IOP PUBLISHING JOURNAL OF MICROMECHANICS AND MICROENGINEERING

J. Micromech. Microeng. **18** (2008) 125010 (9pp) doi:10.1088/0960-1317/18/12/125010

Miniaturized protein separation using a liquid chromatography column on a flexible substrate

Yongmo Yang and Junseok Chae

Electrical Engineering, Arizona State University, Tempe, AZ 85287, USA

E-mail: yongmo.yang@asu and junseok.chae@asu

Received 20 August 2008, in final form 2 October 2008
Published 30 October 2008
Online at stacks.iop.org/JMM/18/125010

Abstract

We report a prototype protein separator that successfully miniaturizes existing technology for potential use in biocompatible health monitoring implants. The prototype is a liquid chromatography (LC) column (LC mini-column) fabricated on an inexpensive, flexible, biocompatible polydimethylsiloxane (PDMS) enclosure. The LC mini-column separates a mixture of proteins using size exclusion chromatography (SEC) with polydivinylbenzene beads (5–20 μm in diameter with 10 nm pore size). The LC mini-column is smaller than any commercially available LC column by a factor of ∼11 000 and successfully separates denatured and native protein mixtures at ∼71 psi of the applied fluidic pressure. Separated proteins are analyzed using NuPAGE-gel electrophoresis, high-performance liquid chromatography (HPLC) and an automated electrophoresis system. Quantitative HPLC results demonstrate successful separation based on intensity change: within 12 min, the intensity between large and small protein peaks changed by a factor of ∼20. In further evaluation using the automated electrophoresis system, the plate height of the LC mini-column is between 36 μm and 100 μm. The prototype LC mini-column shows the potential for real-time health monitoring in applications that require inexpensive, flexible implant technology that can function effectively under non-laboratory conditions.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The ability to diagnose and monitor chronic health conditions like heart disease, diabetes, high blood pressure, cholesterol and cancer [1, 2] is a key concern in medicine. Patients with these conditions are initially diagnosed with bloodwork and, once diagnosed, require regular blood monitoring [3–5] of the many health index markers found in blood. For reliability, convenience, and efficiency, implantable monitoring devices would be an obvious solution. Existing implant technology, however, has several drawbacks, including its large size, material stiffness and high cost [6–10]. For example, current glucose sensors for diabetics demonstrate the need for improved tools and unobtrusive monitoring that could be achieved with implants: residual sugar on a diabetic's hand, for instance, can confound test results, and providing enough blood to cover a test pad and/or having to take several painful measurements each day can be problematic [11–13].

Despite extensive research on detectors and fluid control devices [14–17], the separation/pre-concentration stage for analyzing mixed samples has generally been overlooked because researchers often use pre-treated samples for their laboratory-based detectors [18–20]. Viable implant devices, however, must be able to detect and separate untreated protein samples with high selectivity and sensitivity. Some detectors have achieved high sensitivity [21, 22]; the prototype LC mini-column presented here is a critical first step toward practical implant application, because its high selectivity, coupled with an on-chip detector with high sensitivity, represents a new horizon for medical implant application. In this paper, we focus on a prototype for an implantable protein separator that uses micromachining technologies on flexible, biocompatible material, PDMS (polydimethylsiloxane). This

prototype protein separator achieves miniaturize size exclusion chromatography (SEC) in liquid chromatography (LC). The uniqueness of this LC mini-column protein separator is that it uses a biocompatible, isocratic mobile phase with significantly lower flow rate than any of its macro sized columns, including normal phase, reverse phase and ion exchange chromatography [23]. Drastic LC column size reduction also shows the potential for portable LC systems [24].

Several miniaturized LC and high-performance liquid chromatography (HPLC) columns have been developed, but until this prototype LC mini-column is developed, none had been successfully miniaturized on a flexible substrate [25–28]. Manz *et al* proposed the first microfabricated LC chip in 1990. It was an open-tubular column on a silicon wafer; complete testing results were not presented, but great potentials for miniaturization were discussed [25]. Oleschuk *et al* designed a cavity formed by two weirs in the sample channel to trap the stationary phase [26]. In this study, octadecylsilane (ODS)-coated silica beads were introduced by an electro-osmotic pump and filled in the cavity. BODIPY and fluorescein were also loaded by the electro-osmotic pump and successfully separated. Reichmuth *et al* demonstrated a microchip reverse phase HPLC column that separated peptides and proteins on a fused silica substrate [27]. A porous polymer monolith (C-18 side-chain) was defined by contact lithography instead of actual beads; a mixture of peptides and proteins were successfully separated and delivered by an external syringe pump. Shih *et al* presented a micro-HPLC chip using parylene and silicon substrate as a structural material and C-18 coated beads as the stationary phase [28]. The micro-HPLC chip was integrated with an electrochemical sensor, resistive heater and thermal-isolation structure for on-chip temperature gradient interaction chromatography application; a mixture of derivatized amino acid was successfully separated and detected by an electrochemical sensor. None of them, however, is made of all-flexible, biocompatible materials suitable for unobtrusive human implants; their structural materials (silicon and glass) are stiff and brittle, and likely to cause severe damages on surrounding tissues [8, 29, 30]. The following sections explain the separation mechanism and LC mini-column design, detailing the selectivity, retention factor, column efficiency and specifications. Microfabrication on a flexible substrate, column bead-packing techniques and experimental setups are also described. Polydivinylbenzene beads with 10 nm pore size show separation of two different mixtures of proteins: SeeBluePlus2 (denatured pre-stained proteins) and gel filtration standard (native proteins). Results are presented with implications for future research.

