Stable Microstructured Network for Protein Patterning on a Plastic Microfluidic Channel: Strategy and Characterization of On-Chip Enzyme Microreactors

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Chemical modification of a poly(methyl methacrylate) (PMMA) microchannel surface has been explored to functionalize microfluidic chip systems. A craft copolymer was designed and synthesized to introduce the silane functional groups onto the plastic surface first. Furthermore, it has been found that, through a silicon–oxygen–silicon bridge that formed by tethering to these functional groups, a stable patterning network of gel matrix could be achieved. Thus, anchorage of proteins could be realized onto the hydrophobic PMMA microchannels with bioactivity preserved as far as possible. The protein homogeneous patterning in a microfluidic channel has been demonstrated by performing microchip capillary electrophoresis with laser-induced fluorescence detection and confocal fluorescence microscopy. To investigate the bioactivity of enzymes entrapped within stable silica gel-derived microchannels, the suggested scheme was employed to the construction of immobilized enzyme microreactor-on-a-chip. The proteolytic activity of immobilized trypsin has been demonstrated with the digestion of cytochrome c and bovine serum albumin at a fast flow rate of 4.0 \mu L/min, which affords the short residence time less than 5 s. The digestion products were characterized using MALDI-TOF MS with sequence coverage of 75 and 31% observed, respectively. This research exhibited a simple but effective strategy of plastic microchip surface modification for protein immobilization in biological and proteomic research.

During the past decades, progress has been important in both biotechnology and microfluidic systems.¹³–¹⁵ The main advantages of the micro total analytical systems recently developed for chemical instrumentation are, for example, higher sample throughput and processing rates, reduced manufacturing cost and system integration, and minimized consumption of sample and reagent.²⁶–⁹ Up to now, many microchips have been mainly fabricated on glass and silicon substrates with wet chemical etching,¹⁰ although the sophisticated techniques required are not necessarily adapted for mass production applications. In comparison, polymers are not expensive, usually offer more flexibility, and can be structured to construct microdevices either in a research laboratory environment or in a production plant, the techniques used including injection molding, laser ablation, imprinting, and hot embossing techniques.¹¹–¹⁴ In this way, analytical devices can be produced inexpensively and in bulk,¹⁵–¹⁷ which makes their prospect in commercial applications more likely.

On the other hand, it is clear that precise patterning of functional biomolecules is critical in the development of microanalytical systems. Recent advance in chemical and biological analysis has involved the incorporation of biomolecules onto analytical surfaces of new devices, which is capable of selective and high-affinity binding to analytes of interest.¹⁸ Sol–gel technol-

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ology, being commonly considered as a relatively mild routine for the immobilization, has thus been utilized to generate microstructures on the solid supports, during which process biomolecules are entrapped in the growing gel network rather than being chemically or physically attached to the surfaces. With regard to stability, biomolecules entrapped in sol−gel typically exhibit improved resistance to thermal and chemical denaturation and increased storage and operational stability. Therefore, it is promising to incorporate such micropatterned gels in microfluidic channels for protein immobilization.

However, because not only are the plastic surfaces hydrophobic but also there is no intermolecular interaction between gel matrix and poly(methyl methacrylate) (PMMA) substrate, it is necessary for the development of routine, simple, and well-defined modification protocols on polymer surfaces to improve the membrane adhesion, while it is an easy way to chemically modify the glass surfaces via organosilanes. Approaches for surface derivatization of microfluidic chips have been explored in order to introduce additional purposes such as immunoadsorptions, stationary phases for electrophromatography, and enzyme immobilization. For the mass fabrication of inexpensive disposable devices, the straightforward approach to incorporate a solid phase in a microfluidic system is necessary to derivatize the microchannel walls with molecular reagents that bear the desired functional property. The advantage of this approach is the ease of functionalization, as it is usually accomplished by flowing reagents through the microchannels to be derivatized. Such modification techniques are essential to the development of protein microchips in polymer-based substrates. Dodge et al. immobilized protein A on the wall of a microfluidic chip and used it for heterogeneous immobilization of enzymes on fluid bilayers supported on the microfluidic channels, and the preparation of reactive porous monoliths necessary for the development of routine, simple, well-defined protocols on polymer surfaces to improve the membrane adhesion, while it is an easy way to chemically modify the glass surfaces via organosilanes. Approaches for surface derivatization of microfluidic chips have been explored in order to introduce additional purposes such as immunoadsorptions, stationary phases for electrophromatography, and enzyme immobilization.

