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Microchip bioreactors based on trypsin-immobilized graphene oxide-poly(urea-formaldehyde) composite coating for efficient peptide mapping

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ABSTRACT

Trypsin was covalently immobilized to graphene oxide (GO)-poly(urea-formaldehyde) (PUF) composite coated on the channel wall of poly(methyl methacrylate) microchips to fabricate microfluidic bioreactors for highly efficient proteolysis. A mixture solution containing urea-formaldehyde prepolymer and GO nanosheets was allowed to flow through the channels. The modification layer on the channel wall could further polycondense to form GO-PUF composite coating in the presence of ammonium chloride. The primary amino groups of trypsin could react with the carboxyl groups of the GO sheets in the coating with the aid of carboxyl activating agents to realize covalent immobilization. The feasibility and performance of the novel GO-based microchip bioreactors were demonstrated by the digestion of bovine serum albumin, lysozyme, ovalbumin, and myoglobin. The digestion time was significantly reduced to less than 5 s. The obtained digests were identified by MALDI-TOF MS with satisfactory sequence coverages that were comparable to those obtained by using 12-h in-solution digestion. The present proteolysis strategy is simple and efficient, offering great promise for high-throughput protein identification.

1. Introduction

Proteomics is considered to be the next step after genomics in the study of biological systems. One of the most important tasks of proteomics is to develop high throughput approaches to separating and identifying a large number of proteins from a wide variety of biological sources [1,2]. In proteome research, protein digestion is an important procedure prior to subsequent mass spectrometry (MS)-based peptide mapping [3]. The typical time of the commonly used in-solution proteolysis is in the range of several hours to half a day [4,5]. It is incompatible with the high-throughput identification of proteins. Moreover, the autolysis of proteases generates some peptide fragments that may interfere with the identification of the target proteins. To solve the problems of in-solution proteolysis, proteases were immobilized on various particles, fibers, and the inner surface of microchannels to minimize their autolysis and to increase the amount of proteases during heterogeneous proteolysis [6–8].

Since the pioneering work of Manz and Harrison, microfluidic chips have received more and more attentions owing to their minimal sample/reagent consumption, high performance, portability and high degree of integration [9,10]. Microfluidic devices are powerful platforms for handling small-volume samples (nL to μL) in microchannels to perform enzymatic reactions [11], immunoassay [12], etc. Microfluidic chips can dramatically change the speed and scale of biomedical analysis and should find a wide range of applications in protein identification [13].

Proteases have been immobilized in the channels of microchips by sol–gel encapsulation [14], covalent linking [15], and adsorption [16] to fabricate microfluidic bioreactors for the rapid digestion and identification of proteins in combination with MS techniques. Because the enzymes were immobilized in microchannels, they became much more stable and highly resistant to environmental changes, providing molecular-level interactions between the immobilized proteases and the flowing protein samples. In addition, the autolysis of proteases and the amount of the interfering peptides in the digests were minimized [8].

Since Novoselov and Geim successfully isolated graphene in 2004, it has attracted tremendous scientific and technological attention because of its unique nanostructure and properties [17,18]. Graphene is an important allotrope of carbon with a two-dimensional nanostructure of sp^2-bonded carbon atoms that are arranged in a chicken wire or honeycomb pattern [19,20]. It indicates great promise for a variety of applications such as electronics, sensors, drug delivery, batteries, fuel cells, solar cells,

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supercapacitors, hydrogen storage and functionalized materials because of its excellent electrical and thermal conductivity, strong mechanical strength, and high surface area [21–29].

As a chemical precursor of graphene, graphene oxide (GO) is basically a single atomic layer of carbon covered with epoxy, hydroxyl, carbonyl and carboxyl groups [30]. It can be facilely prepared by chemical oxidation of graphite and subsequent sonication exfoliation. GO has been employed to prepare nanocomposites [31], chemically modified graphenes [32], antibacterial paper [33], and conjugate with proteins [34]. Because hydrophilic GO can be well dispersed in aqueous solution and should find a wide range of applications in the fabrication of microchip bioreactors for highly efficient proteolysis. Proteases can be immobilized in microchips via GO-based materials assembled in the channels. Recently, we immobilized trypsin in the layer-by-layer coating of GO and chitosan on glass fibers by adsorption to fabricate in channel fiber bioreactors for efficient protein digestion [35]. Because GO bears a great amount of carboxyl groups that can be employed to immobilize trypsin via amide bonds, it indicates great promise for the fabrication of microfluidic bioreactors.

As a thermosetting polymer, poly(urea-formaldehyde) (PUF) is made from urea and formaldehyde by polycondensation [36]. To prepare PUF, urea and formaldehyde are usually allowed to react in basic mediums to produce water-soluble prepolymer solution. It can further polycondense to form water-insoluble crosslinked PUF network with the aid of curing catalysts such as ammonium chloride [37,38]. As a reactive polymer mixture, urea-formaldehyde prepolymer solution can be employed to prepare GO-based functionalized materials.