2. Separation mechanism: size exclusion chromatography (SEC) and the LC mini-column design

SEC is often referred to as *gel-permeation chromatography* (GPC) or *gel-filtration chromatography* (GFC); it differs from other chromatographies, however, because the stationary phase surface does not react with mixed samples. SEC separates mixed samples by size based upon analytes penetrating nanopores on the stationary phase. It uses an isocratic biocompatible mobile phase that does not require high-performance pumps, and requires significantly lower flow rate than other techniques (normal phase, reverse phase and ion exchange chromatography [31]). These features make SEC the only technique that would enable practical, biocompatible miniaturization with full functionality. SEC enables the prototype LC mini-column to separate mixed protein samples in non-laboratory conditions. The SEC stationary phase is polydivinylbenzene, 5–20 μm in diameter and has 10 nm surface pores. When mixed samples flow into the bead-packed mini-column, smaller samples tend to diffuse into the pores while larger ones tend to diffuse through interstitial spaces between the beads; separation occurs because larger samples elute first (after a short travel distance) and smaller samples elute later (after a longer travel distance).

2.1. LC mini-column selectivity, retention factor and efficiency

The selectivity and separation range of the SEC column are determined primarily by the beads' pore size [32]. The pore size should be large enough for a target analyte to penetrate. Thus, we can select the separation range or selectivity with different pore size. The retention factor (k_e) in the LC is a parameter to normalize retention, defined as the ratio of retention time of an analyte to an unretained peak [31–33]. In the SEC, k_e can also be expressed by the relative elution volume ratio of the excluded and retained peaks, as shown in equation (1):

$$k_e = \frac{t_R - t_o}{t_o} = \frac{V_R - V_e}{V_e}, \tag{1}$$

where t_R and t_o are the retention time of an analyte and an unretained peak, and V_R and V_e are the volume of the retained and unretained peaks, respectively. The retention factor and time are calculated by equation (1) with the elution volume.

The efficiency of a column is measured by height equivalent to the theoretical plate (HETP) or plate height, H [34]. The plate height, H, is a ratio of the column length, L, and the plate count, N, as $H = L/N$. The plate count determines the separation quality based on a single peak in the chromatogram [35]. The plate count can be calculated where t_R is the retention time, w_p is the width of a single peak, and f is a parameter depending upon the measuring method for the peak width. Half-height is the most common method, and f is 5.545

$$N = \frac{f \times t_R^2}{w_P^2}. \tag{2}$$

2.2. LC mini-column specifications

Figure 1(*a*) shows a conceptual top view of the fabricated prototype LC mini-column. The actual widths, lengths and heights in the LC mini-column prototype are, respectively,

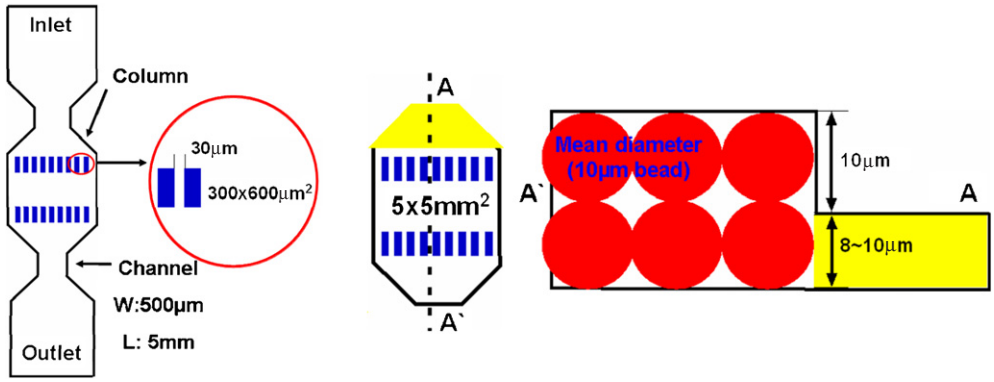

Figure 1. LC mini-column design concept, (*a*) LC mini-column and (*b*) trapped beads in the column.

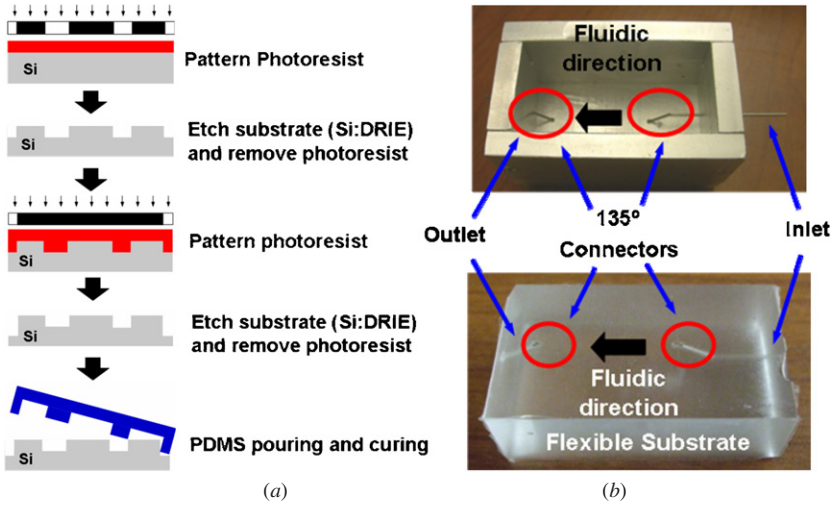

Figure 2. Fabrication process flow, (*a*) PDMS Si master for top layer and (*b*) aluminum mold for customized housing.

5 mm × 5 mm × 20 μm for the column and 500 μm × 5 mm × 10 μm for the channel.

