Published in final edited form as:

Anal Chem. 2009 August 15; 81(16): 6837–6842. doi:10.1021/ac901114k.

Continuous Operation of Microfabricated Electrophoresis Devices for 24 Hours and Application to Chemical Monitoring of Living Cells

Kendra R. Reid[†] and Robert T. Kennedy^{*,†,‡}

[†]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109

[‡]Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48109

Abstract

Microchip electrophoresis is an emerging analytical technology with several useful attributes including rapid separation time, small sample requirements, and automation. In numerous potential applications, such as chemical monitoring or high-throughput screening, it may be desirable to use a system for many analyses without operator intervention; however, long term operation of microchip electrophoresis systems has received little attention. We have developed a microchip electrophoresis system that can automatically inject samples at 6 s intervals for 24 h resulting in collection of 14,400 assays in one session. Continuous operation time of a prototype of the device was limited to 2 h due to degradation of reagents and electrophoresis buffers on the chip; however, modification so that all reagents were continuously perfused into reservoirs on the device ensured fresh reagents were always used for analysis and enabled extended operating sessions. The electrophoresis chip incorporated a cell perfusion chamber and reagent addition channels to allow chemical monitoring of fluid around cells cultured on the chip by serial electrophoretic immunoassays. The immunoassay had detection limits of 0.4 nM for insulin and generated ~4% relative standard deviation over an entire 24 h period with no evidence of signal drift. The combined system was used to monitor insulin secretion from single islets of Langerhans for 6 to 39 h. The monitoring experiments revealed that islets have secretion dynamics that include spontaneous oscillations after extended non-oscillating periods and possible ultradian rhythms.

INTRODUCTION

Microchip electrophoresis has been intensely studied over the past 15 years primarily due to its small sample volume requirements, high speed, efficiency, and potential for integration with other on-chip functions.^{1–5} In numerous applications of microchip electrophoresis, it would be desirable to operate the devices for many cycles of analysis over long periods without requiring operator intervention or re-conditioning of the chip. Nevertheless, most studies of electrophoresis chips report only short-term operation (typically < 2 h) with little reference to longer-term operation. A commercial system (Caliper Life Sciences LabChip 3000) that allows relatively long-term operation is available. This system uses vacuum to constantly pull fresh buffer through the electrophoresis channel. While effective, the use of hydrodynamic flow through the electrophoresis channel limits separation resolution. The system itself is designed specifically for drug screening applications and therefore is not necessarily amenable to other applications. A few studies of longer-term operation of capillary electrophoresis have been reported.⁶ These studies have suggested that performance with repetitive injections over longer

*Corresponding author. Telephone: 734-615-4363. Fax: 734-615-6462. rtkenn@umich.edu.

periods is limited by detector and injector fluctuations, buffer instability, and temperature variations.^{6, 7} In this work we have developed a microfluidic electrophoresis chip that is capable of serial injections at 6 s intervals for 24 h. We further integrated the electrophoresis system with a cell chamber and on-line pre-column reaction to allow continual monitoring of cell secretions during the chip operating period.

The long-term microfluidic device is applied to monitoring insulin secretion from single islets of Langerhans. Islets are microorgans composed of approximately 2000-4000 cells, 70-80% of which are insulin-secreting β -cells.⁸ At elevated glucose concentration, β -cells are stimulated to secrete insulin,⁹ which is crucial for maintaining glucose homeostasis.¹⁰ The most common methods of measuring insulin secretion from islets are radioimmunoassays (RIAs) or enzyme-linked immunosorbent assays (ELISAs); however, these methods are not well-suited for continual monitoring at high temporal resolution for long periods. Our laboratory previously developed a microfluidic electrophoresis chip capable of monitoring insulin secretion at 6 s intervals.¹¹ The system incorporates electrophoretic sampling from islets, on-line immunoreactions, and an electrophoresis-based competitive immunoassay for insulin.¹² The temporal resolution of this system is sufficient to capture dynamics of insulin secretion, such as first phase and oscillations of insulin secretion that have periods of 3-5 min. This device has been extended to parallel operation^{13, 14} and has been used in several applications to study acute insulin secretion.¹⁵⁻¹⁷ While the device has excellent temporal resolution and utility for short experiments, continuous operation for longer than about 2 h results in degradation of performance due, at least in part, to alterations in the buffer, likely because of evaporation and electrolysis.

