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A "plug-and-use" approach towards facile fabrication of capillary columns for high performance nanoflow liquid chromatography

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ABSTRACT

Capillary columns used for nanoflow liquid chromatography play an important role in modern proteomics. High quality columns are needed to provide high peak capacity and highly reproducible separations. This is extremely important when multiple separations were compared in parallel in searching for potential biomarkers. Herein, we introduce a "plug-and-use" fritting technology for fabrication of high quality and highly reproducible capillary columns. Due to the identical length, good permeability, and stability of the prefabricated frits adopted, the capillary columns presented excellent performance consistency in terms of retention time, peak width as well as peak capacity at a column-to-column level (relative standard deviations, RSDs, at 0.4–0.9%, 2.1–3.6%, and 2.7%, respectively, $n = 6$) for separations of complex mixtures of protein digest. For capillary columns packed with 5 μm particles, high separation efficiency was demonstrated by the minimum plate height of 11 μm, approaching the theoretical performance limit of such material. For separations of protein digests, the columns demonstrated excellent peak capacities of 110 and 300 for 20 and 360 min gradients, respectively. The simple fabrication, good performance as well as consistent quality of such columns provide a reliable tool for high throughput separations requiring the use of multiple high performance capillary columns in parallel.

1. Introduction

In 2010, *Nature Biotechnology* published guidelines for column chromatography by Human Proteome Organization's Proteomics Standards Initiative [1], which highlighted the demand for high quality microcolumns towards the standardization of proteomic analysis. Capillary column used for nanoflow liquid chromatography (nanoLC) has become a key separation tool for biomolecules [2,3]. Over the past ten years or so, many developments and improvements for microcolumn technology have been introduced, both in capillary [4–12], and microchip formats [13–18], for normal pressure and recently also ultra high pressure nanoLC separations [19–22].

As an indispensible consumptive material in modern proteomics, capillary columns need to be quality-controlled to a high standard. In practice, however, quality control studies were seldom seen over the microcolumns fabricated and used. A common practice seen in many proteomics laboratories is that, one packs a single column and uses it for nanoLC–mass spectrometry (MS) experiment straight away. The column is used until its performance degradation or column breakage, and then a new capillary is packed and used. Although MS as an information-rich detector can tolerate, to a great extent, the inconsistency of separation performance between columns, the column-to-column irreproducibility may become a crucial issue when large-scale screening proteomics based on parallel use of multiple columns [16–18,23–26] is performed, especially when optical detection is adopted.

At the turn of the century, the introduction of capillary array electrophoresis using 96 or 384 capillaries greatly pushed forward the pace of genomic discovery [27]. From the viewpoint of proteome-wide screening for biological discovery [28,29], there needs innovative developments in separation platforms and related consumable devices. It is to this end that high quality capillary columns, with excellent performance consistency at a column-to-column level, are demanded by, but not limited to, high throughput proteomics.

To date, the most routinely used chromatographic medium for microscale bioseparations is particulate packed capillary columns [2,3], although monolithic [30–35], and open-tubular capillary columns [36], have also been introduced. The aim of the present work is to develop a packed column technology with high standard reproducibility to support high performance microcolumn separations of complex mixtures. In resolving this issue, there are two aspects one needs to take into account: fritting and packing. Although column packing plays an important role in column performance [37,38], the main technical challenge is fabrication of high

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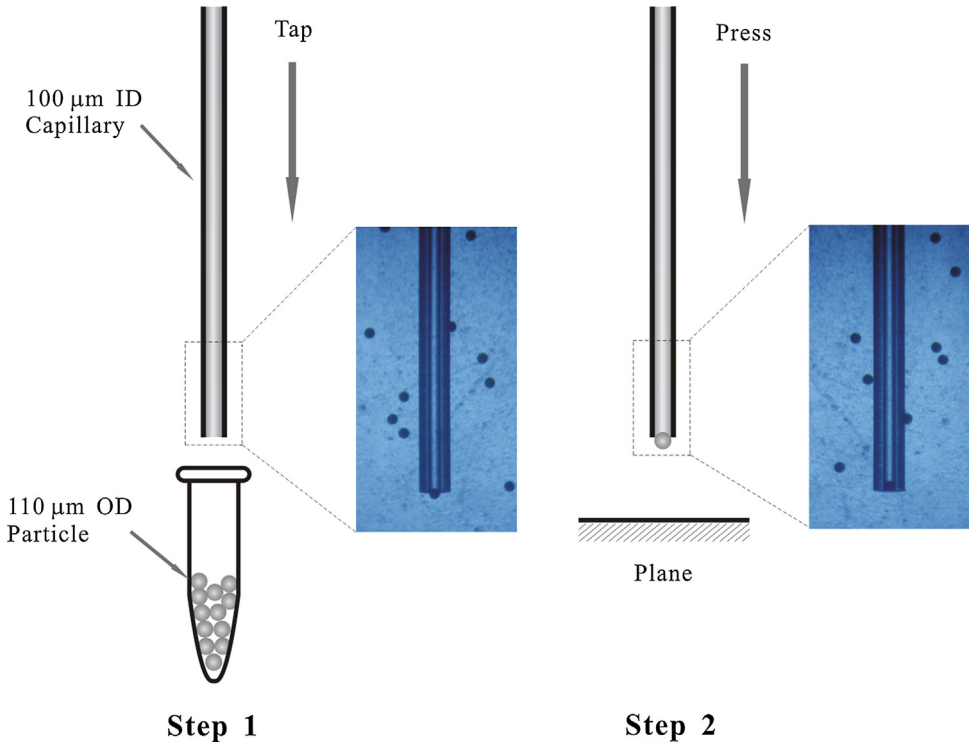