Beads in the stationary phase are retained in the LC mini-column by the height difference between the mini-column and microfluidic channel, in combination with immobilizers (similar to a frit in a 'macro' LC column). The microfluidic channel is 8–10 μm high (figure 1(*b*)), smaller than the mean diameter of the beads (10 μm). Immobilizers located at the mini-column inlet and outlet are 300 × 600 × 20 μm^3 in width, length and height, respectively. The gap between two immobilizers is 30 μm. This gap evenly disperses the applied fluidic pressure and allows beads to be distributed and retained in the LC mini-column.

3. Microfabrication of the LC mini-column

A multi-level soft lithography technique is used to fabricate the prototype LC mini-column using a all-flexible material [36–38]. Polydimethylsiloxane (PDMS) is used for both the substrate and structure material because of its biocompatibility, cost effectiveness and ease of handling [39, 40]. Though previous research efforts have paved the way for this prototype, the LC mini-column is the first miniaturization of its kind.

3.1. All-flexible LC mini-column and enclosure

A multi-level soft lithography technique is used to fabricate the all-flexible column [36–38]. Figure 2(*a*) illustrates the process flow of the PDMS silicon master (top layer) fabrication [41]. To begin, a photoresist is spin-coated on a silicon wafer and patterned for columns and channels. The silicon wafer is then etched 7 μm by deep reactive ion etching to transfer the pattern. Another photoresist layer is then coated, patterned and hardbacked. The 10 μm height difference is etched and the photoresist then removed by a stripper. Finally, PDMS is poured and cured for 10 min at 100 °C on a hotplate, then peeled off from the silicon master.

The PDMS substrate shown in figure 2(*b*) is made of an aluminum master. The aluminum master interfaces between the macro and micro-world and accommodates high pressure flow [42]. The mold consists of two pairs of rods (1/32 inch in diameter) and five aluminum plates; all use screws so that they can be easily disassembled to detach the PDMS substrate. To alleviate high fluidic impedance at the inlet/outlet ports, each pair of rods meets inside the mold to connect the microfluidic channel at an 135° angle.

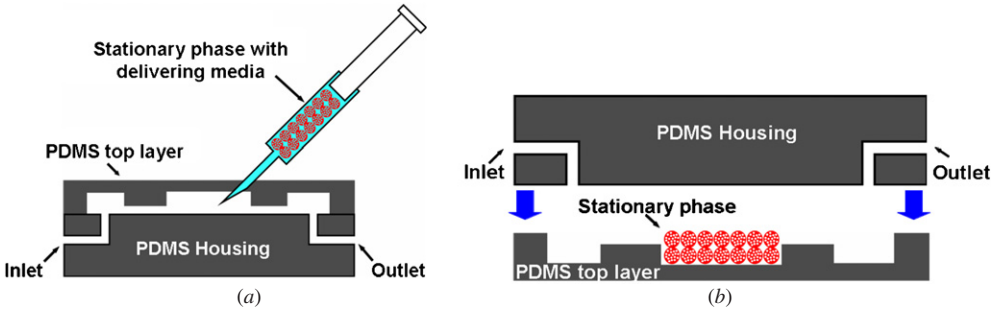

Figure 3. Bead packing methods, (*a*) slurry packing with syringe and (*b*) manual bead packing.

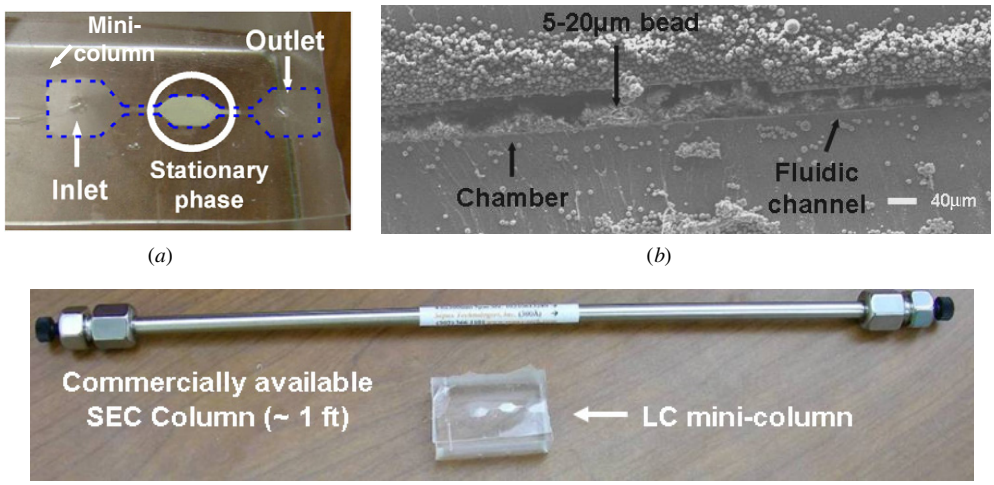

Figure 4. The LC mini-column, (*a*) packed with stationary phase, (*b*) SEM picture of bead packing and (*c*) size comparison between the commercially available SEC column (SRT SEC-300, 4.6 × 300 mm^2) and the fabricated prototype LC mini-column.

3.2. Bead-packing techniques in the LC mini-column

Two techniques can be used to fill the column with beads: (1) syringe-based slurry packing and/or (2) manual bead packing in the column. Overall, manual bead packing is the preferred method because of its high yield and ease of handling. Slurry packing starts with bonding the two PDMS layers using an oxygen plasma for 1 min at 390 mTorr with 100 W [43]. The bonded stack is cured at 80°C in the oven for 20 min, followed by another oxygen plasma treatment to convert hydrophilic fluidic channels. A 31-gauge syringe inserts the stationary phase mixed with delivering media, organic solvent (figure 3(*a*)). Bead immobilizers inside the column retain the stationary phase, and the hole on the PDMS top layer shrinks after pulling out the syringe.