Our interest in extending the operation time of the chip stemmed from indications of longer-term phenomena at islets that are of significance. For example, oscillations of insulin with periods of approximately 2 h (ultradian rhythms)¹⁸ and 24 h (circadian rhythms)¹⁹ have been observed using RIAs and ELISAs both *in vivo* and in collections of isolated islets. These slow oscillations are important for optimal insulin action throughout the body²⁰ and are impaired in diabetics.²¹ Because of the limited ability to study these phenomena, it is not known if isolated, single islets can maintain these slow rhythms, nor is it known if the slow waves are comprised of increases in magnitude of known faster oscillations (~5 min period) or due to periodic fluctuations in other mechanisms of insulin secretion. Another slowly evolving phenomenon at islets is the effect of fatty acids. Acute treatment of islets with fatty acids enhances insulin secretion but longer exposures impair insulin release. The transition from enhancing to impairing and effects on faster fluctuations, such as oscillations, is not well understood. The ability to monitor secretion over longer periods at high temporal resolution would allow such effects to be studied.

In this work, we describe a new chip design that is compatible with continuous operation for over 24 h. With the device, insulin secretion from individual islets was monitored for 24 h resulting in the collection of 14,400 immunoassays for a single experiment. The improved insulin immunoassay capabilities enabled observation of insulin secretion dynamics that are not feasible with other immunoassay techniques. The results presented here demonstrate the potential of extended electrophoresis assay sessions and the utility for long-term monitoring of the chemical environment of cells.

EXPERIMENTAL SECTION

Chemicals and Reagents

Tricine, electrophoresis grade, was obtained from MP Biomedicals (Aurora, OH). Fluorescein isothiocyanate-labeled insulin (FITC-ins) was purchased from Molecular Probes (Eugene, OR), and monoclonal antibody (Ab) to human insulin was purchased from Biodesign

International (Saco, ME). Cell culture reagents were purchased from Invitrogen (Carlsbad, CA). Collagenase type XI, Tween 20, ethylenediaminetetraacetic acid (EDTA), and insulin were obtained from Sigma (St. Louis, MO). All other chemicals were from Fisher (Pittsburgh, PA). All solutions were made using Milli-Q (Millipore, Bedford, MA) 18 M Ω deionized water and filtered using 0.2 μ m nylon syringe filters (Fisher). Stock antibody solution was stored at 4 °C in the manufacturer-provided phosphate-buffered saline. Stock FITC-ins was diluted to 166 μ M in immunoassay reagent buffer and stored at -20 °C.

Balanced salt solution (BSS) contained 125 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl₂, 2.4 mM CaCl₂, 25 mM tricine, and 0.7 mg mL⁻¹ bovine serum albumin (BSA), adjusted to pH 7.4. Immunoassay reagent buffer contained 60 mM NaCl, 1 mM EDTA, 20 mM tricine, 0.1% (w/v) Tween 20, and 0.7 mg mL⁻¹ BSA, adjusted to pH 7.4. Electrophoresis buffer was 20 mM NaCl and 150 mM tricine, adjusted to pH 7.4.

