Microfluidic system for electroelution of proteins from a clinical sampling strip

Sun Min Kim

Abstract A clinical methylcellulose sampling strip is one of the popular means for collecting gingival crevicular fluid (GCF) from dental patients for dental disease diagnosis. In this research, a microfluidic device for protein elution from a sampling strip was fabricated with poly (dimethylsiloxane) (PDMS) polymer. Electroelution experiments were performed with fluorescein isothiocyanate (FITC) dye labeled bovine serum albumin (BSA) and ovalbumin (OVA). The total amount of eluted protein is measured by quantitative fluorescence imaging. About 50% of the initial concentration of BSA and OVA was eluted by the \( \approx 20 \) V/cm electric field. Electroelution is an appealing method for protein elution; however, the thickness of the wet strip (\( \approx 400 \) µm) introduces interesting practical difficulties. During the electroelution process, unsteady electrokinetic phenomena by the pressure driven flow and the pH change of the reservoirs were observed. Several possible solutions to these problems are still under investigation including modifying reservoirs and thin polymer film coating of PDMS channel surfaces. This electroelution device would be a useful component of a fully integrated micro total analysis system for oral fluid samples.

1 Introduction

Development of microfluidic systems for analyzing chemical and biological samples has significantly increased in the past decade (Burns et al. 1998; Harrison et al. 1993; Manz et al. 1990; Sanders and Manz 2000). These microfluidic systems have played an important role in DNA analysis and sequencing (Burns et al. 1998; Schmalzing et al. 1998), drug delivery (Effenhauser et al. 1997), clinical diagnostics (Pal et al. 2005; Schulte et al. 2002), and biological/chemical agent detection (Anderson et al. 2000; Vandenberg et al. 1993; Vanderschoot et al. 1991) due to their advantages over conventional assay systems such as decreased costs for manufacture, use, and disposal; decreased time of analysis; reduced consumption of reagents and analysis; reduced production of potentially harmful by-products; increased separation efficiency; and increased portability (Anderson et al. 2000; Sanders and Manz 2000).

When biological analysis is performed using a microfluidic system in clinical applications, interfacing macroscale sampling methods to microfluidic systems is one of the outstanding challenges. Typically, this involves transporting the sample molecules in a reliable, repeatable manner from an (imperfect) sampling device to a microchannel, and preferably without too much dispersion or dilution; it may also require sample pretreatment to preliminarily separate the analyte of interest from particles, cells, or other interfering compounds, or to enhancing the sensitivity of subsequent analysis and detection by some form of tagging that involves some sort of chemical reaction. Depending on the sampling methods available in a given application, this may be straightforward.

Electroelution have been widely used for extraction of DNA and proteins captured in a gel or other macro scale matrix for biological and proteomic analysis by exploiting electrokinetic mobility of charged molecules under applied high electric voltage (Ahokas 1987; Antal et al. 2007; Chang et al. 2001; Chang et al. 1987).
The aim of this study is to develop a microfluidic device for electroelution of proteins from a clinical methylcellulose sampling strip (so called “sampling strip” hereafter), which is one of the popular means for collecting gingival crevicular fluid (GCF) from dental patients, for analysis of disease biomarkers (Griffiths 2003). Analysis of proteins in oral fluids, especially saliva and gingival crevicular fluid, has great potential for diagnosing oral disease as well as systemic disease, monitoring levels of environmental toxins, and detecting drugs-of-abuse. For example, proteins such as Interleukin-1 (IL-1), endotoxin, and C-telopeptide of type I collagen (ICTP) in oral fluids are putative biomarkers of periodontal diseases (Giannobile et al. 1995). The early diagnosis of periodontal disease may help facilitate intervention into the treatment of patients presenting with subclinical disease. Recently, Herr et al. presented a microfluidic immunoassay system for rapid clinical diagnostics of saliva (Herr et al. 2007). This system demonstrated the rapid measurement of the collagen-cleaving enzyme matrix metalloproteinase-8 (MMP-8) in saliva, which is the biomarkers for the periodontal disease. However, this microfluidic system requires the off-device sample preparation process including centrifugation and chemical pretreatment before the on-chip analysis.

