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Enhanced Protein Digestion through the Confinement of Nanozeolite-Assembled Microchip Reactors

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An on-chip microreactor was proposed toward the acceleration of protein digestion through the construction of a nanozeolite-assembled network. The nanozeolite microstructure was assembled using a layer-by-layer technique based on poly(diallyldimethylammonium chloride) and zeolite nanocrystals. The adsorption of trypsin in the nanozeolite network was theoretically studied based on the Langmuir adsorption isotherm model. It was found that the controlled trypsin-containing nanozeolite networks assembled within a microchannel could act as a stationary phase with a large surface-to-volume ratio for the highly efficient proteolysis of both proteins at low levels and with complex extracts. The maximum proteolytic rate of the adsorbed trypsin was measured to be 350 mM min^{-1} μg^{-1}, much faster than that in solution. Moreover, due the large surface-to-volume ratio and biocompatible microenvironment provided by the nanozeolite-assembled films as well as the microfluidic confinement effect, the low-level proteins down to 16 fmol per analysis were confidently identified using the as-prepared microreactor within a very short residence time coupled to matrix-assisted laser desorption-time-of-flight mass spectrometry. The on-chip approach was further demonstrated in the identification of the complex extracts from mouse macrophages integrated with two-dimensional liquid chromatography-electrospray ionization-tandem mass spectrometry. This microchip reactor is promising for the development of a facile means for protein identification.

Current proteomics research entails an arsenal of methodologies to capture the structural organization of proteins and monitor the dynamics under defined conditions correlating to cells, tissues, or body fluids.[1−3] Over the past decade, proteomics has enjoyed success in the large-scale determination of gene products and cellular function at the protein level, whereas it still remains an open-ended endeavor.[4,5] Effective protein digestion has been generally recognized as an essential technology in this endeavor. Due to the conventional digestion in solution suffering from long reaction times and autolysis interference, it remains a challenging task to achieve the simplicity, robustness, and sensitivity of protein digestion for the identification and characterization of proteins. Several strategies have been developed to improve protein digestion efficiency.[6−9] Chen et al. developed an efficient method using a microwave combined with nanoparticles for the digestion of proteins and other large molecules in a commercial microwave device within 1 min.[6] Kleno et al. proposed an in-gel digestion protocol where sample preparation was performed directly on a matrix-assisted laser desorption (MALDI) probe with prestructured sample support.[7] The protocol consisted of few sample-handling steps and had minimal consumption of reagents, making the protocol sensitive, time-saving, and cost-efficient. Ericsson et al. described an on-target protein digestion in silicon nanovials utilizing piezoelectric dispensing as a continuous and contact-free supply of enzyme and substrate, resulting in digestion times reduced to minutes or even seconds.[8] David and Li developed an automated system for protein digestion after the adsorption of a protein from solution onto a hydrophobic medium that was contained within a microcolumn.[9]

Microfluidic chips have shown promise in performing high-speed analyses with increased throughput and reduced sample and reagent consumption as well as large surface/volume ratio, suitable microstructure confinement, and portability.[10] These attractive features make them promising as microreactors for the biological reactions occurring at the surfaces and interfaces. As a bioreactor, enzymes were immobilized on the surface of the microchannels to exert their activity, while the bioactivity of the immobilized enzymes and the availability of the bioreactor strongly

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rely on the desirable design of a biocompatible stationary surface. On the other hand, the surface tailoring can greatly benefit from the rapid development of microdevices because many emerging applications require the device surface to be decorated with a compatible environment to fulfill the increasing demands for miniaturization, integration, and high throughput of future device architectures. Herein, we put our efforts to develop a versatile and flexible microfluidic platform for protein digestion in proteomics through chip surface coatings.[11−15] Trypsin, as the enzyme of choice, is required to immobilize in the modified channels to form a miniaturized bioreactor. A heterogeneous range of surface coating methods has been developed to fabricate a series of biofunctional microchip systems. Wang et al. developed a microfluidic system that enabled the packing of a cavity in a chip with immobilized enzyme beads through a side channel.[16] They found that this packed bed reactor enabled better protein processing at a higher speed. Honda et al. have developed a modified sol−gel technique for the surface modification of microchannels and its application for the preparation of the microreactor.[17]

