

SUSPENDED MICROFLUIDICS: AN OPEN AND USER-FRIENDLY TECHNOLOGY PLATFORM FOR HIGH-THROUGHPUT METABOLOMIC STUDIES

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ABSTRACT

The fungal and bacterial secondary metabolism provides organisms with a highly versatile and bioactive panel of chemicals affording them both physical and competitive fitness. While powerful tools have been developed for performing a metabolomics analysis of microbial cultures, identification of secondary metabolite profiles resulting from inter- and intra-kingdom interactions remains a significant challenge. Here we present a platform that enables the extraction of metabolites using a novel open microfluidic method for creating bi-phasic interfaces. The simplicity of the method is amenable to integration into the current metabolomics workflow and allows the creation of high-throughput microscaled platforms for screening co-culture interactions and micro-environmental factors.

KEYWORDS

Open microfluidics, Secondary metabolites, Metabolomics, Bi-phasic microfluidics, LC-MS

INTRODUCTION

Secondary metabolites of microbial origin have strong bioactive properties that are the source of important worldwide health and economical issues (e.g. aflatoxin contamination of crops), as well as a treasure chest of putative therapeutic agents (e.g. penicillin, statins, etc...) [1]. In particular, fungi, such as *Aspergillus Fumigatus*, excel at generating a multitude of secondary metabolites in response to microenvironmental factors such as nutrients, stress factors, and competing organisms [2]. In addition, large spectra of metabolites are only generated through symbiotic interactions with other fungi or bacteria [3]. While metabolomic profiling has been the target of intense research, current platforms do not allow an exhaustive exploration of the whole range of microenvironmental growth conditions and co-culture conditions that can yield unknown compounds. Furthermore, most recent microfluidic approaches have focused on gains in precision and sample volume by performing the analysis on-chip [4]. Thus, the lack of available high-throughput platforms allowing metabolite extraction from multi-organism cultures is a limiting factor in the discovery of novel therapeutic metabolites.

Here, we present a novel microfluidic platform technology, allowing the creation of open, pipette-operated, bi-phasic microfluidic systems, named suspended microfluidics. In brief, we developed and validated an analytical model describing the conditions for which capillary fluid flow occurs in channels devoid of both a ceiling and a floor, or more generally any channel containing multiple open interfaces (Fig. 1). This enables the creation of extremely simple and reliable microfluidic systems in which immiscible fluids (e.g. organic solvent and media) can be brought in contact on a platform that can be interfaced with most liquid handlers and HPLC auto-samplers. Furthermore, the technology significantly decreases the fabrication complexity and cost by removing the necessity of bonding and the use of expensive micro-porous membranes. In the context of metabolomics,

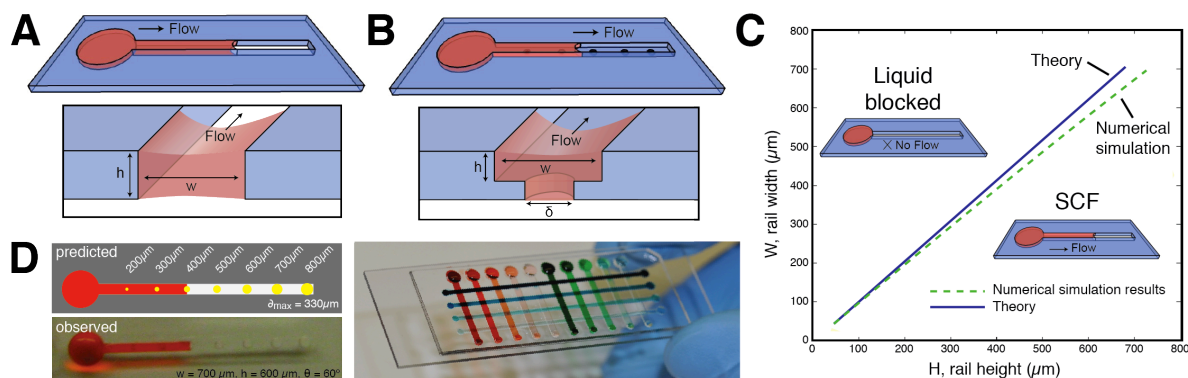


Figure 1. **A.** Schematic of a suspended microfluidic channel, which contains neither ceiling nor floor. **B.** Suspended microfluidic channel in which a U-shaped groove contains apertures in the floor. **C.** Plot of the regions that support spontaneous capillary flow (SCF) and those that don't. The theoretical prediction matches the numerical simulation closely. **D.** Experimental validation of the theoretical conditions developed and demonstration of a suspended microfluidic array.