In this present work, we fabricated a microfluidic device for protein elution from a sampling strip with poly(dimethylsiloxane) (PDMS), and presented the electroelution of two model proteins, bovine serum albumin (BSA) and ovalbumin (OVA). This device could be integrated with the analysis device and sequentially perform the sample preparation and analysis in one device.

2 Materials and methods

2.1 Device fabrication

The electroelution device shown in Fig. 1a was fabricated using PDMS elastomer (Folch et al. 1999; McDonald et al. 2000; McDonald et al. 2001). Molds for casting PDMS are typically produced by the photolithograph method using SU-8 or other photoresist on top of a silicon wafer. However, the sampling strips used here (Periopaper, Pro Flow Inc., Amityville, NY, USA) are quite large (Dimensions of sample collection part: length = 6.54 mm, width = 2.79 mm, and thickness = 160 μm (dry) ~ 400 μm (wet)), so the mold for casting was simply fabricated using laser-cut sheets of 450 μm-thick PEEK plastic (McMaster-Carr, Aurora, OH, USA) instead of photolithography process. The PEEK plastic sheet was cut with a CO₂ laser cutter and the cut part was bonded to the bottom of a Petri dish using epoxy (Loctite® Quickset, Henkel corp., Mentor, OH, USA). A 10:1 mixture of the PDMS prepolymer and the curing agent (Sylgard 184, Dow Corning, Midland, MI, USA) was prepared using a typical electric hand mixer and placed in a vacuum to evacuate any bubbles created during mixing. The uncured mixture was poured into the Petri dish, and cured about 24 hours at room temperature. The size of the channel pattern on the PDMS is 18 mm × 6 mm × 450 μm (Fig. 1b).

After curing, a PDMS slab with the designed pattern was detached from the mold and access holes were punched through the PDMS slab using a metal hole punch (ID = 3 mm) to allow introduction of fluid samples into the channels. This PDMS slab was bonded with another flat PDMS slab after 1 min treatment with air plasma of 100 W RF power in ~300mTorr vacuum using a PlasmaPrepII system (SPI supplies, West Chester, PA, USA) and this bonded object was placed atop a glass microscope slide for structural strength.

Finally, large reservoirs manufactured using PMMA cut to the desired size (16 × 10 × 5 mm) are bonded above the access holes on the PDMS slab using a low fluorescence and transparent UV curing optical adhesive (Optical Adhesive 68, Norland, New Brunswick, NJ) to reduce

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Fig. 1 A 3D schematic drawing of an electroelution device and the shape of a microchannel. a The micro-device is composed of a PDMS slab which has a patterned microchannel, a flat PDMS substrate, a slide glass and two sample reservoirs. b The microchannel is 7 mm—wide, 18 mm—long and 450 μm—deep. A methylcellulose sampling strip (Dimensions of sample collection part: length = 6.54 mm, width = 2.79 mm, and thickness = 160 μm) is inserted to the channel through the insertion port.
optical distortion of fluorescence detection. Reservoirs were designed to be quite wide in order to minimize pressure driven flow generated by head height difference, which arise as a result of large EOF flow rate through the large cavity cross-section (discussed in detail later).

2.2 Sample preparation and experiment

Phosphate buffer (20 mM, pH 7.2) solution was used for the buffer system. Two protein samples, Fluorescein isothiocyanate conjugate BSA (Sigma-Aldrich, St. Louis, MO, USA) and OVA (Molecular Probes, Eugene, OR, USA), were prepared for the electroelution experiments to detect the movement of protein using a fluorescent microscope. All protein samples were kept in a freezer to prevent deterioration and all liquid samples were filtered with a 0.2 μm syringe filter (Whatman, Maidstone, UK) to remove particulates.

Before each experiment, the PDMS device was treated with an air plasma (100 W, *300mTorr) for 1 min to oxidize the surface so that it becomes hydrophilic. The cavity was then immediately filled with phosphate buffer solution. PDMS surface properties are quite unstable when exposed to air, changing significantly in a matter of hours, but they are much more stable when in contact with water (Ren et al. 2001).