Just as heterogeneous as the spectrum of coating methods that have been employed is the range of materials that have been utilized as the enzyme carriers. The immobilization of enzymes onto inorganic materials such as silica, porous glass, alumina, and zeolites has attracted much attention in practical applications for the advantages of their chemical stability, resistance to microbial contamination, and good mechanical and thermal stability. Balkus and Diaz were the first to publish work on the immobilization of trypsin on MCM-41, and they found the stability of trypsin is enhanced by the physical entrapment in MCM-41.[18] Lately, Palmira and Adalgisahave used zeolites as an efficient means to immobilize cytochrome.[19] Zeolite particles at the nanometer scale (nanozeolites) have displayed some characteristics such as large clean surfaces, plentiful and tunable surface properties, and high dispersibility in both aqueous and organic solutions, leading to a very high adsorption capability and a facile self-assembly character. Taking these unique characteristics into consideration, nanozeolites provided us with an ideal candidate for the microfluidic surface modification and enzyme immobilization.[20] A series of biosensors constructed with controlled enzyme immobilization by nanozeolites on the electrode surface has been reported in our group.[21,22]

In this paper, polycation poly(diallyldimethylammonium chloride) (PDDA) and β-zeolite nanocrystals were electrostatically assembled to form a large surface-to-volume ratio network via a layer-by-layer technique in polyethylene terephthalate (PET) microchip channel walls. This well-defined nanozeolite network is easily attached on the channel surface and provides a biocompatible microenvironment for enzyme immobilization. The nanozeolite-derived bioreactor is highly sensitive for the analysis of proteins within few seconds, and the detection limit can reach low femtomole amounts for standard proteins. Furthermore, such a modified setting coupled to MALDI time-of-flight tandem mass spectrometry (MALDI-TOF-MS/MS) or two-dimensional liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) systems has successfully been completed in a preliminary study for a potential application in digesting complex protein mixture extracted from mouse macrophages.

EXPERIMENTAL SECTION

Materials and Chemicals. PET sheets (100 μm thick Melinex) were purchased from Dupont (Geneva, Switzerland). PDDA, poly-(4-styrenesulfonate) (PSS), bovine serum albumin (BSA), Cytochrome *c* (cyt-c) were obtained from Sigma company (U.S.A.). The macrophage cell line AMJ2-C8 (ATCC CRL-2455) was cultured in high glucose Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 g/mL streptomycin and incubated at 37 °C in a 5% CO_2 atmosphere. The grown cells were harvested, washed with ice-cold phosphate-buffered saline (PBS) twice, and stored at −80 °C. The proteins were extracted by two different solvents with protease inhibitor mixture: one was water and the other was 4 M urea. The obtained proteins were digested, separated, and analyzed, respectively. All of other chemicals were of analytical grade and purchased from the Shanghai Chemical Reagent Co. (Shanghai, China).

Nanozeolite Synthesis. Colloidal crystals of β-nanozeolite were synthesized in a mixture solution with the molar composition of $12(TEA)_2O{:}Al_2O_3{:}60SiO_2{:}588H_2O$ according to a literature method.[22] The obtained nanosized zeolites were purified by centrifugation and redispersion in a 0.1 M ammonia solution for three times. The last colloid was adjusted to a solid content of 1.0 wt % and a pH of 10 to make the zeolite nanoparticles stable and negatively charged.

Fabrication of PET Microfluidic Chips. The microchip was fabricated by photoablation and thermal lamination from LEPA.[23] Briefly, the PET sheet was placed on computer-controlled *XY* translation stages (Physik Instrumente, Germany) and scanned to be photoablated by a UV excimer laser (Argon Fluor Excimer Laser at 193 nm; Lambda Physik LPX 205i, Gottingen, Germany) for generating a 2 cm length channel with a trapezoidal cross-section shape of about 42 μm depth and 107 μm width. The straight-line channel has photoablated reservoirs at each end. The PET with microchannels, previously washed and modified, was finally thermally sealed by a PET layer at 110 °C using a lamination machine (Hangzhou Fengling electronic instrument Co. Ltd., Zhejiang, China).

Microchannel Surface Modification and Enzyme Immobilization. After rinsing with distilled water and ethanol, the PET microchip was hydrolyzed using 1 M aqueous NaOH for 16

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min at 60 °C and washed with 0.1 M HCl, distilled water, and ethanol and dried at reduced pressure. Subsequently, the PET microchannel was first incubated in PSS for 30 min followed by rinsing with water. In the next step, PDDA (3 mg/mL, containing 0.13 M sodium chloride and 0.005 M ammonia) was adsorbed for 30 min and the charge polarity on the surface of the film was reversed by the adsorption of a layer of nanozeolite followed by rinsing with water. After the three layers of PDDA/nanozeolite assembly had been deposited, the film was dried at room temperature under reduced pressure. The nanozeolite-modified PET chip was soaked in 5 mg/mL trypsin solution containing 50 mM Tris−HCl buffer (pH 8.0) and 20 mM $CaCl_2$ at 4 °C overnight. Subsequently, the channel of the PET microchip was sufficiently washed by water.

