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In-tip nanoreactors for cancer cells proteome profiling

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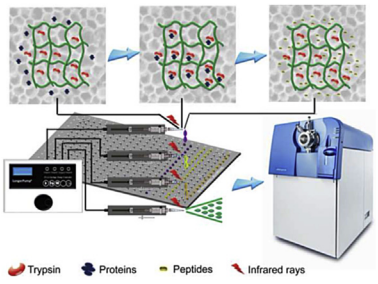
HIGHLIGHTS

- Increased number of identified proteins compared to the 12 h in-solution digestion.
- Enhanced proteolytic performance towards a group of proteins with specific *p*I.
- Desirable throughput consuming ~6 min for proteolysis of bio-samples.
- Automated process in the sample pretreatment for online MS analysis.
- Long term stability after two months storage, reusability with little memory effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Mass spectrometry (MS)-based proteome profiling is essential for molecular diagnostics in modern biomedical study. To date, sample preparation including protein extraction and proteolysis is still very challenging and lack of efficiency. Recently tips-based sample preparation protocols exhibit strong potentials to achieve the goal of "a proteome in an hour". However, in-tip proteolysis is still rarely reported and far from ideal for dealing with complex bio-samples. In this work, nanoreactors encapsulated micropipette tips were demonstrated as high performance devices for fast (~minutes) and multiplexing proteolysis to assist the profiling of cancer cells proteome. Nanoporous silica materials with controlled pore size and surface chemistry were prepared as nanoreactors and encapsulated in micropipette tips for efficient *in situ* proteolysis. The as-constructed device showed desirable sensitivity (LOD of 0.204 ± 0.008 ng/μL and LOQ of 0.937 ± 0.055 ng/μL), selectivity, stability (two months under −20 °C), reusability (at least 10 times), and little memory effect in MS based bottom-up proteomic analysis. It was used for comprehensive protein mapping from cancer cell lines. The number of identified proteins was increased by 18%, 22%, 52%, and 52% dealing with HepG2, F56, MCF7, and HCCLM3 cancer cells, compared to traditional in-solution proteolysis based bottom-up proteomic strategy. With the enhanced performance, our work built a novel, efficient and miniaturized platform for facile proteomic sample preparation, which is promising for advanced biomarkers discovery in biomedical study.

1. Introduction

Profiling of proteome is essential for molecular diagnostics in modern biomedical study, which reveals the physiological and pathological process of selected biological systems [1–5].

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Particularly, mapping of cancer cells proteome plays a key role in the study and management of the versatile disease including diagnosis and prognosis et al. [5–9]. It is desirable to detect as many proteins as possible to comprehensively characterize the proteome, and considerable effort has been devoted to the development of proteome profiling methods, such as two dimensional gel electrophoresis methods [10–12], protein chips [9,13], mass spectrometry (MS) technology [14–18], *etc*.

To date, the bottom-up proteome is the most widely used strategy, which integrates a series of core techniques, such as proteolysis of proteins, chromatographic separation of digested peptides, MS and tandem MS based peptide sequencing and proteome mapping supported with databases. Bottom-up proteome enjoys the unique advantages of antibodies-free, high-throughput, and fast sequencing capability over other methods in large-scale clinical applications [19–21]. Despite the substantial progress in the mass spectrometers and on-line database construction, sample preparation including protein extraction and proteolysis is critical in the workflow of Proteomics, but very challenging and lack of efficiency [22].

Most recently, "a proteome in an hour" [23] has been proposed for advanced MS analysis of a proteome in just over one hour, and tips-based sample preparation protocols displayed strong potentials to achieve the goal, *e.g.* by utilizing stop-and-go tips (StageTip) [22]. Notably, although the in-tip protein extraction has been performed by the StageTip [22], protocols for protein proteolysis are limited when dealing with low concentrated proteins, and 12–24 h are usually needed for the overall sample preparation. Efficient in-tip proteolysis is still rarely explored and far from ideal for dealing with complex bio-samples [24,25].