Sample collection part of the sampling strip was soaked with 2 μL of FITC-labeled protein samples (30 μM BSA or 40 μM OVA) and inserted into the cavity. Then, electric fields were applied across the cavity using a high voltage power supply (PS350, Stanford Research Systems, Sunnyvale, CA) through bright platinum electrodes placed in the reservoirs. The protein elution was monitored by an inverted fluorescence microscopic system (IX-71, Olympus, Japan) equipped with a spectral filter set for FITC (488 nm) and a 100 W mercury lamp. A CCD (Charge-coupled device) camera (Hamamatsu, Japan) was mounted on the microscope for image acquisition and IPlab 3.6 software (Scanalytics, Fairfax, VA, USA) was used for camera control and image processing. To prevent photo-bleaching of FITC, a neutral density filter (ND 1.0, Omega Optical, Brattleboro, VT, USA) was installed to reduce excitation light intensity and, using a shutter, the system was exposed to excitation only during image capture (≈2 s).

For reliable quantification of eluted proteins, nearly all experiments were performed with a fresh new device. Background noise correction was performed with a pure dark image, and a flat-field correction (to correct for the distribution of illumination intensity from the UV lamp) was obtained by imaging fluorescence of a Schott colored glass filter (Model CG-520, Newport Corp., Irvine, CA, USA).

3 Results and discussion

The basic idea for the electroelution device is that the clinical sampling strip is introduced into a cavity, across which an electric field may then be applied and hence remove the proteins from the sampling strip. Electroelution of the protein was observed in the PDMS microfluidic channel after the electric field was applied between two reservoirs. Figure 2 shows a time-lapse sequence of fluorescence images during successful electroelution of BSA from a strip; a comparison experiment (not shown) of the OVA electroelution was performed with the same condition. At 1 min after ~20 V/cm of the electric field is applied, elution of BSA from a sampling strip was observed. During electroelution a relatively small electric field (<20 V/cm) was applied to avoid excessive Joule heating, so the electroelution took approximately 10 min to complete. After 10 min, no more fluorescence was detected at downstream and electroelution was complete. The eluted protein molecules were observed at the anode side reservoirs as expected (not shown).

Figure 3 quantifies the elution of these proteins from a sampling strip using quantitative imaging methods (ensuring no dye bleaching by exposing only momentarily at 1-min intervals). The amount of eluted proteins is quantified by measuring the fluorescent intensity changes of BSA and OVA on the strip with respect to time. This result shows that approximately 50% of the protein can be eluted by weak electric fields in about 10 min. It is noted that the ~20 V/cm of the electric field is much lower than usual electric field strength (~10 kV/m) that is used for electrophoresis; this is required to avoid Joule heating in this extraordinarily thick cavity.

As shown in Fig. 3, the elution speed of OVA is somewhat faster than that of BSA and this difference is caused by the electrophoretic mobility and mass difference of two proteins. This result is not inconsistent with published data at this buffer pH (Basak and Ladisch 1995).

Although these experimental results show that electroelution can be highly efficient at protein elution, there can be numerous complications due to the thickness of the wetted strips (~400 μm). The thickness introduces interesting practical difficulties including significant reservoir filling by relatively large total electroosmotic flow (EOF), as well as pH evolution of buffer in the reservoirs due to the current drawn by the system.

The most obvious complication is caused by bulk fluid flow induced by relatively large total EOF. Microfluidic channel surfaces are constructed with negatively charged PDMS and applied electric voltage causes bulk EOF from anode to cathode reservoir. EOF is adverse to the electrophoretic elution of negatively charged proteins and slows protein elution down. EOF also increases head height on
the cathode side reservoir, generating pressure driven flow (PDF) to the anode side (See Fig. 4).

Figure 5a and b show the bright secondary flow from the right (cathode) to the left (anode) induced by the PDF. At the initial stage of elution, PDF wave did not appear but after 3 min typical parabolic-shape PDF wave suddenly come into view. This PDF is due to the pressure increase on the cathode side reservoir high enough to break up the hydrodynamic equilibrium. Even though PDF takes proteins from a sampling strip and speeds the net motion of proteins to anode side reservoir, but PDF causes the higher dispersion of eluted molecules than the electroelution and the elution process by the PDF is hard to be controlled.