In this work, trypsin was covalently immobilized to the GO-PUF composite coating on the channel walls of poly(methyl methacrylate) (PMMA) microchips for efficient proteolysis. The primary amino groups of trypsin were linked to the carboxyl groups of the entrapped GO sheets in the coating via amide bonds to realize covalent immobilization. Moreover, the novel bioreactors were combined with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the efficient digestion and peptide mapping of bovine serum albumin (BSA), lysozyme (LYS), ovalbumin (OVA), and myoglobin (MYO). The fabrication details, characterization, feasibility, and application of the novel microchip bioreactors are reported in the following sections.

2. Experimental

2.1. Reagent and solutions

Ammonium bicarbonate (NH_4HCO_4), urea, formaldehyde, concentrated ammonia (28%), ammonium chloride, acetonitrile (ACN), graphite powder, sodium nitrate, potassium permanganate, and sulfuric acid (98 wt%) were all purchased from SinoPharm (Shanghai, China). BSA from bovine blood, LYS from chicken egg, MYO from horse heart, OVA from chicken egg, trypsin from bovine pancreas, 4-morpholinoethanesulfonic acid (MES), N-hydroxysuccinimide (NHS), trifluoroacetic acid (TFA), and α-cyano-4-hydroxycinnamic acid (CHCA) were supplied by Sigma (St. Louis, MO, USA). All aqueous solutions were prepared in doubly distilled water. Other chemicals were all analytical grade. The stock solutions of BSA, LYS, OVA, and MYO (1 mg/mL each) were prepared in water and were denatured in a 95 °C water bath for 15 min.

2.2. Fabrication of poly(methyl methacrylate) (PMMA) microchip

The PMMA microchips (16 mm × 75 mm × 2.5 mm) used in this work had a simple cross layout. They consisted of a 67 mm-long main channel and a 10 mm-long injection channel. The channels had a trapezoidal cross section with a top width of ~100 μm, a bottom width of ~40 μm, and a depth of ~35 μm. The channel plates of the PMMA microchips were fabricated by in-situ surface polymerization using a silicon template. It was sealed with PMMA cover plates (16 mm × 75 mm × 1 mm) by plasticizer-assisted

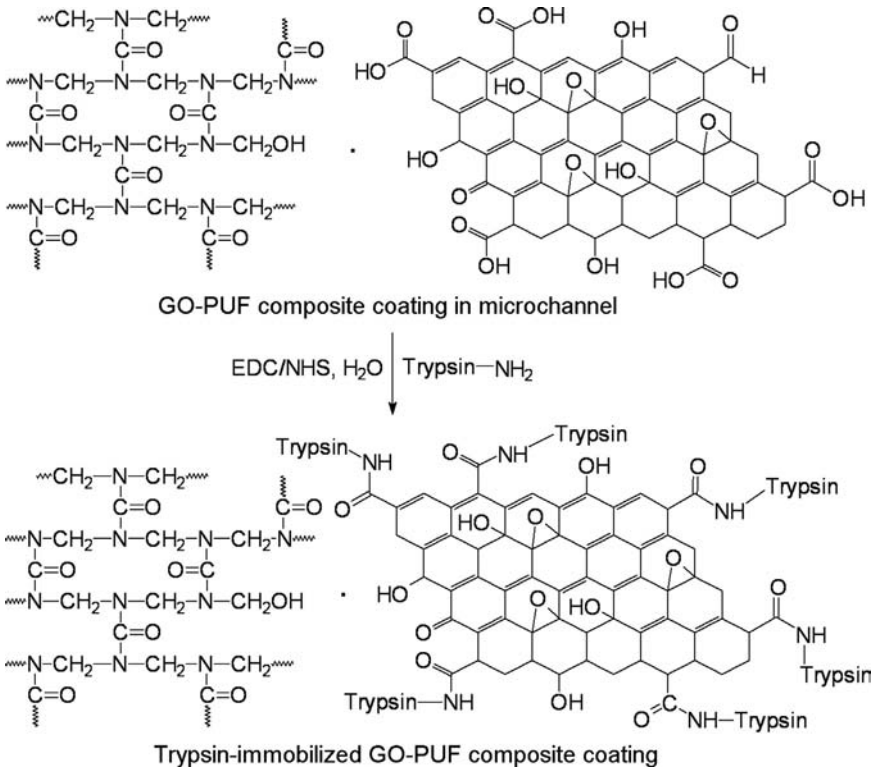

Fig. 1. Reaction routes for the immobilization of trypsin to GO sheets in GO-PUF composite.

bonding approach to form complete PMMA microchips [39]. Prior to use, the channel of the PMMA microchip was flushed with 0.1 M NaOH aqueous solution and doubly distilled water for 10 min each.