Microfluidic Chip Fabrication and Preparation

The microfluidic devices were fabricated using a previously described method.^{22, 23} Briefly, 1 mm thick Borofloat photomask blanks (2.5 cm $\times$ 7.6 cm) were exposed to a collimated UV light source (Optical Associates, Inc., Milpitas, CA) for 5 s through a patterned photomask (Digidat, Pasadena, CA). The blanks, purchased from Telic Co. (Santa Monica, CA), had a 530 nm layer of AZ1518 positive photoresist on a 120 nm chrome layer. The exposed photomasks were developed in AZ915 MIF developer (Clariant Corp., Summerville, NJ) and the exposed chrome was developed in CEP-200 chrome etchant (Microchrome Technologies, Inc., San Jose, CA). The exposed glass was etched in 14:20:66 (v/v/v) HNO₃:HF:H₂O for 20 min to create channels 12 μ m deep. Diamond-tipped drill bits (Tartan Tool Co., Troy, MI) were used to drill 360 μ m diameter access holes. The remaining photoresist was removed with acetone, and the remaining chrome was removed with the CEP-200 chrome etchant. The etched chips and blank coverplates were placed in piranha solution (3:1, v/v, H₂SO₄:H₂O₂) to remove organic oxides, and the plates were subsequently placed in a heated RCA solution (5:1:1, v/v/v, H₂O:NH₄:H₂O₂) to increase glass hydrophilicity. The plates were bonded at 640 °C under vacuum in a Neytech Centurian Qex furnace (Pacific Combustion, Los Angeles, CA). Microfluidic reservoirs (Upchurch Scientific, Oak Harbor, WA) were applied to the device over drilled access holes after bonding. The chip was conditioned prior to experiments by flowing 0.1 M NaOH through the channels, followed by deionized water and then experimental solutions.

Microfluidic Chip Operation

The layout of the microfluidic channels in the device and diagrams illustrating chip operation are shown in Figure 1. All solutions were filtered prior to use to prevent introduction of particulates to the chip, as well as to prevent degradation of the solutions. During chip operation, all solutions were continuously pumped onto the chip via fused-silica capillaries inserted into vials pressurized with 5-30 psi of helium (Figure 1B and C). In this way, fresh buffer was continually provided to all reservoirs. Solutions in excess of 100 μ L in the electrophoresis reservoirs were continuously removed via fused-silica capillaries connected to a vacuum manifold (Figure 1B and C). Negative high voltage (-6 kV) was applied at the waste reservoir of the device, and all other reservoirs were grounded. Solution sampled electrophoretically from the islet reservoir mixed with 50 nM FITC-insulin and 25 nM Ab in immunoassay reagent buffer on a heated reaction channel. Electrophoresis buffer was pumped into the gate and waste reservoirs. The gate reservoir was connected to ground by either a gas-filled (model K61C241) or a vacuum dielectric (model SO5LTB245) high voltage relay (Kilovac, Santa Barbara, CA). Injections were performed by opening the relay as described elsewhere.²⁴ Laser-induced fluorescence (LIF) detection occurred 1 cm downstream the injection cross. Each separation resulted in a zone of FITC-ins bound to antibody (B) zone

followed by a free FITC-ins (F) zone. The ratios of the heights of the bound and free peaks (B/F) were used to quantify insulin by comparison to a calibration curve.

Islets were perfused with BSS containing glucose at $1.0 \mu\text{L min}^{-1}$. For islet experiments, a single islet was placed in the grounded islet reservoir and perfused with BSS while perfusate from the islet was sampled by electroosmotic flow. For calibration, grounding was changed from the islet reservoir to the insulin standards reservoir.

Detection

LIF detection was performed with a Zeiss Axiovert 35M inverted microscope equipped with a Photon Technology International 814 photometer (Lawrenceville, NJ). Excitation light (488 nm) from a 20 mW optically-pumped semiconductor Sapphire laser (Coherent, Santa Clara, CA) was directed onto a 500 nm long-pass dichroic mirror and through a 40X, 0.6 numerical aperture, long working distance objective (Carl Zeiss, Inc., Thornwood, NY). After passing through the dichroic mirror, the emission light passed through a 530 ± 30 nm band-pass filter. The fluorescence emission was further spatially filtered by an iris diaphragm on the photometer. Instrument control and data collection were performed using LabVIEW software written in-house (National Instruments, Austin, TX). High-throughput analysis of collected electropherograms was performed using Cutter software.²⁵

Islet Isolation Protocol

Pancreatic islets were obtained from 20- to 30-g male CD-1 mice as previously described.²⁶ Briefly, mice were sacrificed by cervical dislocation, and collagenase type XI was injected into the pancreas through the main pancreatic duct. The pancreas was removed and incubated in 5 mL of a collagenase solution at 37 °C. A Ficoll gradient was used to separate islets from exocrine tissue. Islets that were used for experiments were 100-200 μm in diameter, had an intact islet membrane, and were oblong to spherical in shape. Islets were placed in tissue culture dishes and incubated in RPMI 1640 containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 $\mu\text{g/mL}$ of streptomycin at 37 °C, 5% CO_2 , pH 7.4. Islets were used 3-7 days following isolation.

