the array (Figure 1B). The resulting magnetic field gradients generate localized capture sites for MNPs to form aggregated particle assemblies suspended between micropillars. This forms a solid-phase extraction (SPE) matrix with MNPs distributed over the entire cross-section of the fluidic pathway, allowing in-flow target capture. We generate multifunctional M-cloud consisting of i) 200 nm immunomagnetic NPs (iMNPs) for specific capture of *E. coli* O157:H7, and ii) 200 nm silica-coated MNPs for subsequent isolation and purification of gDNA upon bacterial lysis to obtain purified sample compatible with qPCR analysis (Figure 1C). Using a programmable syringe pump, the multifunctional M-cloud is first formed by introducing 50 µg of iMNPs in 500 µl of PBS to the capture area using unidirectional flow to form a 3 mm wide immunomagnetic capture band. We then add the silica-coated MNPs from the opposite end of the chip to generate a similar band for the NA extraction matrix. For the subsequent introduction of the *E. coli* sample (heat-killed bacteria spiked in river water), we reverse the flow through the array to capture bacteria in the iMNP segment.

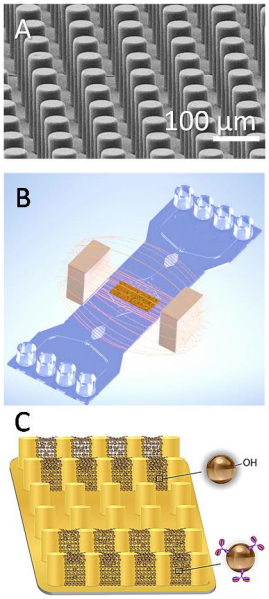

***Figure 1**: A. SEM image of micropillar array. B. Microfluidic device setup. C. M-cloud concept with two capture regions.*

Using a flow rate of 100 µL/min, the capture process takes 10 min to complete for a 1 mL sample aliquot. A PBS buffer wash step is then performed to remove possible impurities from the cloud. The passage of lysis/binding buffer results in the disruption of the cellular membrane for the bacteria trapped in the immunomagnetic segment, while solubilized gDNA is captured downstream in the NA extraction matrix. Following a wash step to remove debris and PCR inhibitors, the purified gDNA sample is eluted and collected for downstream qPCR.

RESULTS AND DISCUSSION

We formed M-clouds from iMNPs conjugated to anti-*E. coli* O157:H7 Ab moieties and Cy5-labeled BSA to observe a capture process that involves heat-killed *E. coli* O157:H7 fluorescently stained with SYTO 9 using confocal microscopy imaging. From Figure 2A, the clouds (red color) span the entire gap between pillars and also extend the complete height of the pillars, while bacteria (green color) are co-localized in and around the iMNP M-clouds. In an attempt to observe the aggregates that formed during the capture process at higher resolution, we dried the capture matrix while maintaining the magnetic field in place so that the array can be imaged using SEM. Figure 2B shows agglomerates of spherical iMNPs intertwined with rod-shaped bacteria bridging the space between the pillars perpendicular to the flow direction, as corroborated by the confocal fluorescence images.

We used qPCR to assess the performance of the device for in-situ bacterial capture, lysis and gDNA isolation in comparison to manual capture and extraction performed in Eppendorf tubes using equivalent reagents, sample volumes, and NP concentrations. As shown in Figure 2C, cell numbers obtained by the qPCR assay correlate linearly with the sample input concentration, making it possible to positively recognize *E. coli* O157:H7 at 10^3 cells/mL, which was found to be the limit of detection (LOD) for the on-chip sample preparation process described herein. The M-cloud method provided a measured cell number for all input bacteria concentrations that was one order of magnitude higher than a 10 min incubation of the sample with iMNPs in tubes (being equivalent to the residence time for 1 mL during on-chip capture at a flow rate of 100 µL/min).

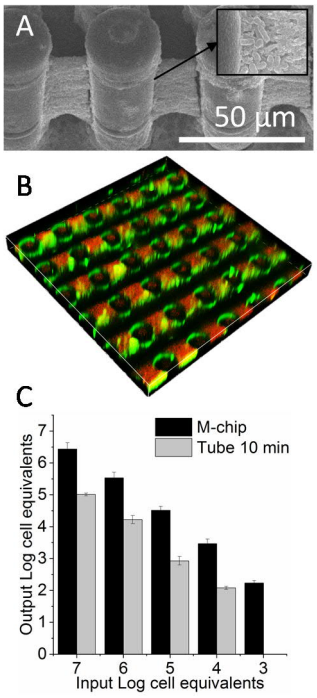

***Figure 2**: M-cloud capture of E. coli as revealed by SEM imaging (A) and confocal microscopy (B). C. Measured log cell number obtained by qPCR for M-chip and tube-based sample preparation at different input E. coli concentrations.*

CONCLUSION

We described a novel concept for microfluidic-based sample preparation which uses multifunctional M-clouds tailored for specific, immunomagnetic capture of target bacteria and isolation of gDNA *in-situ*. The combined LOD of in-flow *E. coli* capture and DNA extraction using environmental water samples was 10^3 bacterial cells/mL. We believe that the presented methodology can be useful for routine assessment of environmental samples by enabling in-line monitoring of microbial contamination.

ACKNOWLEDGEMENTS

We thank National Research Council Canada for financial support.

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