Fig. 1. A two-step "plug-and-use" fritting approach. Step 1: A large permeable porous (perfusive) silica bead is tapped onto one end of a capillary. Step 2: The captured silica bead was forced into the capillary. In the photos, the dark dots are the perfusive silica beads intentionally left in to show the relative size of the beads and the capillary tube. The perfusive silica bead has a nominal outer diameter of ~110 μm and the capillary's inner diameter is 100 μm.

quality frits inside capillary tube [39]. In this study, we introduce in a "plug-and-use" approach, based on prefabricated frits with good permeability and predetermined short length, to facilitate column preparation. We will also interrogate the columns' quality as well as performance consistency in separations of complex mixtures.

2. Experimental

2.1. Materials and apparatus

Polyimide-coated fused silica capillaries were purchased from Yongnian Reafine Chromatography (Hebei China). The porous silica particles ~110 μm in diameter with large throughpores about 1 μm, to be used as prefabricated single particle frits, were provided by X-tec (Bromborough, UK). The packing material Ultimate XB-C18 (5 μm, 300 Å) was obtained from Welch Materials Inc. (Shanghai, China). Thiourea, NH_4HCO_3, methyl-, ethyl-, propyl-, and butylbenzenes of analytical grade, dithiothreitol (DTT), iodoacetamide (IAA) trifluoroacetic acid (TFA), trypsin of sequencing grade, standard protein cytochrome C, lysozyme, ovalbumin, bovine serum albumin, and transferrin were purchased from Sigma–Aldrich (St. Louis, MO). Acetonitrile and acetone of HPLC grade were provided by Merck (Darmstadt, Germany). An Elite P230 high pressure pump from Dalian Elite Analytical Instruments (Dalian, China) was used for column packing.

2.2. Protein digestion

Complex peptide mixtures were prepared by tryptic digestion of standard proteins in solution. Generally, proteins were solubilized in 8 M urea, 50 mM NH_4HCO_3. Then, the sample was reduced by DTT and alkylated by IAA. Finally, trypsin was added at a protein-to-enzyme ratio of 50:1, the digestion was incubated at 37 °C over night.

2.3. Nanoflow liquid chromatography

NanoLC experiments were carried out on an Ultimate 3000 nanoLC system (Thermo-Dionex, Amsterdam, The Netherlands), equipped with an autosampler and a variable wavelength UV–vis detector with a 3 nL flow cell. A 4 nL Valco nanovolume injector (VICI AG, Schenkon, Switzerland) was used for column performance evaluation under isocratic condition. For large volume injections under gradient elution, the autosampler with a 1 μL loop was adopted.

2.4. "Plug-and-use" fritting and column packing

A 25 cm long, fused silica capillary (100 μm I.D., 365 μm O.D.) was chosen as the column tubing. As shown in Fig. 1, one end of the capillary was tapped into a micro centrifuge tube, in which a small number of ~110 μm perfusive silica beads were deposited. A single perfusive silica bead can be captured at the head of the capillary (Step 1, Fig. 1), the single bead was then pushed into the capillary by pressing the end of the capillary against a plane surface (Step 2, Fig. 1). This two-step process can be monitored and confirmed by observation under a microscope. This single silica bead served as the outlet frit of the column. The capillary column was slurry-packed under high pressure. The packing material was suspended in acetone at a concentration of 2 mg/mL and ultrasonicated for 15 min. The slurry was loaded into a reservoir (4.6 mm I.D., 15 cm long) attached to a high pressure pump. The one end fritted capillary tube was connected to the reservoir via the open end. Pressure was increased gradually (upto 6000 psi) until the column was packed. The column was cut to a desired length after the packing system was fully depressurized. Finally, another single perfusive silica bead was forced into the cut end serving as the inlet frit of the column. Before use, the column was mounted on to the