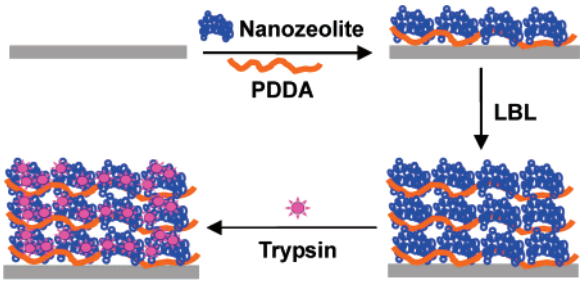
Characterization of the Trypsin-Containing Nanozeolite-Assembled Microchannel. *Scanning Electron Micrographs (SEM) Measurement.* SEM images of the unmodified and multilayer-assembled PET microchannel surfaces were obtained with a Philips XL-30 scanning electron microscope (Philips, Ltd., The Netherlands).

Quartz Crystal Microbalance (QCM) Measurement. The controlled immobilization of trypsin in well-interconnected nanozeolite film was performed with a QCM analyzer (CHI440, CH Instruments, Shanghai, China) and quartz crystals (8 MHz) sandwiched between two Au electrodes (area 0.196 cm^2). The QCM resonator was cleaned in a mixed solution of H_2SO_4 and H_2O_2, followed by rinsing with bidistilled water and alcohol. Then the resonator was immersed into a PSS solution to form a negatively charged surface. PDDA, nanozeolite, and trypsin solution were assembled on the gold surfaces. The adsorption time for each deposition step was 1 h to get to adsorption equilibrium. After each layer deposition, the resonator was taken out, thoroughly rinsed with pure water, and dried by a N_2 blast and then the frequency shift was measured. The QCM frequency change in air was measured.

Proteolytic Efficiency Assay of the Immobilized Trypsin. The activity of the encapsulated enzyme in a microchip was examined by pumping BAEE (BAEE = α-*N*-benzooyl-L-arginine ethyl ester) solution (5−20 mM) in a 50 mM Tris−HCL buffer (pH 8.0) using a 74900 series syringe pump (Cole-Parmer Instrument Co.) through the trypsin-adsorbing PET channel at a flow rate of 100 μL/h. The resulting products were analyzed by a capillary electrophoresis system (P/ACE System 5000, Beckman). The capillary electrophoresis procedure was run in a phosphate buffer solution (pH 2.5) at the temperature of 15 °C with UV detection at 214 nm. The reaction rate was calculated via the flow rate and absorbance difference. Furthermore, in order to further examine the effects of the $(PDDA/nanozeolite)_n$ assembly on the digestion efficiency, BSA as a substrate was digested using the nanozeolite-derived reactors with different assembled layers.

On-Chip Standard Protein Digestion and Identification. Protein solutions of BSA and cyt-c in 10 mM NH_4HCO_3 buffer solution (pH 8.13) were driven through the nanozeolite-modified PET microchannel by a syringe pump at a flow rate of 120 μL/h. Effluents accumulated in the reservoir were collected and then identified by MALDI-TOF MS. All MS experiments were performed on a 4700 Proteomics Analyzer (Applied Biosystems, Framingham, MA). Each volume of 0.5 μL sample solution was dropped on the MALDI plate. After the solvent evaporated, a

Scheme 1. Schematic Illustration of the Microfluidic Chip Coating Protocol Based on the Alternate Deposition of PDDA and Zeolite Nanocrystals



volume of 0.5 μL matrix solution (4 mg/mL α-cyano-4-hydroxycinnamic acid dissolved in 50% aqueous acetonitrile (CAN)/0.1% trifluoroacetic acid was dropped on the dried samples. The MS instrument was operated at an accelerating voltage of 20 kV. A 200 Hz pulsed Nd:YAG laser at 355 nm was used. Before the samples were identified, the MS instrument was calibrated with an external calibration mode by tryptic peptides of myoglobin. All spectra were taken from signal averaging of 800 laser shots with the laser intensity kept in a proper constant. *GPS Explorer* software from Applied Biosystems with *Mascot* as a search engine and *SwissProt* (version of 050303) as a database were used to identify proteins. All proteins were identified using the peptide fingerprint mass spectra combined with tandem mass spectra. The peptide mass tolerance was set to 80 ppm, and the tandem mass tolerance was set to 0.5 Da.

Digestion of the Cell Extracts. The digestion of proteins extracted from murine alveolar macrophage cell line AMJ2-C8 by immobilized trypsin-nanozeolite microreactor was performed with the same procedure as that of standard proteins. Approximately 50 ng of digested peptide mixture was collected. Two-dimensional liquid chromatography (SCX-RPLC) separation and tandem mass spectrometry conditions as described by Washburn et al. were used for the analysis.[24] Mass measurements were performed with a 7 T hybrid quadrupole (Q)-FT-ICR mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with an Apollo electrospray ion source. The ESI flow rate was 50 μL/h, and nebulization was assisted by N_2 gas.