RESULTS AND DISCUSSION

Microfluidic Device for Stable Long-term Electrophoresis

The microfluidic chip developed for long-term electrophoresis-based insulin secretion monitoring is illustrated in Figure 1. Rather than using static reservoirs of buffers and reagents as is typical, this design allowed all buffers and reagents to be continuously perfused into the chip via pressure-driven flow (see Figure 1). In this way, fresh buffer could be continually pulled by electroosmotic flow into the microfluidic channels. In the device, a single islet was placed in a chamber on the chip and continuously perfused with BSS, a physiological saline solution. For long-term operation, it was necessary to build into the device the ability to periodically calibrate the immunoassay, even in the presence of cells, so that the stability of calibration could be monitored. To calibrate the device with the islet present, the electrical connection to the islet reservoir was removed and the insulin standard reservoir was grounded, allowing the electrophoretic sampling of insulin standards while maintaining perfusion of the islet. This separate channel also eliminated the need to flow exogenous insulin through the islet chamber for calibration while the islet was present.

Initial experiments were performed to demonstrate the effectiveness of continuous perfusion of buffers for maintaining long-term electrophoresis stability (Figure 2). If the chip was operated without constant buffer perfusion while collecting electropherograms every 6 s, then the resulting plot of B/Fs calculated from serial electropherograms (Figure 2A) showed that

the immunoassay became unstable within a few hours of operation. After only 2 h, the relative standard deviation (RSD) of B/F was 7%, but after 3 h the RSD had increased to 20% largely due to downward drift. Comparison of electropherograms (Figure 2B) collected at the start of the immunoassay operation and after 3 h of immunoassay operation reveal that after extended operation electroosmotic flow was significantly diminished, resolution between the bound and free peaks was decreased, and the peak heights were significantly lower. In contrast, if the perfusion system was used, the immunoassay stability was much improved with little drift and an RSD of 4% for B/F from the 14,400 electropherograms collected over 24 h (see Figure 2C). An overlay of an electropherogram collected at the start of the immunoassay series and an electropherogram collected after 24 h of continuous chip operation demonstrates that with perfusion, the migration time and peak heights were stable over 24 h (Figure 2D). The results shown in Figure 2C are representative of four identical experiments, wherein the average RSD over 24 h was 5%.

Although the above results show good performance for 24 h, initial experiments had revealed that the B/F was only stable for approximately 12 to 15 h, at which point a gradual decline in both B and F signals occurred (Supporting Information, Figure S1). It was determined the loss of fluorescence signal was a result of failure of the high voltage relay used for controlling injections. Replacement of the gas-filled relay with a vacuum dielectric relay eliminated this problem and allowed attainment of the results shown in Figure 2.

At present, reliability of long-term operation of the chip is most limited by the formation of particulates that appear in the channels and ultimately cause blockage of flow. Approaches to prevent and or clear blockages are the most important changes to make to improve reliability and further extend the operation lifetime.

Calibration of Immunoassay and Limit of Detection of Insulin

We next evaluated the stability of the immunoassay calibration over 12 h (see Figure 3). For this experiment, the chip was calibrated before and after 12 h of continuous operation while sampling a 50 nM insulin standard. Figure 3B shows that calibration curves overlaid well with excellent fits to a variable slope sigmoidal dose response function ($R^2 = 0.999$) after 12 h of continuous operation. While the possibility of drift in the B/F cannot be precluded in any given experiment, the ability to calibrate the device without interrupting the perfusion of cells in the cell chamber allows one to correct for shifts in response.

