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Morelli, Lidia; Serioli, Laura; Centorbi, Francesca Alessandra ; Matteucci, Marco; Demarchi, Danilo; Zor, Kinga; Boisen, Anja

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EXTRACTION AND SERS BASED DETECTION OF BACTERIAL METABOLITES IN MIXTURE ON A CENTRIFUGAL MICROFLUIDIC DEVICE

Lidia Morelli*[1], Laura Serioli[1], Francesca Alessandra Centorbi[2], Marco Matteucci[1], Danilo Demarchi[2], Kinga Zór[1] and Anja Boisen[1]

[1] Department of Micro- and Nanotechnology, Technical University of Denmark, DENMARK
[2] Department of Electronics and Telecommunications, Politecnico di Torino, ITALY

ABSTRACT

In this work we present a centrifugal platform aimed at quantitative screening of genetically modified bacterial strains. The platform combines liquid-liquid extraction (LLE) and surface enhanced Raman scattering (SERS) sensing, enabling automated sample pretreatment and detection of secondary metabolites (e.g. *p*-coumaric acid (pHCA) and cinnamic acid (CA)) in mixture from complex matrix, such as bacterial growth medium.

KEYWORDS: Surface enhanced Raman scattering (SERS), centrifugal microfluidics, liquid-liquid extraction (LLE), *p*-coumaric acid, cinnamic acid.

INTRODUCTION

Standard screening methods in the field of metabolic engineering, such as HPLC or GC/MS, although robust and accurate, are often expensive and time-consuming. Therefore, there is a need for fast, cost efficient, automated analysis methods. SERS-based sensing was proved to be a promising analytical tool, but detection in a complex medium, such as bacterial supernatant, can be challenging due to the presence of interfering compounds and salts [1]. An assay combining LLE and SERS, previously developed in our group [1], already proved successful for quantification of a single analyte [1]. However, in most cases there is more than one compound of interest in bacterial supernatant [4]. Centrifugal microfluidics has developed significantly in the last decades, showing fast and automated implementation of complex assays [2]. However, only few solutions were developed for screening of microbial strains through LLE, such as the system described by Kim et al. [3].

In this work the LLE assay [1] was successfully adapted to the here presented platform and applied for the extraction and detection of two phenolic compounds, pHCA and CA in a mixture from bacterial growth medium. Microfabrication of a disposable platform resistant to dichloromethane (DCM) was achieved through injection molding, whereas assembly was carried out with ultrasonic welding.

EXPERIMENTAL

Figure 1(a) shows 4 microfluidic slides assembled on a disc. Each slide is composed of layers of clear polypropylene (PP) (Borclear RF366MO, Borealis AG), fabricated with injection molding (Victory Tech 80/45, Engel) and bonded through ultrasonic welding (Telsonic USP4700 20 kHz, Telsonic, Erlangen, Germany). 2 mm x 4 mm SERS chips, fabricated with the methods described by Schmidt et al. [5], were glued with a double layer of double-sided tape in a closed sensing chamber in each slide (Figure 1(b)). Figure 1(b) and Figure 1(c) depict the microfluidic design and the spinning protocol for performing the LLE assay [1] on the centrifugal platform.

RESULTS AND DISCUSSION

Typical calibration curves for pHCA and CA, obtained from spiked DCM solutions are shown in Figure 2. Samples of M9 growth medium [4] spiked with 1 mM pHCA, 1 mM CA and 500 µM pHCA and CA were analyzed. As shown in Figure 2(b), SERS spectra of pHCA/CA samples with no prior LLE did not show any characteristic spectral features. When performing LLE, instead, characteristic features at 1634 and 1002 cm^{-1} for CA and at 1603 and 1169 cm^{-1} for pHCA were clearly visible.

CONCLUSION

These results indicate that our setup enables extraction and detection of both pHCA and CA, separately or in a mixture, opening up new possibilities for high-throughput, on-site screening of bacterial strains through SERS based detection.