As one of the most commonly used polymer materials, PMMA substrate has been used to fabricate microfluidic chips by hot imprinting. In the present study, we focused our attention on exploring a simple surface modification and protein immobilization protocol of the PMMA microchannel and thus constructing an on-chip enzymatic microreactor. Following the strategy of silane-based chemistry being employed to introduce a variety of active groups, a craft copolymer has been designed and synthesized, which affords a firm, stable but easy-access silane-functionalized chemical scaffold on a PMMA-based microchannel for the first time. These silane functional groups reacted readily with the sol−gel to form stable bonding. Consequently, because of the properties of sol−gel such as large microstructured surface area, porous morphology, and hydrophilicity, the immobilization of proteins in this matrix could result in high biomolecule loading with a larger extent of bioactivity remaining in the microfluidic device. The PMMA substrate modification by copolymer and silica gel were investigated by X-ray photoelectron spectroscopy (XPS). A confocal fluorescence microscope was adopted to reveal the capability of protein encapsulating, while the stability of the immobilization strategy was further monitored on-line under a continuous flow condition by chip electrophoresis with laser-induced fluorescence (CE-LIF) detection. Furthermore, trypsin employed as a model enzyme was effectively immobilized within the gel-patterned PMMA microchannel to fabricate an enzymatic microreactor-on-a-chip for proteolytic digestion, coupled to matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS). The high surface-to-volume ratio of the constructed microchip has resulted in sensitive response with much shorter analysis time. The gel-derived microstructured networks are efficient for protein patterning, which was considered as the premise of the favorable reproducibility and long-term stability obtained. Such an approach provides an effective means for rapid chemical modification and microfabrication of protein-functionalized microfluidic systems, which might hold considerable promise in drug-screening, clinical diagnosis, immunoadsorption, and proteomic applications.

**EXPERIMENTAL SECTION**

**Materials and Chemicals.** Butyl methacrylate (BMA), 2,2'-azobisisobutyronitrile (AIBN), tetraethyl orthosilicate (TEOS), and other chemicals were purchased from the Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA, fraction V) and trypsin were obtained from Sigma Chemical Co., Ltd. (St. Louis, MO).

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Preparation of BMA/MSMA Copolymer and Silica Sol–Gel for Enzyme Encapsulation. Different amounts of the two monomers, BMA and MSMA, were mixed together according to the weight ratio as [MSMA] to ([MSMA] + [BMA]) being 0.2. AIBN, initiator of the polymerization, was added to the mixture at 0.2% weight ratio to the total reactants. The copolymerization was generated at 70 °C in THF solvent under nitrogen flow purged for 8 h. The product of the copolymerization reaction, expressed as (BMA)\(_x\)–(MSMA)\(_y\), was held in a sealed pot and preserved in desiccators. The chemical reaction is shown in Scheme 1.

Just prior to use, a silica sol solution was obtained by mixing TEOS with 2.5 mM HCl in triple-distilled water, at a molar ratio of 1:8, containing 10% poly(ethylene glycol). The mixture was stirred until a clear solution was obtained and sonicated for 30 min in an ultrasonicator bath (model SCQ 50, 220 V/50 Hz, Shanghai Shenbo Ultrasonic Co., Ltd.).

Fabrication of PMMA Microfluidic chips. PMMA chips employed in this work were made by the method of hot imprinting with the micromachined silicon templates that were fabricated by the Institute of Microanalytical System, Zhejiang University (China). The sectional diagram of the template figured as a trapezoid form with the dimensions as 60–120 \(\mu\)m width and 50 \(\mu\)m depth (see Supporting Information (SI), Figure 1). Before imprinting, both the polymer substrates and glass slides were washed successively with liquid detergent, water, and ethanol in an ultrasonic bath and dried in a clean case. The silicon template was thoroughly cleaned with acetone before use. Channels were fabricated using PMMA Plexiglas pieces. During the fabrication process, a homemade hot-embossing machine was used to apply uniform pressure on the surfaces (see SI, Figure 2). One slice of PMMA and the silicon template were held between two glass slides and then clamped together by two aluminum blocks. PMMA channels were formed by heating the plastic template sandwich assembly at 100 °C for 2 min and then cooling to 80 °C with the additional embossing pressure of \(\sim 2.0 \times 10^5\) N/m\(^2\) for at least 1 min. The assembly was subsequently removed from the machine and allowed to cool completely. The glass slides were removed and the silicon template was pulled away from the plastic piece to reveal the channel. Afterward, the fabricated plate with micro-channel embossed was bonded with another blank PMMA plate between two glass slides as before. The PMMA devices were heated at 80 °C for 18 min while an appropriate pressure, \(\sim 5.0 \times 10^4\) N/m\(^2\), was applied to bond the chips. Prior to bonding, 3-mm-diameter holes were drilled through the cover slide. These holes were aligned to the channel ends in the imprinted slide to create buffer, sample, or waste reservoirs according to detailed requirements. It should be mentioned that thermal bonding would cause the deformation of imprinted channels.