Manual bead packing requires beads to be inserted into the LC mini-column before bonding (figure 3(*b*)). Since the column is rather large, fine-tip tweezers can be used to place the beads. Once the beads have been inserted, the two PDMS pieces are aligned and bonded. Figure 4(*a*) shows the fabricated LC mini-column packed with the SEC stationary phase and (b) shows an SEM picture for LC mini-column height difference and packed beads.

To underscore the degree of miniaturization allowed by the soft lithography technique, figure 4(*c*) demonstrates the size difference between a commercially available 'macro' SEC column (SRT SEC-300, SEPAX Technologies) and the fabricated LC mini-column. The mini-column's width, length and height are only 5 × 5 × 0.02 mm^3, respectively, a reduction factor of ∼11 000 from the macro-scale column's inner diameter and length: 4.6 × 300 mm^2.

4. Experimental setups: testing the LC mini-column

The LC mini-column is tested for its ability to separate mixed proteins. Qualitative results are observed and collected at timed intervals. Additional detection equipment provides quantitative results through HPLC testing with off-chip detector systems.

4.1. LC mini-column test materials: stationary phase, solutions and analytes

The stationary phase for the LC mini-column uses Jordi FLP polydivinylbenzene beads (5–20 μm in diameter) with surface nano-pores of 10 nm. HPLC-grade acetonitrile and water (Fisher Scientific) are used as received. SDS-PAGE running buffer (Invitrogen, Inc.) is diluted with DI water before loading to SDS-PAGE electrophoresis (Invitrogen, Inc.) and used as a mobile phase in denatured protein separation. NuPAGE Mini-gel (Invitrogen, Inc.) is washed

Table 1. GFS proteins.

Gel filtration standard	Molecular weight (kDa)	ng $(\mu L)^{-1}$	μMol
Thyroglobulin (bovine)	670	10 000	14.93
γ-globulin (bovine)	158	10 000	63.3
Ovalbumin (chicken)	44	10 000	227.3
Myoglobin (horse)	17	5 000	294.12
Vitamin B12	1.35	1 000	740.74

out before loading to the electrophoresis. Phosphate buffered solution (PBS) with pH 7.3, (Fisher Scientific) is used as the mobile phase for separating a native protein mixture. Two different mixed samples are used for the protein mixtures: SeeBluePlus2 (Invitrogen, Inc.) and gel filtration standard (BioRAD). SeeBluePlus2 has 10 denatured and pre-stained proteins ranging in size from 3 kDa to 188 kDa [44]. Different color spectrums on the electrophoresis allow us to have quick qualitative results. Due to denaturation, SeeBluePlus2 is insensitive to the pH of a mobile phase, which allows robust and reproducible separation. BioRAD's gel filtration standard (GFS) is a mixture of five standard native proteins [45]. Table 1 lists these native proteins and the concentration of each in the vial. This study focuses on the three highlighted proteins in table 1 (myoglobin (17 kDa), ovalbumin (44 kDa) and γ-globulin (158 kDa)) because they are within the detection range (10–260 kDa) for the instrument used to confirm protein separation in the LC mini-column.

4.2. LC mini-column testing methods: instrumentation setup

A syringe pump 33 (Harvard apparatus) delivers the mobile phase and protein mixtures (SeeBluePlus2 and GFS proteins) into the LC mini-column with 3 μl min^{-1} flow rate. Two syringes contain mixed proteins and mobile phase and are switched manually. Separated analytes are collected at the outlet of the LC mini-column and loaded to three different off-chip detectors: SDS-PAGE electrophoresis (Invitrogen, Inc), HPLC (Agilent 1100 series) and Experion (BioRAD). SDS-PAGE electrophoresis shows qualitative results with SeeBluePlus2 as mixed proteins. Separated SeeBluePlus2 samples are collected from the outlet and loaded on the SDS-PAGE electrophoresis (Invitrogen, Inc.) with an SDS-PAGE running buffer to meet the minimum loading amount, 10 μl. Two control groups are initially loaded at the left and right wells. For further verification of qualitative results, the eluted SeeBluePlus2 proteins are analyzed using a commercially available HPLC (Agilent 1100 series). SDS-PAGE running buffer is added to the separated SeeBluePlus2 sample to meet the minimum HPLC sample, 5 μl [46]. A mixture of HPLC-grade water and acetonitrile (1:1 ratio) is used as a mobile phase with 2 ml min^{-1} flow rate. Experion (BioRAD) is used to evaluate the LC mini-column's separation capability. Experion is a miniaturized automated electrophoresis system that analyzes eluted GFS proteins with a detectable range from 10 kDa to 260 kDa. This is why the resulting protein analysis is focused on the three proteins in the table 1 (myoglobin (17 kDa), ovalbumin (44 kDa) and γ-globulin (158 kDa)).

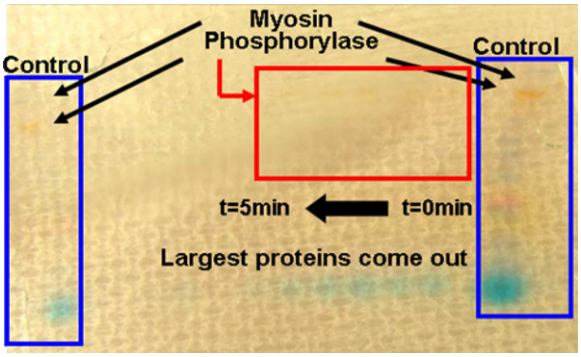

Figure 5. SDS-PAGE electrophoresis result.