suspended microfluidics enables novel functionalities for culturing microorganisms, increases the scope of experimental conditions that can be screened for metabolite production, and reduces the time and cost of doing so (Fig. 2). Specifically, suspended microfluidics has applications for the high-throughput identification of secondary metabolite profiles from intra- and inter-kingdom cultures for the identification of novel metabolites and the discovery of micro-environmental regulation of cryptic secondary metabolite gene clusters.

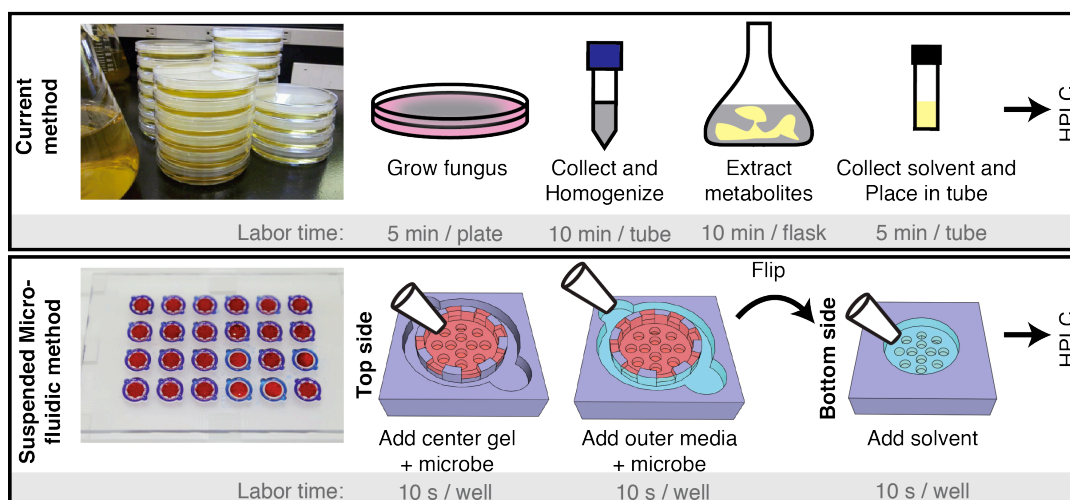


Figure 2. Comparison of the current workflow used for extracting *A. Fumigatus* metabolites and the novel microfluidic workflow. Beyond the gains in time and space conferred by the suspended microfluidic platform, the device enables the co-culture of microorganisms in a high-throughput approach with a 96-well plate footprint.

EXPERIMENT

In order to design open microfluidic systems that can create stable liquid-air and liquid-liquid interfaces we developed an analytical model for open capillary flow in a microchannel containing any number of free open interfaces. The analysis results in a design guideline that states that the ratio of the length of the cross-section of the open channel that represents the wetted hydrophilic material, called the wetted perimeter (P_w), and the length of the cross-section of the open channel that represents the open interface, called the free perimeter (P_f), must be lower than the cosine of the contact angle of the fluid. Using these design considerations we show that suspended flow over apertures in the floor of an open microfluidic channel is reliably achievable, allowing for the creation of bi-phasic pipette-operated microfluidic systems (Fig. 1). Open suspended microfluidic platforms thus enable the creation of aqueous-organic solvent interfaces allowing for the extraction of small molecules from culture media in a