PMMA Microchannel Surface Modification and Protein Immobilization. Scheme 2 summarized the suggested protocol that permitted protein-encapsulated silica gel on the modified PMMA surface. The as-synthesized copolymer solution was aspirated rapidly through the microfluidic channels with a vacuum pump to form a thin layer on the PMMA surfaces. Two aliquots of 5 mg/mL FITC–BSA solution in 20 mM phosphate buffer solution (pH 7.0) and standard sol solution were mixed completely and then pumped into plastic channels that had been modified as above. Then the chip was stored at 4 °C for more than 24 h for the polycondensation between the sol–gel solution and the silanol groups on the modified surface, during which process solvent evaporation and gelation occurred. For the microreactor fabrication, the immobilization procedure was similar except that the concentration of trypsin used was 10 mg/mL.

XPS was used to investigate (BMA)\(_x\)–(MSMA)\(_y\) modification and the subsequent attachment of silica gel on the PMMA chip, while the control experiment was made on primary PMMA substrate for comparison. After being degassed in the pretreatment chamber for 2 h, samples were then removed into the test chamber for XPS examination. The spectra were obtained by using Al K\(_\alpha\) radiation (1486.6 eV) on a Perkin-Elmer PHI 5000C ESCA system with a base pressure of \(1 \times 10^{-9}\) Torr. Peak positions were normalized to the carbon peak at 284.5 eV.

Scheme 2. Process Summary of the Functional PMMA Surface Modification Followed by Silica Sol–Gel Entrapped Enzyme Immobilization
A confocal system (TCS NT, Leica) was used to take confocal fluorescence microscope (CFFM) images of PMMA microchannels immobilized with BSA–FITC. The immobilization stability was explored by CE-LIF detection (see SI, Figure 3). The employed LIF instrument was fabricated by Shanghai Institute of Optics and Fine Mechanics (Chinese Academy of Sciences). The chip was placed on an x–y–z translation stage. Through a prism, an aspheric lens, and the regulation of an x–y–z translation stage, the beam from a laser (473 nm, 15 mW, Changchun Institute of Optical Machinery, China) could be focused on one point of the microchannel. Any point of the channel could be selected and thus acted as the detection point optionally through adjusting the x–y–z stage. The detection area equaled to the dimension of the laser faculous region, $\sim 20 \mu m$ in diameter. The fluorescence emission at 520 nm was collected and measured by a photomultiplier tube after passing the confocal lens and the filters. The signals were digitized by a specially designed A/D converter (24 bits) and the data handled by homemade software. Chip-capillary electrophoresis was carried out with high-voltage power (Shanghai Institute of Applied Physics, Chinese Academy of Sciences). The fluorescence signals from the detection point were monitored continuously while a 20 mM phosphate buffer solution (pH 7.0) was used as the running solution propelled by electroosmotic flow with 900-V high voltage applied.

Caution: The electrophoresis uses high voltage and special care should be taken when handling the electrophoresis electrodes.

**On-Chip Tryptic Digestion and Identification.** At a flow rate of 240 $\mu L/h$ with a syringe pump (74900 series, Coleparmer Instrument Co. Ltd.), two protein solutions cytochrome c (Cyt-C) and BSA (0.20 $\mu g/\mu L$) in 10 mM NH$_4$HCO$_3$ buffer solution (pH 8.3) were respectively driven through the microreactors that had been prepared with trypsin-encapsulated silica gel. Effluents accumulated in the waste reservoir were collected by a digital pipet (2–20 $\mu L$, Eppendorf Research) and then determined with MALDI-TOF MS. MS experiments were performed on an Applied Biosystems 4700 Proteomics Analyzer with TOF/TOF Optics. Sample solutions were mixed with CHCA matrix solution (containing four parts 0.1% TFA in water and six parts 0.1% TFA in acetonitrile) at a 1:1 ratio on the MALDI plate. The mass spectra data were submitted to a database for protein identification using GPS Explore Software with the integrated Mascot search engine software. For comparison, the digestions of Cyt-C and BSA were also performed by free trypsin in solution according to the conventional procedure (trypsin/protein mass ratio 1:40, digestion time 12 h) and by the silica gel-modified microreactor without trypsin entrapped, respectively. Reflector mode was used to detect low-mass peptides while linear mode was selected for high-mass proteins.