2.3. Preparation of graphene oxide (GO) solution

Oxidized graphite (OG) was prepared by the oxidation of graphite using potassium permanganate and sodium nitrate based on a modified Hummers method [28]. To prepare GO solution (3 mg/mL), 150 mg OG powder was dispersed in 50 mL water and sonicated in an ultrasonic cleaner (SKQ-2200, frequency 56 kHz, 100 W, Shanghai Sonxi Ultrasonics Instrument Co., Ltd., Shanghai, China) for 1 h to exfoliate OG particles to GO sheets.

2.4. Preparation of urea-formaldehyde prepolymer solution

Concentrated ammonia (28% (w/w), about 1.5 mL) was added to a volume of 35 mL of formaldehyde aqueous solution (37% (w/w)) to adjust pH to be 7.5–8.0. After 11.4 g urea was dissolved in the solution, the mixture was heated in a 60 °C water bath for 15 min. Subsequently, 0.6 g additional urea was dissolved in the solution. The reaction was allowed to continue at 95 °C for 1 h to obtain urea-formaldehyde prepolymer solution The solid content in the solution was approximately 50% (w/v). Supplementary Fig. S1 displays the reaction routes for the preparation of the urea-formaldehyde prepolymer.

2.5. Preparation of GO-PUF coating on the channel wall

Prior to channel modification, 200 μL of urea-formaldehyde prepolymer solution was mixed with 5 mL of 3 mg/mL GO solution with the aid of sonification. Subsequently, the prepared mixture solution was injected into the channel with a syringe at a flow rate of 2 μL/min for 2 min and was allowed to stay in the channel for 5 min. After the filled solution was withdrawn, the microchip was stored at room temperature for a minimum of 3 h. Finally, the channel was filled with 0.4 M ammonium chloride aqueous solution for 10 min. As illustrated in Fig. S1, the urea-formaldehyde prepolymer on the channel wall cured in the presence of ammonium chloride to form GO-PUF composite coating.

2.6. Immobilization of trypsin

To immobilize trypsin in the GO-containing coating, 50 mM MES buffer (pH 6.1) containing 10 mM EDC and 5 mM NHS was continuously pumped into the channel at a flow rate of 1 μL/min for 15 min at room temperature to activate the carboxyl groups of GO. After rinsing with water (1 μL/min, 5 min), the channel was filled with 2 mg/mL trypsin solution prepared in a 50 mM MES buffer (pH 6.1) containing 10 mM $CaCl_2$ at a 4 °C for 6 h. The non-covalently adsorbed trypsin was removed by flushing with 50 mM MES buffer (pH 6.1) at a flow rate of 2 μL/min for 10 min. The prepared microchip bioreactors were stored in a 4 °C refrigerator. The surface morphologies of GO sheets, PMMA plate, and trypsin-immobilized GO-PUF composite coating were measured by using a scanning electron microscope (PHILIPS XL 30, Eindhoven, The Netherlands).

2.7. On-chip protein digestion and MS identification

The proteolysis system used in this work consisted of a syringe pump and a GO-based microchip bioreactor. At 37 °C, the sample solution of BSA (20 and 200 ng/μL), LYS (20 and 200 ng/μL), OVA (200 ng/μL), and MYO (200 ng/μL) in 20 mM NH_4HCO_3 buffer solution (pH 8.1) were driven through the channel of the microchip bioreactor by the syringe pump at a flow rate of 2 μL/min for 2 min via the hole near the cross, while the other two holes beside the main channel were sealed with small silicone rubber stoppers. Effluents (~4 μL) accumulated in the product hole on the microchip were collected by a pipette and then identified by MALDI-TOF MS. After each digestion, the holes connected with the inlet and outlet of the main channel needed to be rinsed with water. And then, the channel was flushed with 20 mM NH_4HCO_3 buffer solution (pH 8.1) (2 μL/min, 5 min). All MS experiments were performed on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA). Prior to MALDI-TOF-MS analysis, 0.5 μL of each digest was spotted on a MALDI plate. After the solvent evaporated, 0.5 μL of matrix solution (4 mg/mL CHCA dissolved in 50% aqueous acetonitrile containing 0.1% trifluoroacetic acid) was dropped on the dried samples. The MS instrument was operated at an accelerating voltage of 20 kV. A 1000-Hz pulsed Nd:YAG laser at 355 nm was used. Prior to use, the MS instrument was calibrated with the tryptic digest of MYO in an internal calibration mode. Proteins identification was