5. Results and discussion: characterization of the LC mini-column

5.1. Validation of separation through qualitative testing (SDS-PAGE electrophoresis)

Manual electrophoresis provides quick and visible qualitative data. Two reference groups of SeeBluePlus2 are loaded into an electrophoresis gel at the very left and right wells, diluted by SDS-PAGE running buffer (Invitrogen, Inc.) by 1:10 ratio. SeeBluePlus2 is separated by the LC mini-column and collected at the outlet at timed intervals, every single minute. Figure 5 shows the manual electrophoresis result of samples loaded from right to left. Color spectrums are severely subdued. Results from 0–5 min are highlighted by the rectangular box. This time interval shows only large protein bands (myosin and phosphorylase). Samples after 5 min show no large proteins at all.

5.2. Validation of protein separation through quantitative testing (HPLC)

Preliminary visual results confirm that protein separation occurs, but precise mini-column characterization requires additional analysis using an off-chip detector: HPLC (Agilent series 1100).

Figure 6 shows data from the HPLC analysis of the collected test samples (time versus normalized intensity): (*a*) is the control group, diluted SeeBluePlus2 with the mobile phase, (*b*) and (*c*) are the eluted samples from 0–3 min and from 10–12 min, respectively. The control group (figure 6(*a*)) shows many sharp peaks for the first 10 min corresponding to large proteins such as myosin, phosphorylase and BSA. The peaks at 27 min show small proteins such as aprotinin and myoglobin. All numerical data (including intensity and area ratios) are summarized in table 2. There are clear differences between the control and test variable results, but these are a direct result of diluted mixed samples. Peaks in the test sample for large proteins, for instance, are not as clear as peaks in the control group (5 μl, 0.5 μl of SeeBluePlus2 and 4.5 μl of mobile phase). This is unavoidable; the minimum loading sample volume for the HPLC is 5 μl and the raw eluted protein sample (1 μl) is five times more diluted with the mobile phase, SDS-PAGE running buffer (figure 6(*b*)). For this reason, large

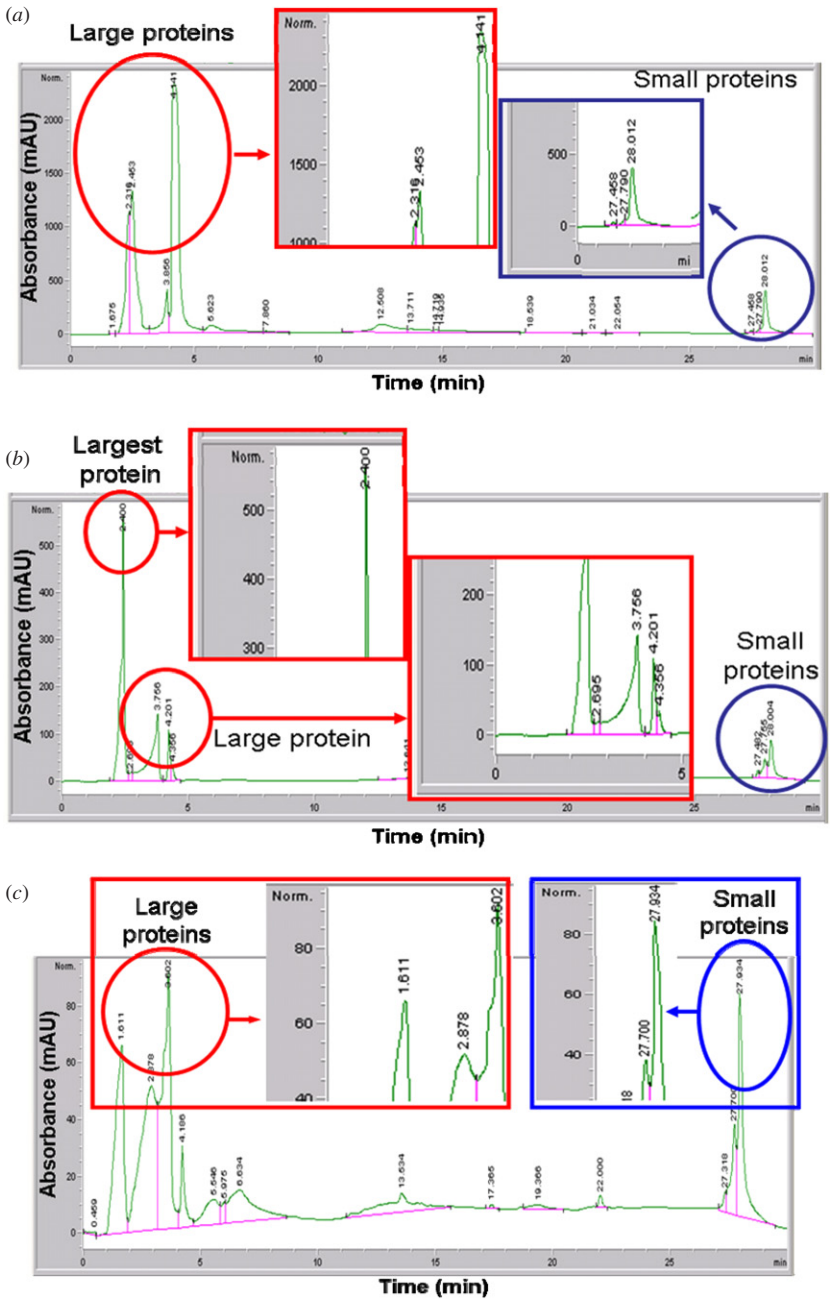

Figure 6. HPLC (Agilent 1100 series) analysis data, (*a*) control group (diluted with a mobile phase), (*b*) collected sample at t_0 (0–3 min) and (*c*) collected sample at t_5 (10–12 min). Mobile phase: 50% of HPLC grade water and 50% of acetonitrile with 2 ml min^{-1} flow rate.