**RESULTS AND DISCUSSION**

**Characterization of (BMA)$_x$–(MSMA)$_y$ Copolymer and Silica Gel Assembled onto a PMMA Surface.** To the hydrophobic polymer-based chips, chemical modification procedures for a plastic surface are essential to improve the compatibility of biomolecules, which is important in biochemical analysis. To this end, the problem is typically overcome with increasing surface energy of the materials by introducing polar functionality using chemical surface modification or reactive gas discharge treatment such as plasma and corona or by surface graft polymerization of polar monomers. However, improvement of the hydrophilicity of a polymer surface with the plasma method is temporary because prolonged exposure to air results in reorientation of the relative hydrophilic hydroxyl groups into the bulk of the polymer.

Here we introduced a simple derivation method to graft silane-functionalized copolymer from BMA and methacryloxypropyltri-

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methoxysilane on a PMMA surface. As a polyacrylate, poly(butyl methacrylate) (PBMA) segments, the main component of the copolymer, has a structure similar to PMMA and is normally adopted as a kind of adhesive agent in macromolecular research. For the affinity between PBMA and PMMA, the as-synthesized copolymer molecules were supposed to link to PMMA substrate easily and firmly. In this way, the BMA portion of the copolymer copolymer molecules were supposed to link to PMMA substrate easily and firmly. In this way, the BMA portion of the copolymer could adhere to the surface of the PMMA film serving as the binding site while the majority of the MSMA moieties would be exposed to the outer surface due to phase segregation. Being hydroxy-terminated through hydrolization of the MSMA, this derived polymer became hydrophilic and sol-gel active. The silanol groups could also take part in the condensation reactions with sol-gel and provide chemical anchorage to the polymeric network. Therefore, the effective and stable adhesion of sol-gel matrix on PMMA was achieved, which allowed the encapsulation of proteins in the microfluidic channels (shown in Scheme 2).

To explore (BMA)$_x$-(MSMA)$_y$ adhesion and construction of a silica gel network on the PMMA surfaces, XPS was used to analyze the modified layers and the certain chemical elements compared with the native PMMA. Figure 1 showed the XPS spectra recorded from different surface coating layers. As for the (BMA)$_x$-(MSMA)$_y$ modified surface, five main peaks, belonging to silicon (Si 2s), silicon (Si 2p), oxygen (O$_{\text{KL}}$), oxygen (O 1s), and carbon (C 1s), respectively, were revealed with the molar ratio as C 70.6% O 26.2% and Si 3.20% (Figure 1b). From the molecular formula (C$_{6}$H$_{14}$O$_{5}$Si)$_{x}$-(C$_{10}$H$_{20}$O$_{5}$Si)$_{y}$, molar composition of the two monomers was calculated as $xy = 1.61$, smaller than that calculated from theoretical prescription and element analysis (about 6–7:1) of the copolymer, which indicated that the outside superficial layer contained a higher amount of MSMA. Under the affinity between BMA and PMMA, rearrangement of copolymer molecules might be produced on the PMMA surface, which would result in the enrichment of most MSMA portions to the exterior where their density was increased dramatically. The enhanced silicon (Si 2s) and silicon (Si 2p) and decreased carbon (C 1s) peaks were observed in XPS spectra after the silica gel formed (Figure 1c). The mole ratio of silicon (16.8%) and oxygen (56.2%) matched the incomplete polycondensation state of the sol-gel. Therefore, the silica gel could be immobilized on the PMMA surfaces intermediating with a robust silane-functionalized scaffold.