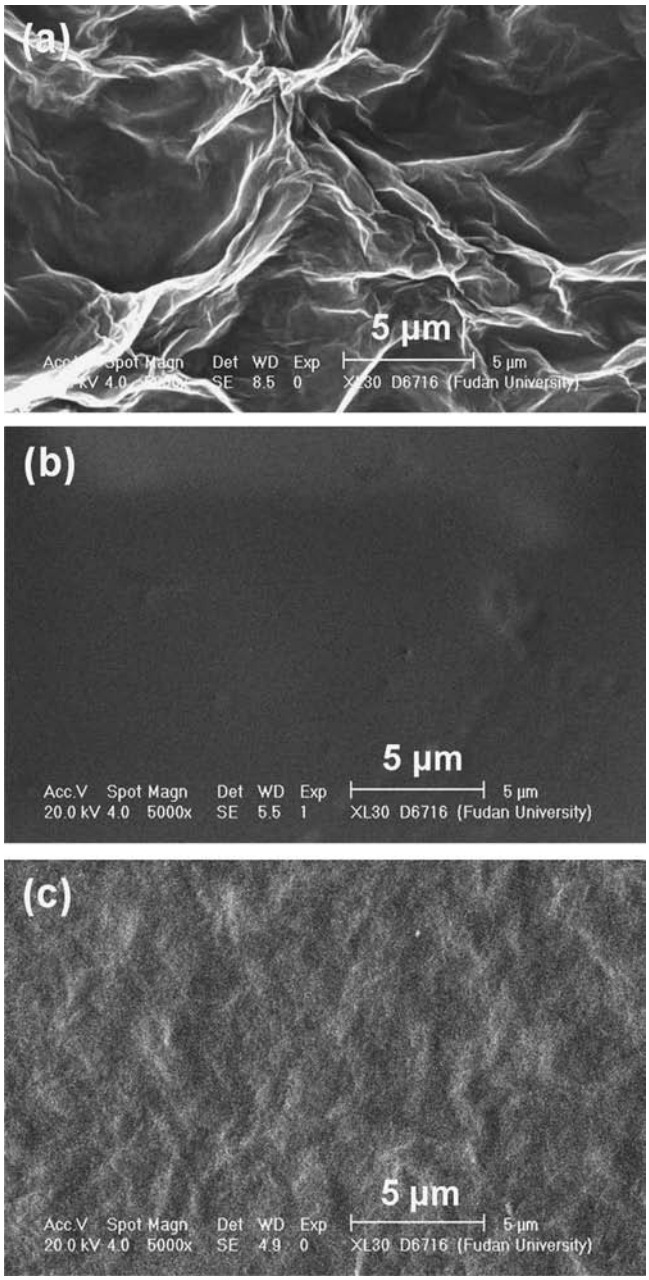

Fig. 2. SEM images (a) GO, (b) PMMA plate, and (c) trypsin-immobilized GO-PUF composite coating on a PMMA plate. Magnification, 5000 ×.

performed by using MASCOT, a web-based tool for predicting protein sequences from peptide mass fingerprinting (PMF) spectra and MS/MS data (www.matrixscience.com). The search was done based on the monoisotopic MH^+ mass values of peptides. The peptide mass tolerance was $\pm$100 ppm while the missed cleavages of peptides were allowed up to 1. Other parameters were set as follows: taxonomy, all entries; enzyme, trypsin; fixed and variable modifications, none selected. For comparison, BSA and LYS (200 ng/μL) were also digested by using conventional in-solution proteolysis in 20 mM NH_4HCO_3 (pH 8.1) at 37 °C for 12 h (trypsin-substrate ratio, 1:40 w/w).

3. Results and discussion

Because GO owns high specific surface area and a great deal of carboxyl groups, it is an ideal material for immobilizing trypsin to fabrication proteolysis bioreactors. In this work, GO-PUF composite coating was prepared on the channel wall of PMMA microchips by the in situ polycondensation of urea-formaldehyde prepolymer in the presence of GO sheets. Based on the fact that GO sheets bear abundant oxygen-containing functional groups such as hydroxyl, epoxy, carbonyl and carboxyl groups [30], they are hydrophilic and can be well dispersed in the urea-formaldehyde prepolymer solution to form a stable mixture solution for the modification of channel. As shown in Fig. 1, the carboxyl groups of GO sheets in the composite can be linked to the primary amino groups of trypsin with the aid of EDC and NHS (activating agents) to realize the covalent immobilization of trypsin.

The prepared GO-based microchip bioreactors take the advantages of both the trypsin-immobilized GO-PUF composite coating and microfluidic chips, offering a fast, flexible, and reliable enzymatic methodology for highly efficient proteolysis and other biological applications with enzymes involved. The trypsin-immobilized microchannel offers the possibility to perform microfluidic protein digestion. When proteins were injected in the channel, they interacted with the immobilized trysin to generate peptides. The digests were collected for the subsequent MS identification.