Table 2. Numerical data of HPLC (Agilent 1100 series) analysis with the eluted SeeBluePlus2, (*a*) control group, (*b*) SeeBluePlus2 collected sample at t_0 (0–3 min) and (c) collected sample at t_5 (10–12 min).

(*a*) 1:9 diluted SeeBluePlus2 sample			(*b*) t_0 (0–3 min)			(*c*) t_5 (10–12 min)		
Peak	Intensity ratio (large/small proteins) (%)	Area ratio	Peak	Intensity ratio (large/small proteins) (%)	Area ratio	Peak	Intensity ratio (large/small proteins) (%)	Area ratio
1	2.83	2.37	1	6.85	4.74	1	0.02	0.02
2	3.33	3.88	2	0.22	0.14	2	0.84	1.42
3	1.02	1.03	3	1.72	2.70	3	0.65	2.07
4	5.82	8.52	4	1.31	0.67	4	1.15	1.88
5	0.15	0.403	5	0.41	0.20	5	0.37	0.29
6	0.19	0.902	9	0.21	0.10	15	1.00	1.00
18	1.00	1.00	10	0.52	0.43			
			11	1.00	1.00			

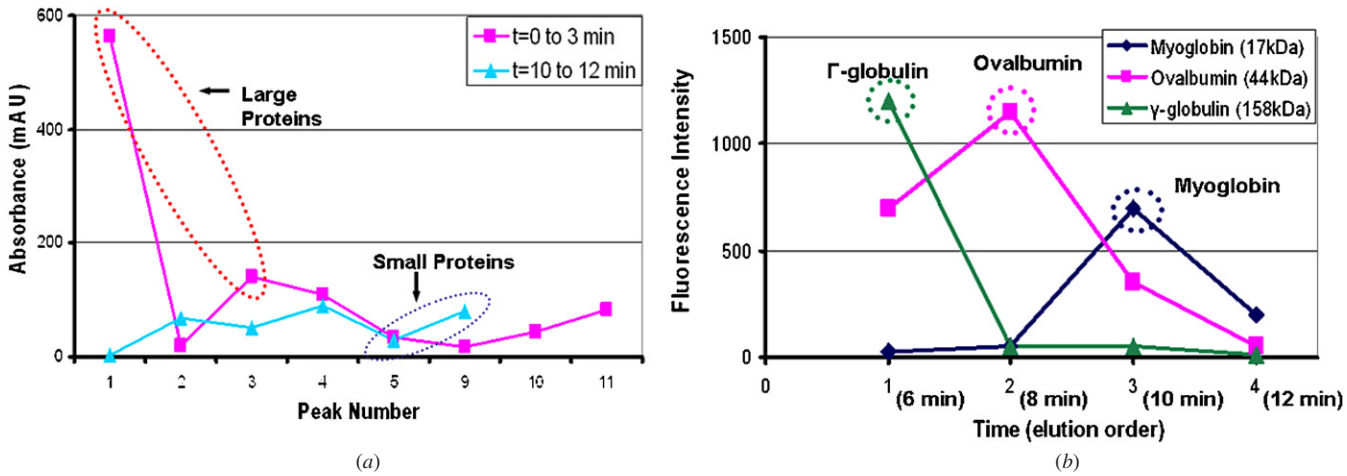

Figure 7. Fluorescence detection, (*a*) intensity in HPLC of t_0 and t_5 samples and (*b*) automated electrophoresis analysis data of fluorescence intensity (1.3 μl of GFS and PBS for mobile phase with 3 μl min^{-1} flow rate).

protein intensity is reduced by a factor of more than 4, and the intensity ratio of large and small protein peak is reduced by a factor of more than 20 (for instance, peak no 5 in table 2 (*b*) and peak no 1 in (*c*)). HPLC quantitative data provide conclusive evidence that large proteins elute from 0–3 min in the prototype LC mini-column. HPLC further confirms that the t_5 sample consists of many small proteins.

Figure 7(*a*) shows the intensities (mAU) of the two chromatography analysis for peak numbers. The 10–12 min sample shows fewer peak numbers than the 0–3 min sample because most of the large proteins are already eluted; peaks on the graph represent only smaller proteins.

We believe that several peaks in each collected sample come from a manual handling: the collected sample amount and time are so large and long that it may overlap several elution orders. The addition of an on-chip detector could enable a more precise evaluation of the LC mini-column.

5.3. LC Mini-column separation efficiency: results of electrophoresis and plate height

An automated electrophoresis system (Experion) determines the LC mini-column's separation efficiency, identifying three GFS proteins. Figure 7(*b*) shows eluted sample results based on the fluorescence intensity. The first collected sample (6 min) shows the highest fluorescence intensity of the largest protein (γ-globulin, 158 kDa) compared to the other two proteins. The second largest protein (ovalbumin, 44 kDa) shows the highest fluorescence intensity in the second collected sample (8 min). As expected, the smallest protein (myoglobin, 17 kDa) elutes with the highest fluorescence intensity at the third collected sample (10 min).

Plate height, H, is used to evaluate separation efficiency. We use 5 μl and 1.3 μl of GFS proteins to characterize the commercially available HPLC and the LC mini-column, respectively. Due to manual and discrete handling of separated proteins in the LC mini-column, we assume that the peak width, w_p in equation (2), is 2 min when we calculate the plate count, N. The plate count of HPLC is 600, while the LC mini-column is 138. Although the dimension of the LC mini-column is not yet to be optimized and it is in the early stage of implant health monitoring, we strongly believe that the LC mini-column shows great separation capability with plate height ranging from 36 μm to 100 μm. At this early stage, we cannot compare the LC mini-column to HPLC or LC macro column only with plate height because column comparison consists of several factors such as reproducibility, resolution and injection volume [35, 47, 48].

