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Three-dimensional ordered titanium dioxide-zirconium dioxide film-based microfluidic device for efficient on-chip phosphopeptide enrichment

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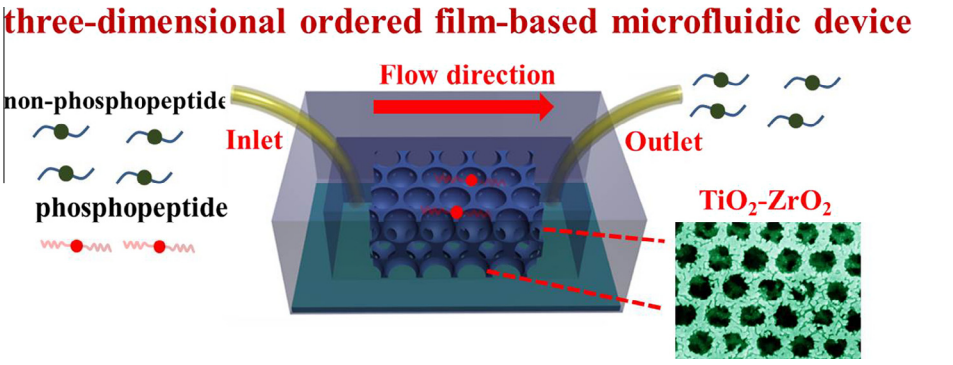
HIGHLIGHTS

- Inverse opal titanium dioxide-zirconium dioxide film was firstly fabricated.
- Inverse opal composite films were firstly introduced into microfluidic device.
- The films-based microfluidic device enriches mono-/multi-phosphopeptides efficiently.

GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: Microfluidic technology plays a significant role in separating biomolecules, because of its miniaturization, integration, and automation. Introducing micro/nanostructured functional materials can improve the properties of microfluidic devices, and extend their application. Inverse opal has a three-dimensional ordered net-like structure. It possesses a large surface area and exhibits good mass transport, making it a good candidate for bio-separation. This study exploits inverse opal titanium dioxide-zirconium dioxide films for on-chip phosphopeptide enrichment.
Experiments: Titanium dioxide-zirconium dioxide inverse opal film-based microfluidic devices were constructed from templates of 270-, 340-, and 370-nm-diameter poly(methylmethacrylate) spheres. The phosphopeptide enrichments of these devices were determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.
Findings: The device constructed from the 270-nm-diameter sphere template exhibited good comprehensive phosphopeptide enrichment, and was the best among these three devices. Because the size of opal template used in construction was the smallest, the inverse opal film therefore had the smallest pore sizes and the largest surface area. Enrichment by this device was also better than those of similar devices based on nanoparticle films and single component films. The titanium dioxide-zirconium dioxide inverse opal film-based device provides a promising approach for the efficient separation of various biomolecules.

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1. Introduction

With the rapid development of lab-on-chip technology, microfluidic devices are playing increasingly important roles in biological and medical research [1–4]. Proteomics explores the role of proteins in the life activities of organisms, and has received enormous attention. Proteomics provides a broad discipline for the analytical capabilities of microfluidic devices to be demonstrated [5–8]. Microfluidic devices support miniaturization, integration, and automation. They could potentially meet current analysis requirements well, while simplifying operational procedures and reducing sample costs [9,10]. Hence, microfluidic devices are good candidates for the continuous and efficient separation and enrichment of proteins, both in scientific research and clinical diagnosis.

Nowadays, nanostructured materials are widely used to improve the performance of microfluidic devices. For example, nanostructured functional materials such as nanorods [11,12], nanotubes [13], nest-like structures [14], and carbon nanotubes based on silicon pillars [15], have been incorporated into the channels of microfluidic devices, and the resulting devices have exhibited effective bio-separation. Integrating nanostructured functional materials with microfluidic technology is receiving more and more attention, and should be further explored to meet the growing demands of proteomics research.

Inverse opal and opal are typical colloidal crystals. Opal is the natural prototype of a colloidal crystal, and consists of a cubic-close-packed array of uniform colloidal spheres. If the space between close-packed spheres is filled with a certain material, and the spheres are then removed, then a three-dimensionally ordered porous material (inverse opal) is formed [16,17]. Inverse opal exhibits interesting optical properties and a large specific surface area, so has been widely applied in optical sensors [18], solar cells [19], lithium ion batteries [20], and supercapacitors [21]. The holes in inverse opal are interconnected, which is beneficial for fluid flow. Its large specific surface area and good mass transport characteristics make inverse opal an excellent candidate for microfluidic bio-separation.