Fig. 2a shows the scanning electron microscopy (SEM) image of GO sheets. As a characteristic profile of graphene sheets, crumpled silk waves-like morphology was observed, indicating that GO sheets were well exfoliated. The trypsin-immobilized GO-PUF composite coating is the crucial part of the present microchip bioreactors. As displayed in Fig. 2c, its surface morphology was much different from those of GO sheets (Fig. 2a) and PMMA plate (Fig. 2b). The roughness of the GO-based coating was much higher than that of pristine PMMA surface, offering an interface with high surface area for the efficient interaction between the immobilized trypsin and proteins. Fig. 2c indicates that the surface morphology of GO sheets still existed after they were embedded in PUF substrate and functionalized by trypsin. The PUF in the composite could adhere GO sheets to form a layer of insoluble film on the channel wall. A great amount of small wrinkles and edges of GO sheets were observed on the surface of the trypsin-immobilized GO-PUF composite coating, indicating GO sheets were well dispersed and connected throughout the composite to form an interconnected GO network.

The feasibility and performance of the prepared microchip bioreactors were demonstrated by the digestion and peptide mapping of several standard proteins in combination with MALDI-TOF MS. Fig. 3a and c illustrates the PMF spectra for the digests of BSA and LYS (200 ng/μL each) obtained by using a microchip bioreactor. The time of the interaction between the protein samples and the immobilized trypsin was estimated to be less than 5 s at a flow rate of 2.0 μL/min based on the volume of

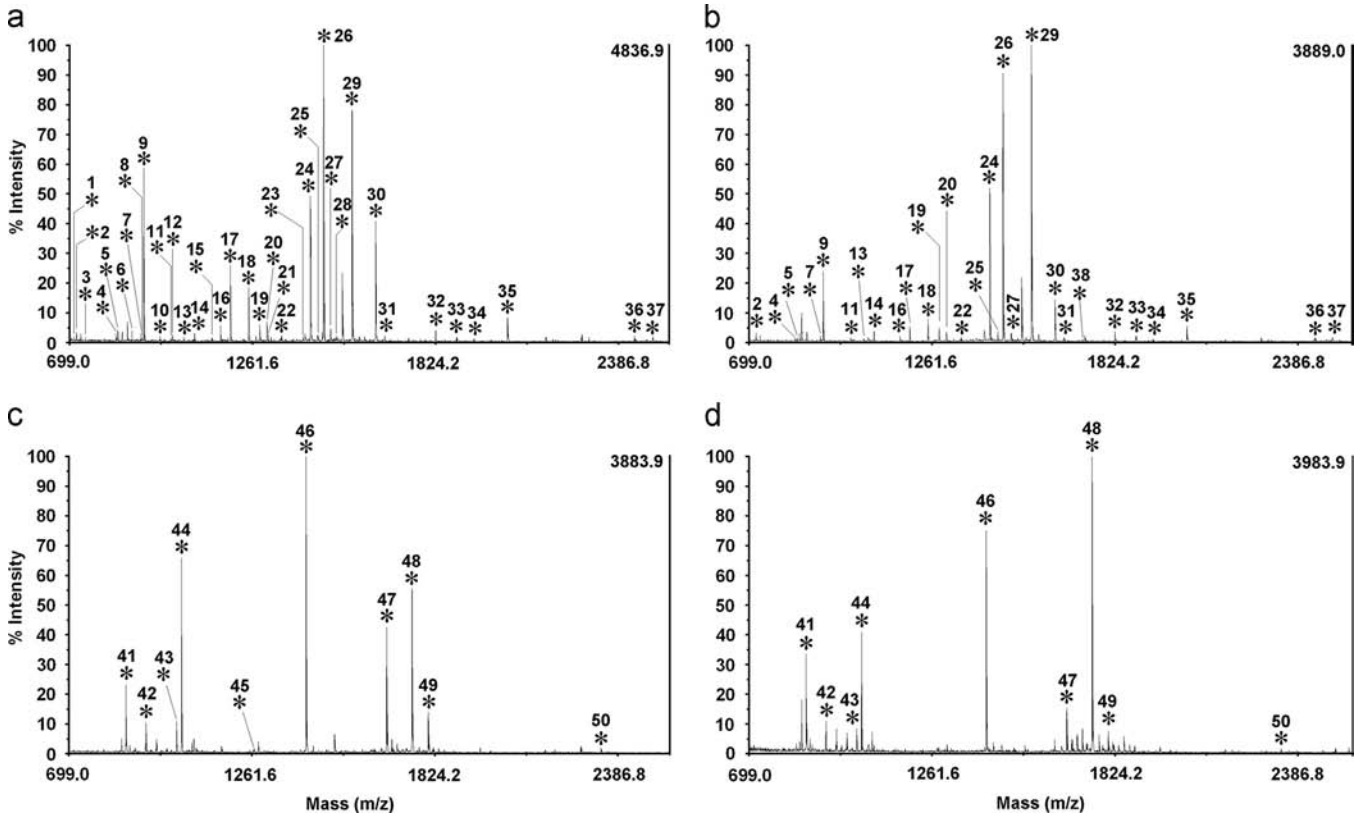

Fig. 3. MALDI-TOF mass spectra for the digests of (a and b) BSA and (c and d) LYS (200 ng/μL each) obtained by using GO-based microchip bioreactors (a and c; flow rate, 2.0 μL/min) and 12-h conventional in-solution proteolysis (c and d; trypsin-substrate ratio, 1:40 (w/w)) in 20 mM NH_4HCO_3 buffer (pH 8.1).

the channel and the flow rate of the sample solutions. All the identified peptides in both digests are presented in Tables 1 and 2. The numbers of the matched peptides and the amino-acid sequence coverages were 37% and 59% for BSA and 10% and 70% for LYS, respectively. In addition, 363 out of the 607 possible amino acids of BSA and 103 out of the 105 possible amino acids of LYS were identified. The results indicated that both protein samples were well digested and positively identified.