6. Conclusion

The functionality and flexibility of the LC mini-column make it a promising first step in developing more cost-effective, flexible, biocompatible component for potential use in human implant health monitoring. The fabricated mini-column uses SEC to separate a mixture of proteins and is characterized by both denatured and native proteins; it uses macro HPLC and the automated electrophoresis system as an off-chip detector. The HPLC analysis clearly shows that 10 denatured proteins are separated. Further evaluation using the automated electrophoresis system shows that the plate height of the mini-column is between 36 μm and 100 μm. It is hard to precisely characterize the mini-column unless an on-chip detector is available. An on-chip detector may overcome the limitations and is currently under development in our group. For separation capability, the design of the mini-column is not optimized, yet. However many applications including on-site health care monitoring may demand a separation unit in an extremely small form factor.

The all-flexible-polymer column shows great potential for practical applications: it could integrate an array of existing micro-detectors on a chip for parallel detection, reducing stringent selectivity requirements and analysis time with high throughput of the micro-detectors. The separator also shows possibility for a cost-effective microTAS (total analysis system) with great portability. In its current state, the mini-column requires an off-chip detector for characterization. Developing this separator in tandem with an on-chip detector to

fully characterize results is a rich area for continued research, and would increase the separator's potential for practical use in medical applications like implantable health monitoring systems.

7. Acknowledgments

The authors acknowledge Mr Sang-Soo Je, Ms Sunhee Hwang, Ms Rhonda Steele and Dr Philip Bernick for numerous and valuable discussions, and staff in CSSER (Center for Solid-State Electronics Research) at Arizona State University. The authors also acknowledge Michael Dodson and Dr Peiming Zhang in Biodesign Institute at Arizona State University for valuable help and support.

References

[1] Wallace D J, Hahn B and Dubois E L 2006 *Dubois' Lupus Erythematosus* (Los Angeles, CA: Lippincott Williams & Wilkins)
[2] Yanowitz F G 1992 *Coronary Heart Disease Prevention* (New York: Dekker)
[3] Viktorov V A, Matveev E V, Gundarov V P and Varin A N 1999 Medical methods and devices for outpatient monitoring of human health *Biomed. Eng.* **33** 1–3
[4] Shakhar G and Ben-Eliyahu S 2003 Potential prophylactic measures against postoperative immunosuppression: could they reduce recurrence rates in oncological patients? *Ann. Surg. Oncol.* **10** 972–92
[5] Cho Y, Kim H, Jeon M, Kang D, Choi H, Lee J, Kim I and Kim S I 2002 ECG acquisition and management system for knowledge discovery in DataBase: data modeling, design, and implementation *Medical Data Analysis Third International* vol 2526 pp 79–85
[6] Frodel J L and Lee S 1998 The use of high-density polyethylene implants in facial deformities *Arch. Otolaryngol. Head Neck Surg.* **124** 1219–23
[7] Engh C A and Bobyn J D 1988 *Non-Cemented Total Hip Arthroplasty* (New York: Raven Press)
[8] Krijnen M R, Smit T H, Strijkers G J, Nicolay K, Pouwels P J and Wuisman P I 2004 The use of high-resolution magnetic resonance imaging for monitoring interbody fusion and bioabsorbable cages: an *ex vivo* pilot study *Neurosurg. Focus* **16** 3–4
[9] Kandziora F, Pflugmacher R, Scholz M, Schnake K, Putzier M, Khodadadyan-Klostermann C and Haas N P 2003 Biomechanical comparison of expandable cages for vertebral body replacement in the cervical spine *J. Neurosurg.* **99** 91–7
[10] Lee K, He J, Singh A, Massia S, Ehteshami G, Kim B and Raupp G 2004 Polyimide-based intracortical neural implant with improved structural stiffness *J. Micromech. Microeng.* **14** 32–37
[11] Dixon N 2001 Monitoring and screening for diabetes *Prim. Care Pharm.* **2** 17–9
[12] Kilo C Sr, Dickey W T Jr, Joynes J O, Pinson M B, Baum J M, Parkes J L and Parker D R 2006 Evaluation of a new blood glucose monitoring system with auto-calibration for home and hospital bedside use *Diabetes Res. Clin. Pract.* **74** 66–74
[13] Talen D J and Thome S P 1997 ActLife LLC USA
[14] Handal M and Ugaz V 2006 DNA mutation detection and analysis using miniaturized microfluidic systems *Expert Rev. Mol. Diagn.* **6** 29–38
[15] Kim J, Kim B, Nam H, Park D, Yun K, Yoon J, You J and Yoon E 2002 A disposable DNA sample preparation microfluidic chip for nucleic acid probe assay *IEEE MEMS 2002* pp 133–6
[16] García Reyes J F, Llorent Martínez E J, Ortega Barrales P and Molina Díaz A 2004 Continuous-flow separation and pre-concentration coupled on-line to solid-surface fluorescence spectroscopy for the simultaneous determination of *o*-phenylphenol and thiabendazole *Anal. Bioanal. Chem.* **378** 429–37
[17] Efendioğlua, Yağan M and Batı B 2007 Bi(III)4-methylpiperidinedithiocarbamate coprecipitation procedure for separation–pre-concentration of trace metal ions in water samples by flame atomic absorption spectrometric determination *J. Hazardous Mater.* **149** 160–5
[18] Pinnaduwage L A, Boiadjiev V, Hawk J E and Thundat T 2003 Sensitive detection of plastic explosives with self-assembled monolayer-coated microcantilevers *Appl. Phys. Lett.* **83** 1471–3
[19] Levitsky I A, Euler W B, Tokranova N and Rose A 2007 Fluorescent polymer-porous silicon microcavity devices for explosive detection *Appl. Phys. Lett.* **90** 041904
[20] Mak K K W, Law A W C, Tokuda S, Yanase H and Renneberg R 2005 Application of cyanide hydrolase from Klebsiella sp. in biosensor system for detection of low-level cyanide *Appl. Microbiol. Biotechnol.* **67** 631–6
[21] Qi P, Vermesh O, Grecu M, Javery A, Wang Q, Peng S, Cho K J and Dai H 2003 Toward large arrays of multiplex functionalized carbon nanotube sensors for highly sensitive and selective molecular detection *Nano Lett.* **3** 347–51
[22] Yang Q *et al* 2007 A Raman probe for selective wrapping of single-walled carbon nanotubes by DNA *Nanotechnology* **18** 1–5
[23] Meyer V R 1988 *Practical High-Performance Liquid Chromatography* (New York: Wiley)
[24] Xie J, Shih J, Miao Y, Lee T D and Tai Y C 2005 Complete gradient-LC-ESI system on a chip for protein analysis *IEEE MEMS 2005* pp 778–81
[25] Manz A, Miyahara Y, Miura J, Watanabe Y, Miyagi H and Sato K 1990 Design of an open-tubular column liquid chromatography using silicon chip technology *Sensors Actuator* B **1** 249–55
[26] Oleschuk R D, Shultz-Lockyear L L, Ning Y and Jed D 2000 Harrison trapping of bead-based reagents within microfluidic systems: on-chip solid-phase extraction and electrochromatography *Anal. Chem.* **72** 585–90
[27] Reichmuth D S, Shepodd T J and Kirby B J 2005 Microchip HPLC of peptides and proteins *Anal. Chem.* **77** 2997–3000
[28] Shih C, Chen Y, Xie J, He Q and Tai Y 2006 On-chip temperature gradient interaction chromatography *J. Chromatograph.* A **1111** 272–8
[29] Kandziora F, Pflugmacher R, Scholz M, Schnake K, Putzier M, Khodadadyan-Klostermann C and Haas N P 2003 Biomechanical comparison of expandable cages for vertebral body replacement in the cervical spine *J. Neurosurg.* **99** 91–7
[30] Lee K, He J, Singh A, Massia S, Ehteshami G, Kim B and Raupp G 2004 Polyimide-based intracortical neural implant with improved structural stiffness *J. Micromech. Microeng.* **14** 32–7
[31] Meyer V R 1988 *Practical High-Performance Liquid Chromatography* (New York: Wiley)
[32] Neue U D 1997 *HPLC Columns* (New York: Wiley)
[33] Bidlingmeyer B A 1992 *Practical HPLC Methodology and Applications* (New York: Wiley)
[34] Giddings J C 1965 *Dynamics of Chromatography, Part I, Principles and Theory* (New York: Marcel Dekker)
[35] Rand W G and Mukherji A K 1981 Comparative study of efficiency and reproducibility of two high-performance gel