Protein phosphorylation is an important protein post-translational modification in nature. It helps regulate various cellular processes, such as cellular proliferation, growth, regulation, and differentiation [22,23]. Thus, phosphoproteomics is an important area of proteomics research. Mass spectrometry (MS) is a powerful tool for analyzing protein phosphorylation, because it can identify phosphorylation sites and quantify their dynamic changes, to explore and understand cellular activities. The abundance of phosphopeptides is low, and their signals are often suppressed by those of non-phosphorylated peptides. Thus, the selective enrichment of phosphopeptides from complex mixtures is vital for MS-based phosphoproteome analysis [24]. Phosphopeptide enrichment is typically achieved by liquid chromatography [25,26], magnetic separation [27–32], and on-plate enrichment [33,34]. However, enrichment by liquid chromatography requires large and expensive instrumentation. Magnetic separation and on-plate enrichment are convenient and efficient methods for phosphopeptide enrichment, but are unable to provide the continuous enrichment of phosphopeptides. Integrating nanomaterials with microfluidic technology is potentially a good strategy for addressing this problem.

Metal oxide affinity chromatography is an efficient method for phosphopeptide enrichment, and TiO_2 [35–38], ZrO_2 [39,40], SnO_2 [41,42], and Al_2O_3 [43,44] have been used as affinity probes. Most of these metal oxides exhibit a complementary preference for mono-phosphopeptides or multi-phosphopeptides. Integrating two or more metal oxides can potentially achieve comprehensive phosphopeptide enrichment. Several such systems proposed for phosphopeptide enrichment have exhibited excellent performance, including TiO_2-ZrO_2 microspheres [45], Fe_3O_4@TiO_2-ZrO_2 [46], SnO_2-$ZnSn(OH)_6$ [47], Fe_3O_4/Graphene/(Ti-Sn)O_4 [48], and magG/PD/(Zr-Ti)O_4 [49]. However, there are few reports of microfluidic devices for comprehensive phosphopeptide enrichment. TiO_2 and ZrO_2 exhibit superior selectivity for multi-phosphopeptide and mono-phosphopeptide, respectively, so TiO_2-ZrO_2 has become a typical composite for comprehensive phosphopeptide enrichment. TiO_2 and ZrO_2 in the form of inverse opal could be integrated with microfluidic technology, for phosphopeptide enrichment. This could exploit the advantages of a large specific area, integrated features, and controllable construction.

In the current study, evaporation-induced self-assembly and sandwich infiltration were used to prepare TiO_2-ZrO_2 binary inverse opal films. Laser etching and oxygen plasma grafting were then used to construct TiO_2-ZrO_2 inverse opal film-based microfluidic devices. The devices were then applied in phosphopeptide enrichment, and offer an alternative approach for separating and enriching biomolecules.

2. Experimental

2.1. Regents and materials

β-casein (from bovine milk), trypsin (from bovine pancreas, TPCK treated), ammonium bicarbonate (ABC, 99.5%), aqueous ammonia solution (28%), acetonitrile (ACN, 99.9%), and trifluoroacetic acid (TFA, 99.8%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals and reagents were purchased in the highest grade commercially available, and were used without further purification. Deionized water (18.2 MΩ cm) was used in all experiments, and was obtained from a Milli-Q system (Millipore, Bedford, MA).

2.2. Characterization

To characterize the morphologies of the inverse opal films, field-emission scanning electron microscopy (FE-SEM) images were collected on a S-4800 FE-SEM instrument (Hitachi, Japan). Transmission electron microscopy (TEM) and high-resolution TEM (HR-TEM) images were obtained using a JEM-2100F instrument (JEOL, Japan). Energy dispersive spectroscopy (EDS) maps of the inverse opal film were obtained using a JSM-6700F FE-SEM equipped with an Oxford Instruments EDS detector. The crystal structures of as-prepared films were investigated using X-ray diffraction (XRD) (D/max 2550 V, Rigaku, Japan) with Cu K_α radiation ($\lambda = 1.54$ Å). X-ray photoelectron spectroscopy (XPS) measurements were recorded using a SECA Lab 220i-XL spectrometer, with non-monochromatic Al K_α (1486 eV) radiation. The specific surface areas and pore size distributions of the as-prepared films were measured using an automatic volumetric sorption analyzer (ASAP 2020, Micromeritics Inc., USA). Samples were prepared by scraping off of glass slides.

2.3. Construction of inverse opal film-based microfluidic devices

The microfluidic devices were constructed as shown in Fig. 1, and as described in detail in the following sections.

2.3.1. Preparation of PMMA opal template

Monodisperse poly(methyl methacrylate) (PMMA) spheres with different diameters were synthesized by emulsifier-free emulsion polymerization [50]. Subsequently, PMMA opal templates were