RESULTS AND DISCUSSION

Trypsin Accommodating Nanozeolite-Assembled Microchip Reactor. This research is to develop a general protocol for on-line protein digestion in a PET microchip and primarily to assess its effective capability. Schematic illustration of the microreactor with multilayer-assembled zeolite nanoparticles for the adsorption of trypsin is shown in Scheme 1. The employed β-nanozeolite is a type of alumino-silicate nanocrystal with a diameter of 80 nm. Figure 1 displays the microchannels and their surface morphologies without and with $(PDDA/nanozeolite)_n$ (n = 3) assembly. Obviously, the uniform nanozeolite-modified layers compactly adhere to the surface of channel which is important for the long holding of the enzyme in the microchip.

It is well-known that the performance of a microreactor through a nanoparticle-modified microchip surface is dependent upon the

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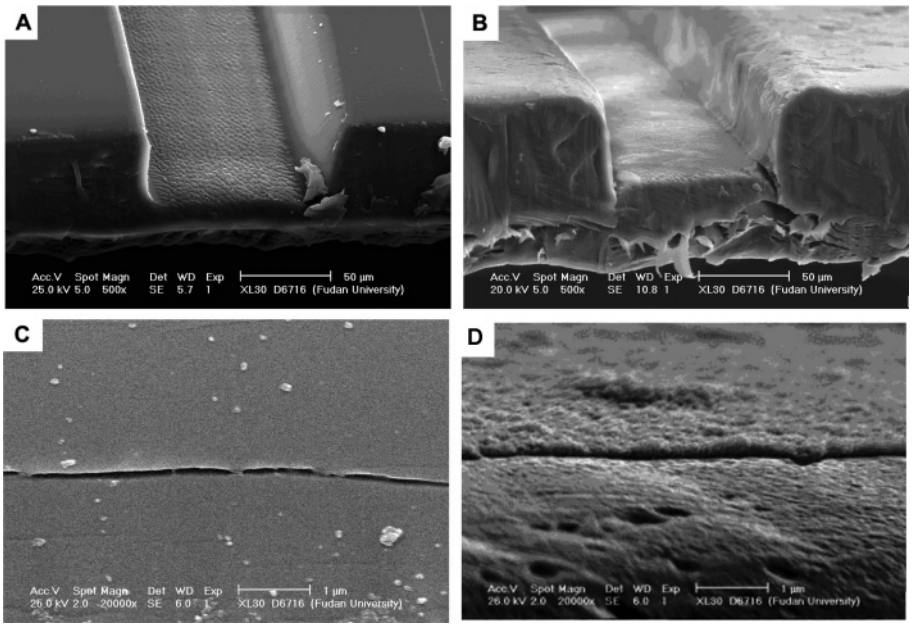

Figure 1. SEM images of the microchannel (A and B) and its side face (C and D). A and C are the unmodified microchip. B and D are the microchannels with (PDDA/nanozeolite)$_n$ ($n = 3$) assembled layers.

physicochemical characteristics of nanoparticles and their interactions with the guest species. Adsorption isotherm is a measurement of the relationship between the equilibrium concentrations of bound and free guest over a certain concentration range. In order to get a better understanding of the behavior of enzyme adsorption onto the multilayer-assembled nanozeolites, adsorption processes monitored by QCM measurements have been modeled. The QCM measurements were performed on the modified QCM resonators to determine the resulting surface coverage of trypsin that corresponds to each enzyme solution concentration. The Langmuir adsorption equation, which relates the concentration of analyte adsorbed on the surface Γ to the one in solution C at equilibrium, is expressed by eq 1[25]

$$\frac{\Gamma_{eq}}{\Gamma_{max}} = \frac{KC^{\circ}}{1 + KC} \tag{1}$$

where Γ_{eq} is the surface concentration at equilibrium, Γ_{max} is the initial concentration of the active sites, K is the thermodynamic constant of adsorption, and C° is the initial concentration of the solution. The adorption isotherm equation (eq 1) is linearized as follows:

$$\frac{C^{\circ}}{\Gamma_{eq}} = \frac{1}{K\Gamma_{max}} + \frac{C^{\circ}}{\Gamma_{max}} \tag{2}$$

The experimental isotherm of trypsin adsorption is shown in Figure 2, with the linearized isotherm in the inset. Reporting C°/Γ_{eq} versus C°, Γ_{max} and K are provided as the respective reciprocals of the slope and the intercept, giving the fitted values $\Gamma_{max} = 1.4 \times 10^{-6}$ mol·m^{-2}. This maximum surface coverage means that