Characterization of Proteins Stably Immobilized in the Modified PMMA Microchannels. Being adopted as the tracing mark, FITC conjugate BSA was gel-encapsulated in the microchannels. Confocal fluorescence microscopy and CE-LIF detection were used to study the reliability and stability of protein immobilization. Before the experiments, the channels were successively washed with running water for 5 min and then dipped in 20 mM phosphate buffer solution (pH 7.0) three times for 15 min to remove protein molecules either excessive or adsorptive nonspecifically. Fluorescence images of the microchannel were displayed at the excitation of 488 nm in Figure 2, indicating that the protein molecules could be homogeneously encapsulated. Four photos were pictured from four normal sections of microchannel with different depths by faultage scan paralleling to the microchannel, which showed the trapezoid shape of the channel, i.e., wider at the top and narrower at the bottom.

On the other hand, the stability of proteins embedded in gel-derived microstructures was an essential property for analytical applications, which was thus investigated by CE-LIF detection. Under the wavelength of excitation at 473 nm, the corresponding fluorescence signals were recorded at 520 nm where the characteristic emission of FITC is located. First, when the chip was removed vertically to the channel from one edge to another side, the fluorescence spectrogram, recorded in Figure 3A, revealed that proteins had been immobilized in the microchannel. Second, in respect that the fluorescence intensity represented the quantity of the corresponding fluorescent biomolecules immobilized, the fluorescence signals were monitored on-line from one detection point while pH 7.0 running buffer solutions propelled by electrosomatic flow with 900-V were applied. A laser beam was focused on one point of the channel that acted as the detection point with a similar operation in Figure 3A. The recorded fluorescence intensity was examined as a function of migration time (Figure 3B), and the result showed that the fluorescence intensity was stable and no obvious decrease appeared during the 20-min process period. It can be concluded that the (BMA)$_x$-(MSMA)$_y$ modification scaffold followed by the silica gel could provide stable microstructured matrixes for protein patterning on a plastic microfluidic channel. For comparison, similar experiments of sol-gel entrapment were carried out on pristine PMMA microchannels without (BMA)$_x$-(MSMA)$_y$ modification. As depicted in Figure 3B (2), the fluorescence intensity decreased to ~10% of the original value after the 20-min period, with 85% loss in the first 6 min. The different results showed that only after the modification of silane functional copolymer, could biomolecules

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Encapsulated within sol-gel matrixes be stably realized on the PMMA microchannel.

**Enzymatic Microreactor-on-a-Chip for Protein Digestion.**

After the characterization made above, the proposed technology was introduced to the fabrication of a microchip-based enzymatic reactor, a powerful tool for proteomic research. Trypsin was gel-encapsulated in the modified microchannels with a total reaction volume of 135 nL. Two larger proteins with multiple cleavage sites, Cyt-C (MW = 12,384) and BSA (MW = 66,000), were used as model substrates for the proteolytic digestion. For comparison, the digestions of the same proteins were performed by free trypsin in solution and by the silica gel-modified microreactor without trypsin entrapped, respectively. At a flow rate of 4.0 μL/min, these two proteins of 0.20 μg/μL in 10 mmol/L NH₄HCO₃ buffer solutions (pH 8.0). Digestion time is less than 5 s with a flow rate of 4.0 μL/min. Eluent/matrix ratio is 1:1. Reflector mode was used to detect low-mass peptides while linear mode was selected for high-mass proteins.

Figure 3. (A) Fluorescence intensity vs time measured across the channel. (B) Fluorescence intensity from the FITC–BSA immobilized PMMA microchannels with (1) and without (2) modification by (BMA)ₓ–(MSMA)ᵧ copolymer versus electrophoresis time (excited at 473 nm and recorded at 520 nm).

Figure 4. MALDI-TOF mass spectrum of digests of (1) Cyt-C and (2) BSA using the silica gel-modified microreactor-on-a-chip (a) with encapsulated trypsin and (b) without encapsulated trypsin. Proteins are 0.20 μg/μL in 10 mmol/L NH₄HCO₃ buffer solutions (pH 8.0). Digestion time is less than 5 s with a flow rate of 4.0 μL/min. Eluent/matrix ratio is 1:1. Reflective mode was used to detect low-mass peptides while linear mode was selected for high-mass proteins.
Table 1. Summary of MALDI-TOF MS Results Obtained from Digests by Trypsin-Immolized On-Chip Microreactors\(^a\) Compared with In-Solution Digestiona