The PDF effect can be avoided using large cross sectional area reservoirs to reduce the head height difference
and thin film surface modification with polymers to suppress the zeta potential of the PDMS surface and EOF (Hu et al. 2002; Kirby et al. 2003). At the same time, the surface modification can reduce the adsorption of protein on the PDMS channel surface, which could have deleterious effects on the sensitivity and quantitative accuracy of the system.

The second, more interesting complication results from buffer depletion in the reservoirs, which results in sudden changes in pH. Because of hydrolysis in the reservoirs, the anode reservoir becomes more acidic and the cathode reservoir becomes more basic. The pH changes in each reservoir are expressed by

Anode side: \[ 2\text{H}_2\text{O}(l) \rightarrow \text{O}_2(g) + 4\text{H}^+ + 4e^- , \text{pH decrease} \]

Cathode side: \[ 2\text{H}_2\text{O}(l) + 2e^- \rightarrow \text{H}_2(g) + 2\text{OH}^- (aq) , \text{pH increase} \]

H\(^+\)s are produced at the anode, which is buffered by converting HPO\(_4\)\(^{-2}\) to H\(_2\)PO\(_4\)\(^{-}\) in phosphate buffer until the HPO\(_4\)\(^{-2}\) gets used up. The moles of consumed buffer during electroelution can be calculated by

\[
\text{Moles of consumed buffer} = \frac{I \cdot t}{F}
\]

where \(I\) is current, \(t\) is time, and \(F\) is Faraday constant (96,500 C/mol). The measured current during electroelution with 20 V/cm electric field was 0.883 mA and the reservoir was filled with 0.6 ml of buffer solution. The calculated time for buffer depletion is about 11 min and this time period limits the running of electroelution.

Buffer depletion caused very interesting and unsteady stirring phenomena in the channel as shown in Fig. 5c and d. Since OH\(^-\) and H\(^+\) are very high mobility ions, this results in a relatively fast propagation of a wave of relatively high or low pH through the channel. The dark stirring wave from the left (anode) to the right (cathode) shows the unsteady pH wave propagation. Unsteady pH wave was observed around 11 min after electric field was applied and this time is quite similar with the buffer depletion time. The dependence of wall zeta potential and electrophoretic mobility of the fluorescent proteins (plus the pH dependence of their fluorescence) complicates the situation. The overall dynamics of the system can be closely related to a phenomenon known as Teorell’s oscillations (Pastushenko 1997).

One solution to this problem is to simply make large reservoirs that hold a large amount of buffer. However, this is not always practical. More reasonable solution to this problem is to use a split-reservoir design similar to a standard electrode, using an ion-selective membrane to prevent pH evolution in the fluid surrounding the platinum electrode from being transmitted immediately into the electroelution reservoir.
4 Conclusions

A microfluidic device for protein elution from a methylcellulose sampling strip used for collecting oral fluids was developed and elution experiments with two model proteins (BSA and OVA) were performed. A microfluidic device was fabricated using PDMS polymer for easy fabrication and rapid prototyping. About the 50% of the initial concentration of the sample proteins (BSA, OVA) was eluted by the ~20 V/cm electric field. This result showed that electroelution is a useful method for introducing analytes from macroscale sampling strip into microfluidic system.

Electroelution is an appealing method for protein removal, however, the thickness of the wet strips (~400 μm) introduces interesting practical difficulties. During this elution process, the PDF wave (the direction of PDF is from cathode to anode) caused by relatively large total EOF and the unstable wave (the direction of unstable wave is from anode to cathode) by the pH change in the reservoirs resulting from the buffer depletion were observed. These problems can be solved by coating PDMS channel surface with thin polymer film and modifying reservoirs: large cross sectional area reservoirs and split-reservoir using ion selective membrane. This electroelution device would be integrated with a protein concentrator and a separation column to analyze proteins in a sampling strip. This integrated device will be developed by following research in the future.

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References