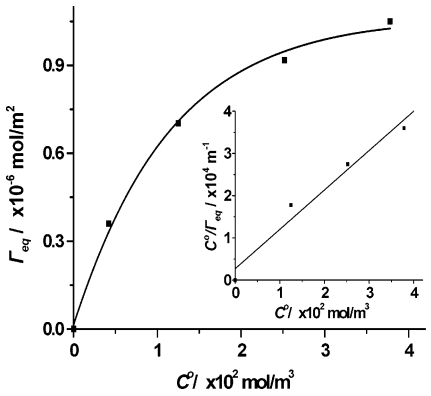

Figure 2. Isotherm of trypsin adsorption in the nanozeolite-assembled PET microchannel obtained from the QCM experimental results. Inset: Linearization of the adsorption isotherm.

there is one active enzyme molecule in only per 1.2 nm^2 area of microchannel if the enzyme immobilized in the nanozeolite networks is spread into an equivalent compact monolayer. This result clearly shows that the nanozeolite-assembled network provides a large surface-to-volume ratio favoring the high enzyme loading.

The reaction efficiency of the immobilized trypsin with different layers of nanozeolite-modified microreactor is quantitatively testified by the values of the maximum velocity (V_{max}), which is derived from a linearized form of the Michaelis−Menten equation: $[S]/v = (K_m)/V_{max} + (1/V_{max})[S]$. The plot of kinetic characteristics for the conversion of BAEE in the microreactors prepared by different layers of PDDA/nanozeolite is shown in Figure 3a (dotted line). As can be seen, trypsin immobilized in the (PDDA/nanozeolite)$_n$ ($n = 3$) matrix possesses the higher V_{max} value, showing that a bioreactor with three nanozeolite-modified layers has a faster tryptic reaction. On account of the value of V_{max}, the bioreactor with three PDDA/nanozeolite-modified layers has the optimum performance for protein digestion. Combined with QCM measurements for the amount of trypsin adsorbed on the nanozeolite

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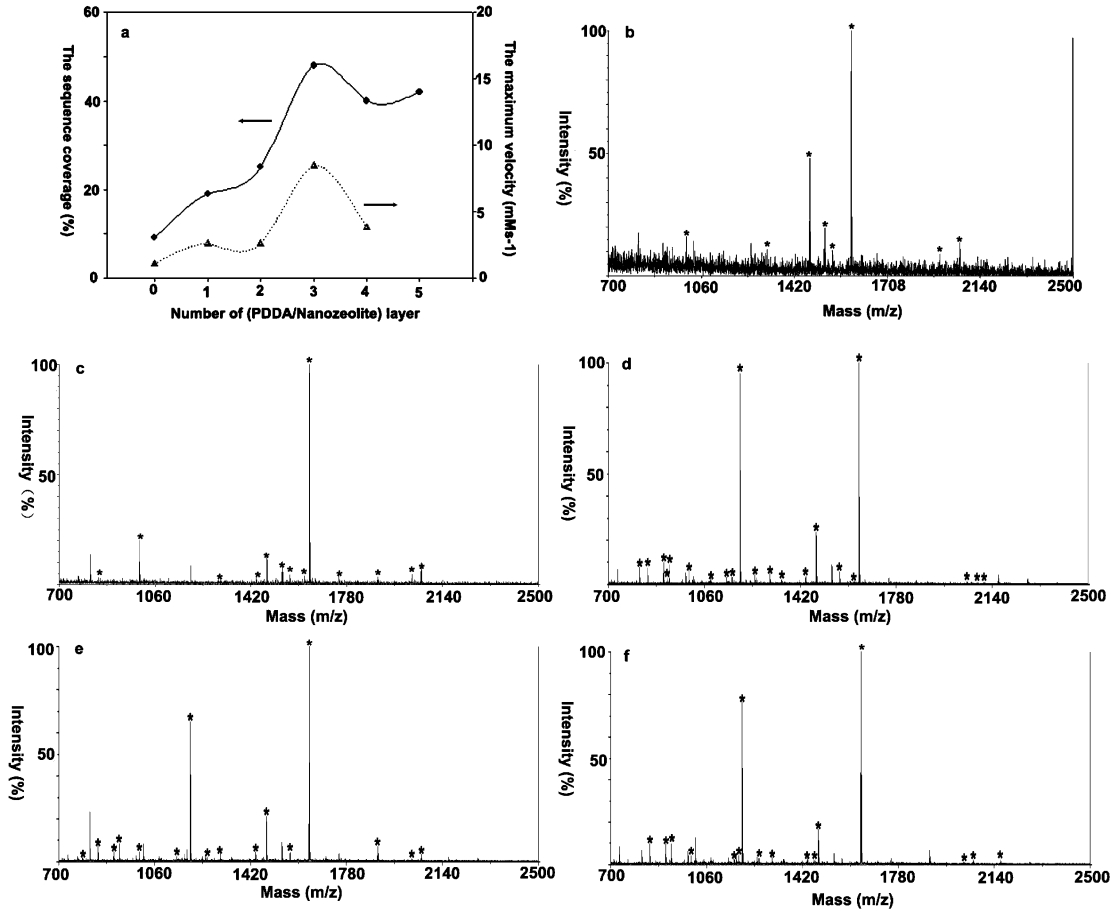