<table>
<thead>
<tr>
<th>protein</th>
<th>Cyt-C</th>
<th>BSA</th>
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<tbody>
<tr>
<td>amino acids identified(^b)</td>
<td>microreactor 71–78</td>
<td>in-solution 94</td>
</tr>
<tr>
<td>total no. of fragments</td>
<td>microreactor 22</td>
<td>in-solution 22</td>
</tr>
<tr>
<td>sequence coverage,(^b) %</td>
<td>microreactor 68–75</td>
<td>in-solution 90</td>
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<tr>
<td>digestion time</td>
<td>microreactor (&lt;5) s</td>
<td>in-solution (\geq 6) h</td>
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<td>peptides matched</td>
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<td>in-solution 18</td>
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<td>with microreactors</td>
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<td>microreactor 101–117 VASLRTEYGDACCEK</td>
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<td>microreactor 198–204 GACLPLK</td>
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<td>microreactor 437–451 KVQVSTPTLVEVSR</td>
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\(^a\) Five spot replicates were taken in the experiments. \(^b\) The difference in amino acids identified and sequence coverage from chip to chip (n = 6). \(^c\) P00004 and P02769 mean cytochrome c and serum albumin precursor, respectively.

Detailed identification results were listed in Table 1. The observation corresponded to the detection of fragments containing 78 out of the 104 possible amino acids of Cyt-C and 178 out of the 583 possible amino acids of BSA. The sequence coverages of 75% for Cyt-C and 31% for BSA from the database were obtained. The identification results could be compared with those by in-solution digestion that required a reaction time longer than 6 h and those from other microfluidic devices reported that required a reaction time of 1–15 min.\(^{39–42}\) Since the silica gel matrix has a large surface area and the microreactor has a very narrow space, the high concentration of immobilized enzyme would be achieved at a certain volume. Thus, the substrate might be in contact with trypsin for longer periods of time compared with the soluble system.\(^{40,41}\) Due to the high surface area-to-volume ratio of the gel-modified microchannels, it is likely that there is a short distance for the substrate to diffuse from the solution to the microstructured gel network where trypsin was located. The fast digestion might also be attributed to the modified hydrophilic surfaces, which could effectively reduce the driving force for surface-induced denaturation and the attractive protein—surface interaction.\(^{42–46}\)

In addition, the relative standard deviation calculated from the sequence coverage was less than 6% (n = 6) by chip-to-chip analysis, which showed a good reproducibility. After protein digestion, the bioreactor was washed and stored at 4 °C to test the stability of the immobilized enzyme. The proteolytic reaction was done repeatedly with the prepared microreactor during 1 week’s storage. Similar identification results witnessed by MALDI-TOF MS demonstrated that the stability of protein molecules and their bioactivity were preserved well in the microsystem. However, a soluble trypsin stored in the buffer of 50 mM Tris-HCl at 25 °C almost completely lost its activity within 1 day.\(^{43}\) The stability of the biomolecules might be enhanced when they were immobilized in a gel-derived micropatterned network because of the suppression of enzyme autolysis by the matrix.\(^{32}\) An additional advantage of our approach lies in its simplicity, which is expected of the automated high-throughput analysis using a parallel channel microchip platform. Although in the current work, only trypsin-immobilized microreactor has been exemplified, the versatile applications of this modification protocol in bioanalytical devices are promising.

CONCLUSION

A primary silane-functionalized scaffold has been prepared within a PMMA-based microfluidic channel through chemical modification of a certain synthesized copolymer. Subsequent attachment of silica sol–gel with the silane-functionalized groups...
makes biomolecules encapsulated onto the hydrophobic plastic-based microchip realized. By reason that this modification strategy resembles the classical silanization of glass material, there is not much difficulty in looking forward to its potential applications in many aspects, not just limited to the sol–gel entrapment of protein that we have explored in this experiment. Other functional groups thus might also be introduced according to the requirements, such as what was frequently used in a fused-silica capillary and packing column of HPLC. Considering the experimental results obtained, several advantages are associated with the suggested design. Since PMMA chips could be fabricated to interface with analytical instruments such as MS, this modification technique is thus appropriate to be applied to microfabricated systems coupled to on-chip ESI-MS, an efficient analytical means in the biological field. Other applications in biosensors and immunoassay were performed within the microfluidic analytical devices, and the detailed work will be further reported. The current development of practical investigation is being applied to the large-scale proteomic studies undertaken in our laboratory. Foreseeable utilization in enzyme microreactors, antigen–antibody interactions, biosensors, etc., will provide a powerful platform for proteomic analysis integrated with the micromachined technique and bioanalysis.

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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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