permeation chromatography columns with microparticulate packings *J. HRC CC* **4** 627–34
[36] Chang-Yen D A, Myszka D and Galea B K 2006 Novel PDMS microfluidic spotter for fabrication of protein chips and microarrays *JMEMS* **15** 1145–51
[37] Rogers J A and Nuzzo R G 2005 Recent progress in soft lithography *Mater. Today* 50–6
[38] Xia Y and Whitesides M 1998 Soft Lithography *Ann. Rev. Mater. Sci.* **28** 153–84
[39] Sasoglu F M, Bohl A J and Layton B E 2007 Design and microfabrication of a high-aspect-ratio PDMS microbeam array for parallel nanonewton force measurement and protein printing *J. Micromech. Microeng.* **17** 623–32
[40] Li M, Ai H, Mills D K, Lvov Y M, Gale B K and McShane M J 2002 Culturing smooth muscle cells on modified PDMS substrates *EMBS/BMES Conf.* vol 1 pp 388–9
[41] Yang Y and Chae J 2007 Miniaturized HPLC column with nano-pore beads for protein separation on flexible medical implants *IEEE MEMS 2007* pp 421–4
[42] Fredrickson C K and Fan Z H 2004 Macro-to-micro interfaces for microfluidic devices *Lab Chip* **4** 526–33
[43] Olah A, Hillborg H and Vancso G J 2005 Hydrophobic recovery of UV/ozone treated poly(dimethylsiloxane): adhesion studies by contact mechanics and mechanism of surface modification *Appl. Surf. Sci.* **239** 410–23
[44] Invitrogen, Inc., accessed Oct. 2007 https://catalog.invitrogen.com/index.cfm?fuseaction=viewCatalog.viewProductDetails&productDescription = 805&catname = North%20America%20Main
[45] BioRad, Gel Filtration Standard, accessed Oct. 2007 http://www.bio-rad.com/cmc_upload/Literature/12697/MSLIT-102E.pdf
[46] Agilent Technologies, Introducing the new HP 1100 series HPLC system, accessed Oct. 2007 http://www.chem.agilent.com/cag/peak/peak3–95/introducing.html
[47] ACE Comparison Guide, http://www.ace-hplc.com/assets/downloads/ACE_comparison_guide_2006.pdf
[48] Haghedooren E, Nemeth T, Dragovic S, Noszal B, Hoogmartens J and Adams E 2008 Comparison of two column characterisation systems based on pharmaceutical applications *J. Chromatogr.* A **1189** 59–71