Figure 3. (a) Maximum velocity (V_{max}) (▲, dotted line) of immobilized trypsin in the different (PDDA/nanozeolite)$_n$ assembled layers within the PET microchannel which was obtained from the digestion of BAEE (5−20 mM) and the tendency of the amino acid sequence coverage (•, solid line) according to the spectra (b−f). (b-f) MALDI-TOF mass spectra of digests of 200 ng/μL BSA in the microreactors with different (PDDA/nanozeolite)$_n$ assembled layers (b, $n = 1$; c, $n = 2$; d, $n = 3$; e, $n = 4$; f, $n = 5$) at the flow rate of 120 μL/h. Eluent/matrix ratio is 1:1.

surface, the biocatalytic activities of the immobilized enzyme in the reactor and the free enzyme in bulk solution were compared. Enzymes in the bioreactor with three nanozeolite-modified layers showed a V_{max} value of 8.5 mM s^{-1}, i.e. the value of V_{max} per unit trypsin was calculated to be about 350 mM min^{-1} μg^{-1}, much higher than that in solution. It indicates that compared with the free typsin, the biological activity of the enzyme in the confinement is not compromised. Immobilized enzyme is dispersed and uniformly distributed on the surface of channel, which increases its stability, reduces its autolysis, and facilitates reactant accessibility. On the other hand, the significantly increased velocity in the functionalized microchannel might be attributed to a high surface area provided by the well-defined nanozeolite-assembled scaffold for enzyme immobilization. All these addressed issues provide us an incentive to design a robust network to figure out effective protein digestion. In order to further examine the effects of the (PDDA/nanozeolite)$_n$ assembly on the tryptic efficiency, BSA as a substrate protein was digested using the nanozeolite-derived reactors with different assembled layers as shown in Figure 3b−f. The results show that the numbers of identified peptides increase with increasing layers for the first three layers ($n = 1, 2, 3$) and gradually no significant difference from 3 to 5 layers derived reactors is observed (Figure 3a, solid line). Twenty-one peptides have been identified in the reactor with (PDDA/nanozeolite)$_n$ ($n = 3$) assembly, while only eight and four peptides can be obtained by using the one-layered reactor and unmodified PET microchannels, respectively. Therefore, we select the microchip reactor assembled with three nanozeolite-assembled layers for the following protein digestion.

Efficient On-Chip Digestion of Proteins. This work is to design an on-line proteolytic microreactor, which is well-suited for the mapping of proteins, followed by the identification of the resulting peptides using MALDI-TOF MS. The availability of this as-prepared system for protein digestion was investigated by using the standard proteins and the complex extracts from mouse macrophage as substrates, respectively.

Standard Proteins. BSA with a concentration of 100 ng/μL was incubated through the miniaturized microchip reactor at a flow rate of 120 μL/h, which afforded a very short residence time of less than 5 s. The effluents were analyzed by MALDI-TOF MS for the identification of digested fragments. Nineteen tryptic peptides were identified from BSA, corresponding to the amino acid sequence coverage of 44%, as shown in Figure 4a. As a comparison, the same BSA sample was digested in solution with an incubation time of 6 h and the result is shown in Figure 4b. Twelve tryptic peptides were assigned with corresponding amino acid sequence coverage of 26%. Thus, trypsin entrapped in the nanozeolite-coated PET microchannels could entail a good biocatalyst and show a more rapid reaction rate.