To enhance the proteolysis for proteomic study, immobilized enzymatic reactors are intensively studied by many research groups using diverse platforms, such as microfluidic chips [26–28], capillary columns [29–31], and nanoporous materials [32–35]. Due to the unique nano-confinement effect of uniform pore channels, nanoporous materials have been employed to host and accelerate proteolytic reactions, and show superior performance to other platforms. For the first generation of nanoreactors, mesoporous materials with the pore size <20 nm were employed to load trypsin for proteolysis [36]. Nevertheless, the free movement of biomolecules is always restricted because both enzymes and substrates are also in the scale of a few nanometers [37,38], resulting in decreased reaction efficiency. Hence, for the second generation of nanoreactors, macroporous materials with pore size >50 nm were developed to provide a confined but not restricted nano-space for proteolysis, which has dramatically improved the proteolysis efficiency for bottom-up Proteomics [39–42]. It should be noted that the performance and function of nanoreactors also relied on their surface chemistry properties, which can provide affinity towards a group of proteins with specific isoelectric points (*p*I) [39,42] or post-translation modifications (PTM) [32,33,43]. However, the engagement of nanoreactors in proteomic workflow is not well established. Consequently, the use of nanoreactors is still limited in the profiling of real case cancer cells proteome, especially for large-scale applications.

Herein, for the first time we have developed nanoreactors encapsulated tips for proteolysis and MS profiling of cancer cells proteome (Fig. 1). The tip device was constructed by loading trypsin-immobilized macroporous ordered siliceous foams (MOSF) into an Axygen® micropipette tip. Through electrostatic interaction, *in-situ* enrichment of enzymes (trypsin) and proteins into the nanopores of MOSF could be realized for subsequent fast proteolytic reaction because of the nano-confinement and enrichment effect [44]. The tips were used for the fast proteolysis of proteins extracted from HepG2, F56, MCF7, and HCCLM3 cancer cell lines. It was found that larger number of proteins could be identified from the sample processed by the tips. Therefore, an effective sample preparation device was demonstrated.

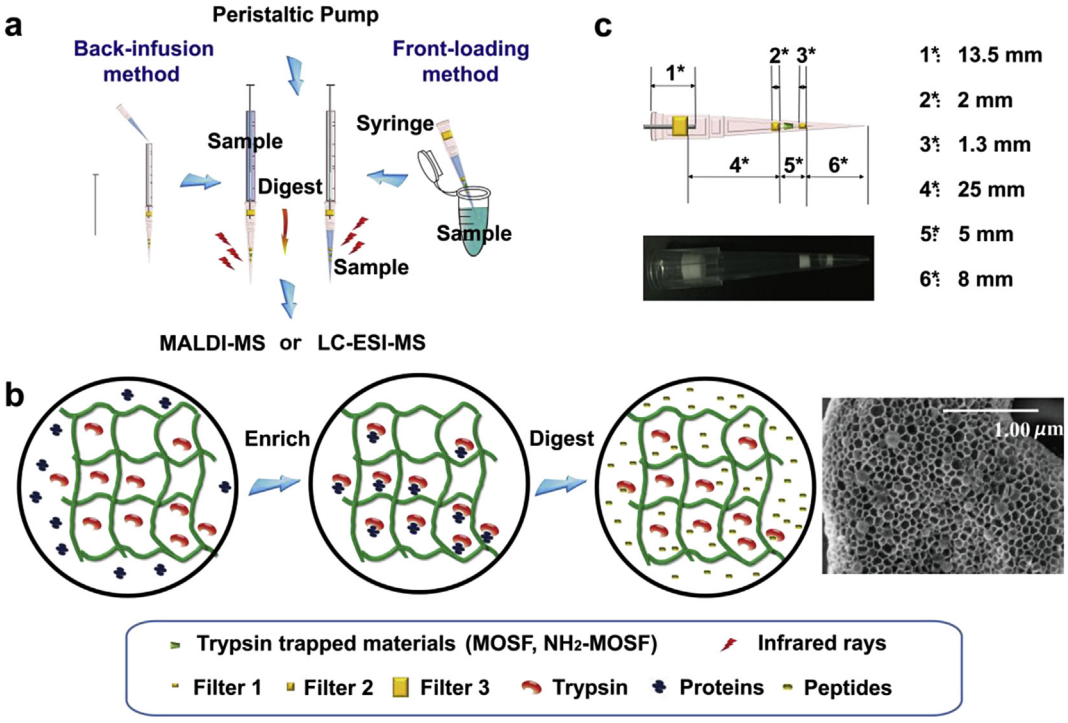

Fig. 1. Nanoreactors-loaded micropipette tips and the applications in MS analysis. a) Schematic illustration of the experimental flow. Protein solution was infused (the back-infusion method) or sucked (the front-loading method) into the tip. Peptides were formed at the end of the tip for subsequent MS analysis. Infrared rays functioned in keeping the temperature at around 37 °C for optimal trypsin activity. b) Schematic illustration of proteolysis with trypsin immobilized nanoreactors and FE-SEM image of the porous silica nanoreactor. c) Structure of the loaded tip and key dimensions of the device.