The calibration data allow calculation of detection limits and evaluation of sensitivity. The detection limit, calculated as the concentration required to give a B/F that was at least 3 standard deviations less than the B/F for 0 nM insulin, was 0.4 nM. The assay was most sensitive in the range of 10 to 125 nM insulin which matches the majority of insulin fluctuations that are detected from islets. As with other competitive immunoassays, the sensitivity range can be adjusted by changing the antibody and tracer concentrations.²⁷

Islet Culture

We also evaluated the stability of cells housed on chip for 24 h. Because islets on the chip are maintained at physiological temperature and constantly perfused with fresh BSS they should remain viable on the chip during 24 h experiments. To investigate the viability of perfused islets cultured in the microfluidic device, islets were placed in 11 mM glucose in BSS in the islet reservoir maintained at 37 °C for 24 h. For some experiments ($n = 3$) the islet was not perfused while for others ($n = 3$) the islet was continuously perfused with fresh buffer. After 24 h with no perfusion, the islet membrane was disrupted and the islet began to lose its integrity. In contrast, constantly perfused islets appeared unchanged after the 24 h perfusion. Furthermore, the islets did not exhibit darkening of the core, which is indicative of necrosis.

(For photographs, see Supporting Information, Figure S2.) These results illustrate that, based on morphology, the islets appear healthy for a 24 h perfusion. It may be necessary to perfuse the islet with cell culture media, rather than BSS, which contains no nutrients other than glucose, for longer-term (> 24 h) culture.

Long-term Insulin Secretion

To illustrate the potential of the system for long-term cell monitoring, we used it to record insulin secretion from single islets following a step change from 3 to 11 mM glucose. We monitored 6 islets for up to 39 h. Of the tested islets, 4 were successfully monitored for 12 h or more, while others failed more quickly, typically due to clogs in the chip. Figure 4A illustrates the averaged data from these experiments, which include over 60,000 assays. Clearly, performing so many assays would require too much time, labor, and cost to be performed using conventional RIAs or ELISAs.

The averaged result shows an initial burst (see inset) of insulin secretion (first phase of release) followed by a lower plateau or second phase. The RSD of these experiments were typical of previous insulin secretion measurements on chips.¹⁴ These results also show that the second phase of insulin secretion can be maintained over this period despite the minimal nutrients available in the perfusion media.

More interesting information can be obtained by examining individual traces, such as those shown for islet recordings of 6 h (Figure 4B) and 12 h (Figure 4C), which illustrate differences in insulin secretion dynamics that can exist between islets. Although all of the islets showed an initial burst of insulin secretion, more variability was seen with regard to oscillations. We observed 3 islets that displayed distinct oscillations (3-5 min period) immediately after the first phase and throughout the trace (example in Figure 4B), 2 that had no initial oscillations but developed oscillations over 1 h after the step change in glucose (Figure 4C and Figure 5), and 1 that never showed oscillations (example not shown). For islets that oscillated, the size of individual bursts varied over the course of the measurement as shown in Figure 4B and 4C. Conclusions about the prevalence of these different patterns would require further study with a larger sample size. Nevertheless, these initial observations prove that islets have capability of maintaining periodic insulin secretion for at least 24 h in minimal media. Furthermore, they show oscillations can spontaneously generate even after a few hours of non-oscillatory release.

These observations can be extended by considering an islet that was successfully monitored for 24 h (see Figure 5A). In this case 3 point calibrations of the assay were performed at 0.0, 9.9, 12.0, 21.5, 22.9, and 23.9 h (Figure 5B). Insulin standards for the calibrations were chosen to fall on the linear portion of the dose response curve. The similar calibrations show that the calibration of the immunoassay system was stable over the course of the 24 h experiment and confirm the results obtained with standards shown in Figure 3 and the stability tests shown in Figure 2.

As shown by Figure 5C, this islet did not immediately begin to oscillate after the initial phase. Examining 1 h sections taken at different points in the recording (see Figure 5D) reveals that the islet repeatedly transitioned between oscillating release and non-pulsatile release. These transitions could be indicative of ultradian rhythms of secretion that occur in isolated islets. In such a case, the slower pulses with ~2 - 4 h period appear to be made up of bursts of more rapid 4-6 min oscillations. Although more replicates are necessary to reach firm conclusions about the presence and nature of ultradian oscillations in isolated islets, the data presented here demonstrate the capability of the long-term electrophoresis chip for monitoring the chemical environment of cells for long term at high temporal resolution.