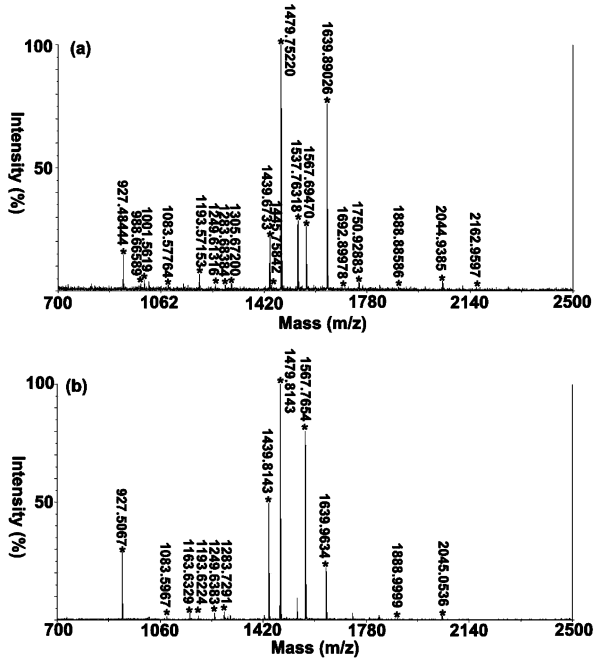

Figure 4. MALDI-TOF mass spectra of digests of 100 ng/μL BSA (a) using a nanozeolite-modified microchip reactor with a flow rate of 120 μL/h and (b) in solution with free trypsin. Eluent−matrix ratio is 1:1.

Low-Concentration Proteins. Desirable proteomic objectives include extending the detection to low-level proteins. The features of both good bioactivity preserved and ultrafast reaction of the nanozeolite-assembled trypsin microreactor are very favorable for the analysis of proteins at low concentration, which is the key problem encountered in proteomics. With the use of the same aforementioned steps within the microreactor, 14 fragments of peptides were identified from 5 ng/μL BSA corresponding to the amino acid sequence coverage of 23%, while only 8 tryptic peptides were observed in solution digestion (Figure 5a). Peptide mass fingerprinting (PMF) of 2 ng/μL cyt-c digestion is shown in Figure 5b, and 14 fragments of peptides were obtained. With the continuous decrease of the concentration of cyt-c down to 0.5 ng/μL (16 fmol), 11 peptides with the sequence coverage of 47% were still obtained, as shown in Figure 5c. However, no peptide could be observed for 0.5 ng/μL cyt-c after digestion in solution (Figure 5c inset). This implies that the nanozeolite-derived microreactor is robust for the sensitive analysis of proteins at low concentration levels.

Complex Proteins Extracted from Mouse Macrophages. With the current drive to characterize the more comprehensive pattern, we apply complex protein mixtures from macrophage cells to evaluate the performance of the as-prepared microchip reactor. Macrophages are distributed throughout the human body. They are involved in several important biological processes, and their functions are tightly regulated. Many studies have demonstrated the crucial role played by macrophages in complex and multifactorial human diseases, such as atherosclerosis, diabetes, and inflammatory diseases.[26] Proteomic analysis takes on the burden of elucidating cellular processes.[27−30] The protein extracts (2 μg/μL) were on-line digested through the microchip reactor at the flowing rate of 100 μL/h, and the effluents were followed to be separated and identified by SCX-RPLC-ESI-MS MS with a 135 min

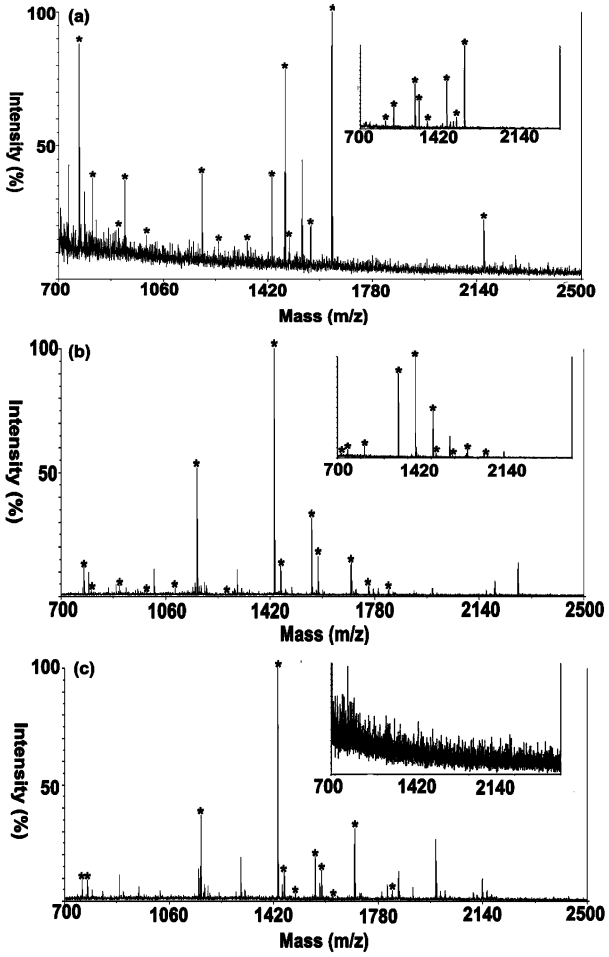

Figure 5. MALDI-TOF mass spectra of digests of (a) 5 ng/μL BSA, (b) 2 ng/μL cyt-c, and (c) 0.5 ng/μL cyt-c in nanozeolite-modified microchip reactor with a flow rate of 120 μL/h. The inset spectra in a−c are those of their corresponding digestions in solution, respectively. Eluent/matrix ratio is 1:1.