For comparison, BSA and LYS (200 ng/μL each) were also digested by using conventional in-solution proteolysis in 20 mM NH_4HCO_3 buffer (pH 8.1) at 37 °C for 12 h. Figs. 3b and d illustrate the mass spectra of the peptides in the obtained digests. The results indicated that 28 and 9 peptides were found matched with the sequence coverages of 47% (identified amino acid, 288) and 61% (identified amino acid, 91) for BSA and LYS, respectively. Tables 1 and 2 summarize the identification results of the digests obtained by using different digestion approaches. The performance of the microchip bioreactor was comparable to that of conventional in-solution digestion while the digestion time was significantly reduced to less than 5 s.

In proteome research, the concentration and amount of proteins in real samples are usually limited. It is of high interest to evaluate lower protein concentration to determine if the chip-based bioreactor would be applicable to low protein amounts. Fig. 4 illustrates the PMF spectra for the digests of BSA and LYS (20 ng/μL each) obtained by using a GO-based microchip bioreactor. All matched peptides were summarized in Tables 1 and 2. When the protein concentration was 20 ng/μL, 24 and 10 peptides were found matched with the amino-acid sequence coverages of 34% (identified amino acid, 209) and 55% (identified amino acid, 82) for BSA and LYS, respectively. Both proteins were positively identified at this lower concentration.

Besides BSA and LYS, the GO-based bioreactor was also employed to digest OVA and MYO (200 ng/mL each) to further evaluate its feasibility and performance. The measured PMF spectra of the digests were illustrated in Supplementary Fig. S2. All the identified peptides in the digests were presented in the insets. Both protein samples were well digested and positively identified. A total of 20 and 11 peptides were identified with the sequence coverages of 58% (identified amino acids, 225) and 67% (identified amino acids, 104) for OVA and MYO, respectively.

To test the stability and reproducibility of the GO-based microfluidic bioreactor, a solution of 200 ng/mL LYS in 20 mM NH_4HCO_3 buffer solution (pH 8.1) was continuously driven

Table 1
Peptide mapping results for the tryptic digests of BSA obtained by using microchip bioreactor and 12-h in-solution digestion in combination with MALDI-TOF MS.

No.	Position	Peptide	Microreactor		In solution (200 ng/μL)
			200 ng/μL	20 ng/μL	
1	29–34	SEIAHR	●[a]	●	–
2	581–587	CCAADDK	●	●	●
3	341–346	NYQEAK	●	–	–
4	483–489	LCVLHEK	●	●	●
5	242–248	LSQKFPK	●	●	●
6	131–138	DDSPDLPK	●	–	–
7	221–228	LRCASIQK	●	–	●
8	249–256	AEFVEVTK	●	–	–
9	161–167	YLYEIAR	●	●	●
10	123–130	NECFLSHK	●	●	–
11	413–420	QNCDQFEK	●	–	●
12	310–318	SHCIAEVEK	●	–	–
13	588–597	EACFAVEGPK	●	●	●
14	161–168	YLYEIARR	●	●	●
15	223–232	CASIQKFGER	●	–	–
16	66–75	LVNELTEFAK	●	●	●
17	25–34	DTHKSEIAHR	●	●	●
18	35–44	FKDLGEEHFK	●	●	●
19	361–371	HPEYAVSVLLR	●	●	●
20	402–412	HLVDEPQNLIK	●	●	●
21	558–568	HKPKATEEQLK	●	–	–
22	76–88	TCVADESHAGCEK	●	–	●
23	298–309	LKECCDKPLLEK	●	●	–
24	360–371	RHPEYAVSVLLR	●	●	●
25	456–468	VGTRCCTKPESER	●	●	●
26	421–433	LGEYGFQNALIVR	●	●	●
27	549–561	QTALVELLKHKPK	●	●	●
28	139–151	LKPDPNTLCDEFK	●	–	–
29	347–359	DAFLGSFLYEYSR	●	●	●
30	437–451	KVPQVSTPTLVEVSR	●	●	●
31	469–482	MPCTEDYLSLILNR	●	●	●
32	508–523	RPCFSALTPDETYVPK	●	–	●
33	89–105	SLHTLFGDELCKVASLR	●	–	●
34	264–280	VHKECCHGDLLECADDR	●	–	●
35	168–183	RHPYFYAPELLYYANK	●	–	●
36	45–65	GLVLIAFSQYLQQCPFDEHVK	●	–	●
37	469–489	MPCTEDYLSLILNRLCVLHEK	●	●	●
38	347–360	DAFLGSFLYEYSRR	–	–	●
39	205–211	IETMREK	–	●	–
40	233–241	ALKAWSVAR	–	●	–
Sequence coverage (%)			59	34	47
Peptides matched matched			37	24	28
Amino acids identified			363	209	288

[a] The matched peptides are labeled with "●".