Previously, the more time- and labor-intensive RIAs and ELISAs did not allow for the observation of such long-term insulin secretion phenomena at single islets. Results shown in Figure 4 and Figure 5 demonstrate the potential for the long-term electrophoresis device to serve as a system for characterizing long-term insulin secretion phenomena. The device could be used to investigate and optimize islet culture conditions, to examine ultradian and perhaps circadian rhythms of insulin secretion, and to examine other slowly developing effects. Results of such studies may further elucidate pathways that link impaired insulin secretion and type 2 diabetes.

CONCLUSIONS

We have developed a microfluidic chip capable of long-term unattended electrophoresis operation. With the automated device, 14,400 serial electrophoretic insulin immunoassays could be completed in 24 h enabling novel observations of insulin secretion dynamics. The device resulted in considerable time and cost savings compared with the conventional insulin assay techniques. With modifications, the long-term measurement device may be used for other applications requiring long term monitoring or high-throughput assays.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH R37 DK046960 (R.T.K.).

Literature Cited

- (1). Huang WH, Ai F, Wang ZL, Cheng JK. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 2008;866:104–122.
- (2). Ohno K, Tachikawa K, Manz A. *Electrophoresis* 2008;29:4443–4453. [PubMed: 19035399]
- (3). Roman GT, Kennedy RT. *J. Chromatogr. A* 2007;1168:170–188. [PubMed: 17659293]
- (4). Viskari PJ, Landers JP. *Electrophoresis* 2006;27:1797–1810. [PubMed: 16645944]
- (5). Wu DP, Qin JH, Lin BC. *J. Chromatogr. A* 2008;1184:542–559. [PubMed: 18207148]
- (6). Mayer BX, Muller M. *Lc Gc North America* 2000;18:694. +
- (7). Mayer BX. *J. Chromatogr. A* 2001;907:21–37. [PubMed: 11217027]
- (8). Kulkarni RN. *Int. J. Biochem. Cell Biol* 2004;36:365–371. [PubMed: 14687913]
- (9). Henquin JC. *Diabetes* 2000;49:1751–1760. [PubMed: 11078440]
- (10). Muoio DM, Newgard CB. *Nat. Rev. Mol. Cell Biol* 2008;9:193–205. [PubMed: 18200017]
- (11). Shackman JG, Dahlgren GM, Peters JL, Kennedy RT. *Lab Chip* 2005;5:56–63. [PubMed: 15616741]
- (12). Schultz NM, Huang L, Kennedy RT. *Anal. Chem* 1995;67:924–929. [PubMed: 7762828]
- (13). Dishinger JF, Kennedy RT. *Anal. Chem* 2007;79:947–954. [PubMed: 17263320]
- (14). Dishinger JF, Reid KR, Kennedy RT. *Anal. Chem* 2009;81:3119–3127. [PubMed: 19364142]
- (15). Gerin I, Dolinsky VW, Shackman JG, Kennedy RT, Chiang SH, Burant CF, Steffensen KR, Gustafsson JA, MacDougald OA. *J. Biol. Chem* 2005;280:23024–23031. [PubMed: 15831500]
- (16). Ueki K, Okada T, Hu J, Liew CW, Assmann A, Dahlgren GM, Peters JL, Shackman JG, Zhang M, Artner I, Satin LS, Stein R, Holzenberger M, Kennedy RT, Kahn CR, Kulkarni RN. *Nat. Genet* 2006;38:583–588. [PubMed: 16642022]
- (17). Morioka T, Asilmaz E, Hu J, Dishinger JF, Kurpad AJ, Elias CF, Li H, Elmquist JK, Kennedy RT, Kulkarni RN. *J. Clin. Invest* 2007;117:2860–2868. [PubMed: 17909627]
- (18). Simon C, Brandenberger G. *Diabetes* 2002;51:S258–S261. [PubMed: 11815489]