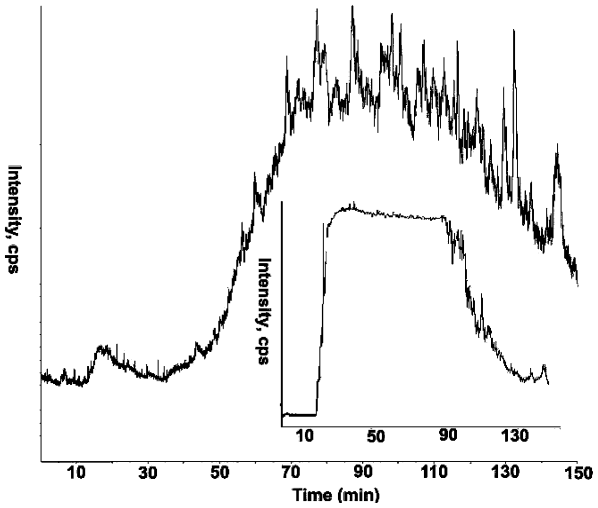

Figure 6. Base peak chromatogram for the separation of digests of murine macrophage proteins from a microchip reactor with a flow rate of 100 μL/h, and the inset is the corresponding one from the in-solution protocol for 12 h.

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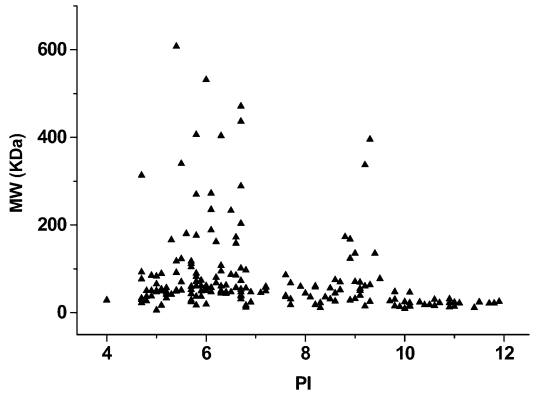

Figure 7. pI distribution of the identified proteins as a function of molecular weight.

gradient elution. The base peak chromatogram for separation of the resulting digests by using SCX-RPLC is displayed in Figure 6. Numerous peaks could be observed in Figure 6, which indicates that the protein mixture is efficiently digested in the nanozeolite/PDDA multilayer-assembled enzymatic microchip. The acquired MS/MS spectra were searched using a *SwissProt* database using *Mascot* MS/MS search software. And a total of 584 unique peptides were identified, which corresponds to 191 distinct proteins (Table SI-1, Supporting Information). The molecular weight of the majority of identified proteins was between 10 000 and 200 000, and their isoelectric points (pI) ranged from 4 to 12 (Figure 7). As an example, the PMF spectrum of membrane-organizing extension spike protein and the MS/MS spectrum of the m/z 903.45 are shown in Figure SI-1 (Supporting Information). For comparison, the chromatogram from the bulk solution digestion of the sample is shown in the Figure 6 inset, and 160 unique proteins are identified with the sample digested in solution for 12 h. The results illustrate that the digestion occurring in the microreactor is not compromised and the consuming time is significantly shortened. Therefore, it is evident that the nanozeolite-assembled microfluidic reactor is efficient for the digestion of proteins from real biological samples.

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CONCLUSION

This research exhibits a simple surface modification protocol in PET microchips for enzyme immobilization and efficient protein digestion. On account of the biocompatible and hydrophilic interface self-assembled by the alternate deposition of zeolite nanocystals and PDDA, trypsin was concentrated in the confined microchannel and the time-consuming sample digestion procedure was significantly shortened. The detection limit can reach low femtomole amounts, and 0.5 μg/mL cyt-c is successfully identified per analysis. Such an on-line microchip reactor coupled to MALDI-TOF-MS/MS or two-dimensional LC-ESI-MS/MS systems was employed to monitor the complex protein extracts from mouse macrophages, and 191 proteins were identified. According to the requirements for the systematic identification and characterization of all the proteins expressed in a cell, future studies can in turn lead to a more targeted strategy to optimize the conditions for large-scale all-protein analysis and development of the automatic platform.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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