Table 2
Peptide mapping results for the tryptic digests of LYS obtained by using microchip bioreactor and 12-h in-solution digestion in combination with MALDI-TOF MS.

No.	Position	Peptide	Microreactor		In solution (200 ng/μL)
			200 ng/μL	20 ng/μL	
41	33–39	HGLDNYR	●[a]	●	●
42	80–86	WWCNDGR	●	●	●
43	32–39	RHGLDNYR	●	●	●
44	135–143	GTDVQAWIR	●	●	●
45	40–51	GYSLGNWVCAAK	●	–	–
46	52–63	FESNFNTQATNR	●	●	●
47	116–130	IVSDGNGMNAWVAWR	●	●	●
48	64–79	NTDGSTDYGILQINSR	●	●	●
49	115–130	KIVSDGNGMNAWVAWR	●	●	●
50	92–114	NLCNIPCSALLSSDITASVNCAK	●	–	●
51	133–143	CKGTDVQAWIR	–	●	–
52	40–63	GYSLGNWVCAAKFESNFNTQATNR		●	–
Sequence coverage (%)			70	55	61
Peptides matched matched			10	10	9
Amino acids identified			103	82	91

[a] The matched peptides are labeled with "●".

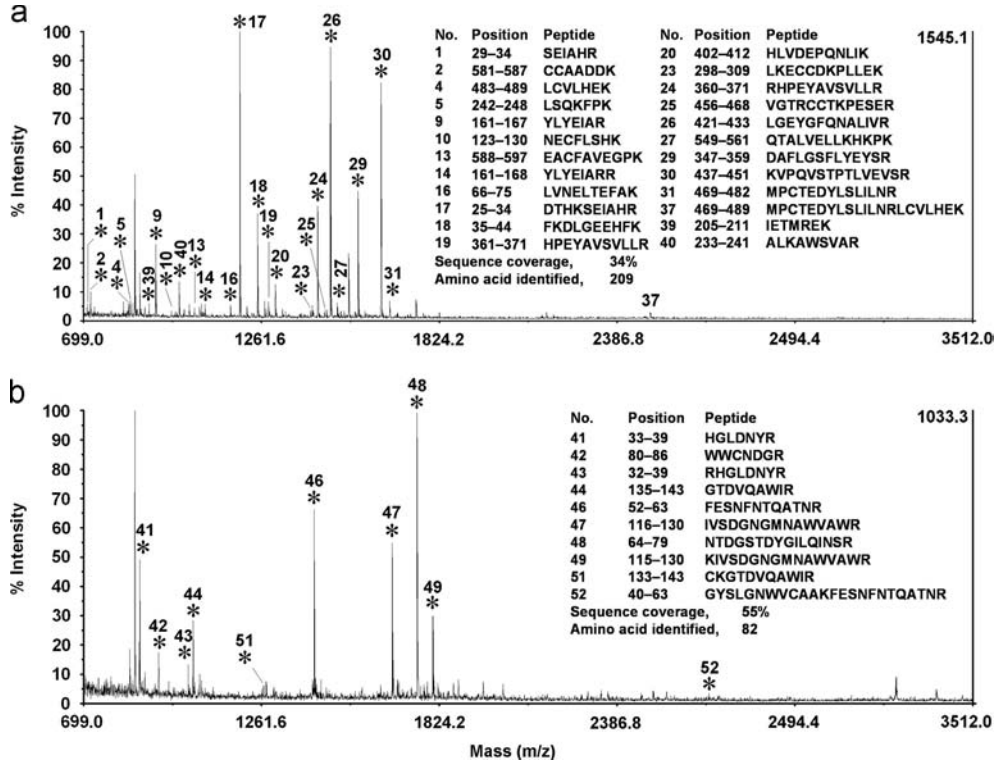

Fig. 4. MALDI-TOF mass spectra of the digests of (a) BSA and (b) LYS (20 ng/μL each) in 20 mM NH_4HCO_3 (pH 8.1) obtained by using GO-based microchip bioreactors at a flow rate of 2.0 μL/min (digestion time, < 5 s; all peptides matched were marked with "*").

through the channel of the bioreactor at a flow rate of 2 μL/min for 5 h. The tryptic digests were collected and analyzed with MALDI-TOF MS every 30 min. The obtained ten PMF spectra (not shown) were identical with the same sequence coverage of 70% except that the peak heights changed to some extent, indicating the satisfactory reproducibility of the GO-based bioreactor. The enhanced stability of trypsin can be attributed to the minimization of autolysis and denaturation in the biocompatible GO-PUF coating, offering a promising platform for highly efficient proteolysis. The satisfactory stability and reproducibility of the microchip bioreactor is crucial for its application in the high-throughput protein analysis of real samples.