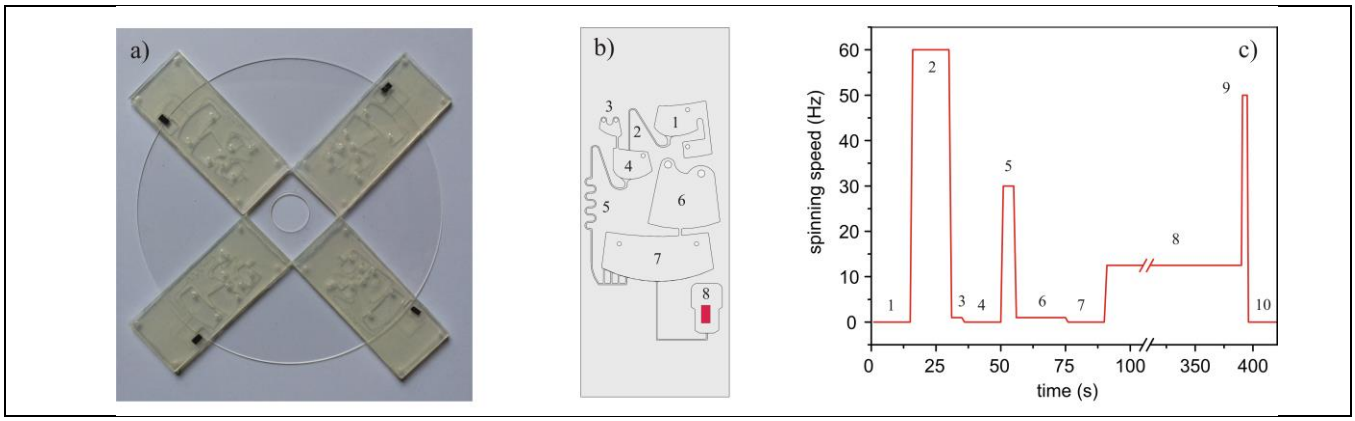

Figure 1: (a) Microfluidic slides assembled on a disc. (b) Top view of a microfluidic slide, with the following elements highlighted: (1) metering chamber, (2) hydrophilic siphon, (3) HCl loading chamber, (4) acidification chamber, (5) serpentine hydrophilic siphon, (6) DCM loading chamber, (7) incubation chamber, (8) SERS chamber. (c) Speed protocol for LLE and sample incubation on the SERS chip, including the following steps: (1) sample loading, (2) metering, (3) siphon priming, (4) HCl loading, (5) acidification, (6) serpentine siphon priming, (7) DCM loading, (8) mixing and incubation, (9) SERS chip wetting, (10) excess sample removal and subsequent SERS acquisition.

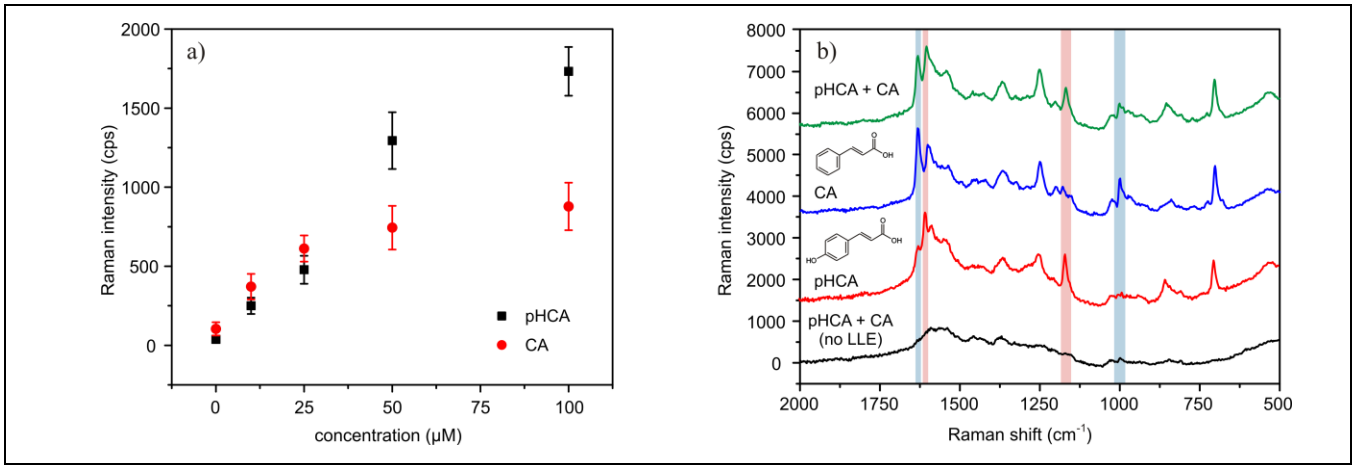

Figure 2: (a) Raman intensity at 1169 cm^{-1} and at 1002 cm^{-1} versus pHCA and CA concentration respectively. 3 maps of 48 points were collected on each SERS chip with a DXRxi Raman Imaging Microscope at 780 nm with a laser power of 2 mW, 10x lens, 50 μm slit. Each point in the graph was performed in duplicates. (b) SERS spectra of extracts of M9 spiked with 1 mM pHCA (red), 1 mM CA (blue) and both 500 μM pHCA and 500 μM CA after LLE (green) and before LLE (black). Characteristic spectral features of pHCA and CA are highlighted in red and blue respectively.

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CONTACT

* L. Morelli; phone: +45 91 73 43 40; lmor@nanotech.dtu.dk