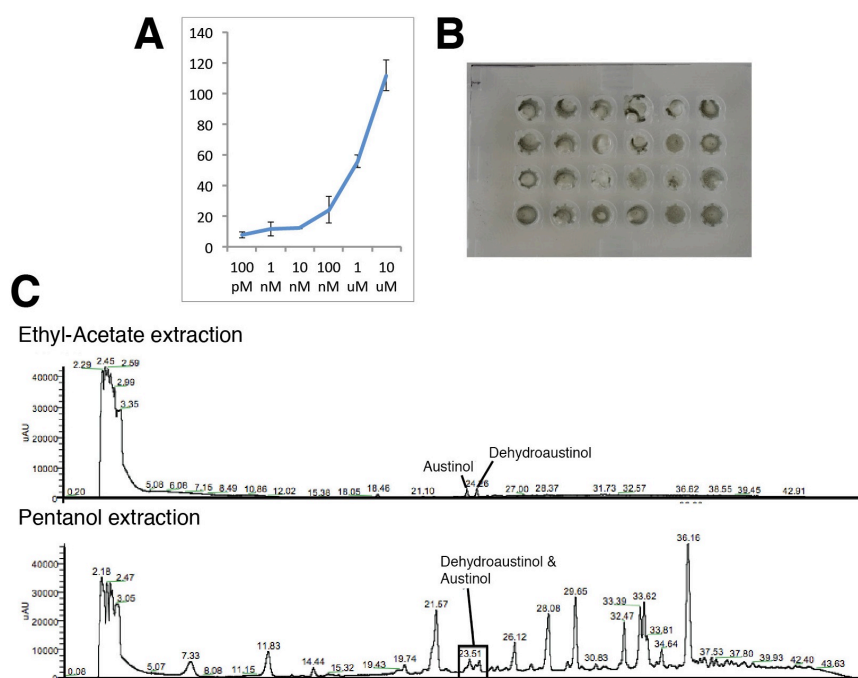


Figure 3. **A.** Extraction of Texas Red dye and quantification on a fluorescent plate reader. **B.** Culture of *A. Fumigatus* on GMM-agar in the 24 micro-well array. **C.** HPLC trace of an *A. nidulans* culture extracted with ethyl acetate and pentanol. Ethyl acetate displays different peaks than the pentanol trace.

simple and scalable operation easily compatible with the traditional metabolomics workflow. We determined geometrical guidelines based on a Bond number analysis enabling a robust and stable interface between aqueous media and different organic solvents used for metabolite extraction, such as chloroform, ethyl acetate, pentanol, and butanol. Furthermore, we developed fabrication methods allowing the creation of solvent resistant microdevices that do not require exotic materials or challenging fabrication methods. Using a combination of polystyrene or PDMS fabrication followed by a parylene C chemical vapor deposition (CVD) step, we show that straightforward solvent-resistant devices are easily manufacturable. Open microfluidic methods are enabling in this context as diffusion-based methods such as CVD are challenging in enclosed environments.

Using suspended microfluidic methods, we developed bi-phasic culture platforms allowing the liquid-liquid and liquid-solid extraction of small molecules into organic solvents. The suspended extraction methods developed are versatile and enable applications for a large range of biological models such as the steroid production of human adrenal cells and the secondary metabolism of filamentous fungi cultures, as well as for a large range of organic solvents. We demonstrate the potential of the platform for mammalian cells metabolomics by culturing human adrenal cells in the device for several days and measuring cortisol, cortisone, and testosterone by LC-MS after extraction in pentanol. We further demonstrate the potential of suspended microfluidic extraction for analyzing the secondary metabolism of microbial cultures by extracting small molecules from a culture of *Aspergillus Nidulans* in a range of solvents of different polarities (e.g. chloroform, ethyl acetate, pentanol) allowing the sampling of a variety of different compounds which may be overlooked in traditional extraction processes. This application illustrates the potential of suspended microfluidic extraction to optimize the extraction process on a large array of micro-environmental conditions and solvents (Fig. 3). Finally, we demonstrate the potential of suspended microfluidic systems for creating simple high-density co-culture extraction platforms. Suspended microfluidic methods were employed to develop a bonding-free microfluidic device in which two organisms are co-cultured in an open channel system on the topside of the platform and extraction is performed on the backside of the platform. The two organisms are in liquid contact through a crenulated wall enabling straightforward access to both microculture areas through the open top interface, and the solvent is placed in contact with one of the two organisms through a horizontal suspended interface. We show that this platform enables the multiplexed study of the metabolite profile resulting from the interaction of a range of different microorganisms, without requiring any electronic or mechanical actuation systems. Provided the simplicity of use and the ease of manufacturing, we expect open microfluidic methods to become an attractive technology for a variety of biological studies. Specifically, in the context of metabolomics, open microfluidic methods allow the creation of platforms that are readily treated for solvent resistance, simple to operate, and easily interface-able with traditional metabolomics tools such as HPLC and LC-MS.

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