To date, a variety of materials have been employed to immobilize trypsin in microfluidic chips for efficient proteolysis [14–16]. Liu et al. coated the channel of a poly(ethylene terephthalate) (PET) microchip with trypsin-containing alumina sol solution. After the coating dried, a layer of trypsin-encapsulated alumina sol–gel network formed on the channel wall. The fabricated microchip bioreactor was applied in the digestion of BSA, cytochrome *c* and biological samples [14]. In 2009, Liu et al. immobilized trypsin in PMMA microchips covalently via silica sol–gel coating to prepare a proteolysis bioreactors. In addition, trypsin was also adsorbed in the charged polysaccharide coating in the channels of PET microchips for efficient proteolysis.

Table 3
The performances of different microchip bioreactors for the digestion of 200 ng/μL BSA.

No.	Immobilized approaches of trypsin	Digestion time (s)	Coverage (%)	Peptides matched	Ease of preparation	Durability	Ref.
1	Encapsulation in alumina sol–gel coating	5	41	22	★★★★★[a]	Excellent	[14]
2	Covalent linking in silica sol–gel coating	10	45	31	★★★★	Good	[15]
3	Adsorption in polymer coating	5	43	28	★★★★	Good	[16]
4	Covalent linking in GO composite coating	5	59	37	★★★★	Excellent	This work

[a] "Five-pointed star" represents the degree of ease.

Table 3 summarizes the performances of the three bioreactors and the present GO-based microreactor for the digestion of 200 ng/μL BSA. Obviously, both the sequence coverage and the number of matched peptides obtained by using the present microchip bioreactors are much higher. In this work, GO was employed to immobilize trypsin covalently on the channel wall of PMMA microchip via its carboxyl groups. GO sheets in the composite offered carboxyl groups for the immobilization of trypsin while PUF acted as a skeleton that adhere the GO sheets together to form a GO-PUF composite. The trypsin-immobilized composite coating illustrated in Fig. 2c owned high specific surface area because it contained GO sheets. The significantly enhanced digestion efficiency of the present microchip bioreactors can be attributed to the high amount of the immobilized trypsin in the channel and the higher surface area of the trypsin-immobilized composite coating, which can increase the frequency of the interaction between trypsin and proteins [14–16]. Among the four immobilization approaches presented in Table 3, encapsulation was the simplest one because trypsin was directly dissolved in the coating solution and modified on the channel wall to prepare bioreactor. As demonstrated above, the stability and durability of the present microreactor was satisfactory because trypsin was covalently immobilized in the biocompatible GO-PUF network.

In this work, BSA, LYS, OVA, and MYO were chosen as model proteins to test the performances of the GO-based microchip bioreactor on the basis of their molecular weight, degree of lipophylicity, isoelectric point, and sources (bovine blood, chicken eggs and horse heart). All the four proteins were well digested and positively identified by using the present bioreactor. However, the measured sequence coverage of each protein (59%, 70%, 58% and 67% for BSA, LYS, OVA and MYO, respectively) was much different at the same concentration of 200 ng/μL. The results indicated that the properties of proteins affected their interaction with the immobilized trypsin and digestion efficiency. For example, the sequence coverages of the higher molecular weight proteins (BSA and OVA) were lower than those of lower weight proteins (LYS and MYO).

4. Conclusions

In summary, GO-PUF composite coating was successfully prepared on the inner surface of microchannels in microchips by the in situ polycondensation of GO-containing urea-formaldehyde prepolymer for the covalent immobilization of trypsin. Insoluble PUF in the composite can adhere GO sheets to form a stable coating with high surface area. The novel microfluidic bioreactors have been employed for the rapid digestion and identification of several standard proteins. The results indicated that the GO-based bioreactors combined with MALDI-TOF MS was a promising strategy for the efficient proteolysis and peptide mapping. The biocompatibility of the GO-PUF coating provided mild environment so that the denaturation and autolysis of the immobilized trypsin was minimized. The unique features of this approach include short digestion time (<5 s) and its minimal sample/reagent consumption. Multiple channel microchips bearing several or even several tens of such channel bioreactors can be fabricated for high-throughput protein analysis. Although GO-PUF composite coating has been employed to immobilize trypsin to prepare microchip bioreactors in this work, it will find more applications in the fabrication of other bioanalytical microdevices.

Acknowledgments

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.08.052.

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