- (19). Peschke E, Peschke D. *Diabetologia* 1998;41:1085–1092. [PubMed: 9754828]
- (20). Sturis J, Scheen AJ, Leproult R, Polonsky KS, Vancauter E. *J. Clin. Invest* 1995;95:1464–1471. [PubMed: 7706450]
- (21). Sturis J, Polonsky KS, Shapiro ET, Blackman JD, Omeara NM, Vancauter E. *Diabetologia* 1992;35:681–689. [PubMed: 1644248]
- (22). Roper MG, Shackman JG, Dahlgren GM, Kennedy RT. *Anal. Chem* 2003;75:4711–4717. [PubMed: 14674445]
- (23). Manz A, Harrison DJ, Verpoorte EMJ, Fettingner JC, Paulus A, Ludi H, Widmer HM. *J. Chromatogr* 1992;593:253–258.
- (24). Jacobson SC, Ermakov SV, Ramsey JM. *Anal. Chem* 1999;71:3273–3276.
- (25). Shackman JG, Watson CJ, Kennedy RT. *J. Chromatogr. A* 2004;1040:273–282. [PubMed: 15230534]
- (26). Pralong WF, Bartley C, Wollheim CB. *EMBO J* 1990;9:53–60. [PubMed: 2403930]
- (27). Taylor J, Picelli G, Harrison DJ. *Electrophoresis* 2001;22:3699–3708. [PubMed: 11699908]

Figure 1.

Microfluidic chip for long-term electrophoresis operation and perfusion scheme. (A) Channel layout of a microfluidic device for monitoring insulin secretion for 24 hours. Solid lines indicate microfluidic channels (12 μm deep and a half-width of 26 μm), while electrical connections are indicated by dotted lines. Circles indicate perfusion inlets and squares indicate reservoirs that are sampled by electroosmotic flow. All fluidic access holes are 360 μm diameter. Arrows indicate direction of perfusion flow. The shaded portion of the chip is heated with a thin film resistive heater. LIF occurred 1 cm past injection point, as indicated by the star. -HV is position of the high voltage applied. (B) Side view of islet chamber. Pressure-driven fluid is perfused into a 0.1 μL chamber and flows into a 100 μL fluidic reservoir. Solution from the chamber is

sampled by electroosmotic flow (EOF) through the sampling channel at a rate of approximately 2 nL min^{-1} . Excess solution is constantly removed by vacuum. (C) Scheme for perfusing reservoirs. Perfusion buffer supply is pressurized and fluid flows into the chip providing a constant supply of buffer that is sampled by electroosmotic flow.

Figure 2.

Immunoassay and electrophoresis stability. (A) B/F from serial injections of 10 nM insulin at 6 s intervals on a chip with no perfusion of reservoirs. (B) Electropherograms collected at 3 min and 3 h of immunoassay operation for the experiment without perfusion. (C) B/F from serial injections of 10 nM insulin at 6 s intervals on a chip with all reservoirs perfused as described in the experimental section. (D) Electropherograms collected at 5 min and 24 h of immunoassay operation for the experiment with perfusion.

Figure 3.

Stability of immunoassay calibration over a 12 h period. (A) B/Fs calculated from consecutive electropherograms collected while perfusing 50 nM insulin into the chip. (B) Overlay of calibration data obtained at 0 h and at 12 h of chip operation. Error bars indicate ± 1 standard deviation. The mean and standard deviation were calculated from 10 electropherograms. (C) Insulin concentrations during 12 h perfusion of 50 nM insulin. Insulin concentrations were calculated from the B/Fs shown in A using the calibration data shown in B.

Figure 4.

Long-term insulin secretion data. (A) Average of insulin secretion from 6 islets. n indicates the number of islets included in the average for the different periods. The error bars indicate ± 1 standard error of the mean. The inset is an enlarged version of averaged insulin secretion for the first hour. (B) and (C) insulin secretion from single islets monitored as glucose was raised from 3 to 11 mM glucose. Time of glucose change is indicated by an arrow.

Figure 5.

Recording of islet insulin secretion for 24 h. (A) Example of 24 h insulin release from a single islet monitored as glucose concentration was raised from 3 to 11 mM. The islet was perfused with BSS and glucose at 1.0 mL min^{-1} . (B) Calibrations of the immunoassay were performed at 0.0 h, 9.9 h, 12.0 h, 21.5 h, 22.9 h, and 23.9 h to measure any drift in calibration over the course of the 24 h monitoring period. Symbols for calibration curve match the time point on the trace in (A) where the calibration was taken. (C) Expanded scale view of first hour of insulin release. (D) Expanded scale of every other hour of insulin secretion dynamics shown in (A).