Published in final edited form as:
Anal Chem. 2008 May 1; 80(9): 3507–3511. doi:10.1021/ac800157q.

Self-Sealed Vertical Polymeric Nanoporous-Junctions for High-Throughput Nanofluidic Applications

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Abstract

We developed a reliable but simple integration method of polymeric nanostructure in a poly-dimethylsiloxane (PDMS)-based microfluidic channel, for nanofluidic applications. The Nafion polymer junction was creased by infiltrating polymer solution between the gaps created by mechanical cutting, without any photolithography or etching processes. The PDMS can seal itself with the heterogeneous polymeric nanoporous material between PDMS/PDMS gap due to its flexibility without any (covalent) bonding between PDMS and polymer materials. Thus, one can easily integrate nanoporous-junction into PDMS microchip in a leak-free manner with excellent repeatability. We demonstrated nanofluidic preconcentration of proteins (β-phycoerythrin) using the device. Because the polymeric junction spans across the entire microchannel height, the preconcentration was achieved with high pressure field or even in large channels, with the dimension of 1000μm width × 100μm depth.

INTRODUCTION

Much attention has recently been drawn to various nanofluidic systems, both in the light of bio/chemical analysis [1-3] and fundamental physics [4-6], enabled by the advances in micro/nanofabrication technologies [7-10]. Compared with microfluidic systems, nanofluidic channels and filters exhibit unique properties such as concentration polarization [3,4,11,12] and ion rectification [13,14] due to electrical double layer overlap, reduced surface drag [15, 16], and various separation / filtration modes of biomolecules [1,2]. The concentration polarization is the generation of concentration gradients of ionic species adjacent to a perm-selective nanochannel (or membrane) under dc bias. Due to the preferential transport of cation through the nanochannel, the concentration of both cations and anions decreased in the anodic side (ion depletion), while it increased in the cathodic side (ion enrichment) in order to meet overall electroneutrality [17]. Most of these nanofluidic systems are built on rigid substrates such as glass or silicon, utilizing standard microfabrication techniques in a clean room facility. However, it is noteworthy that most of these nanofluidic systems are based on the confinement of molecules/fluids in a small channel, which can also be realized using nanoscale beads [18-20], nanocapillary arrays [21] or polymeric nanostructures such as hydrogels [22]. Indeed, polymeric materials such as poly-dimethylsiloxane (PDMS) [23-26], polymethyl methacrylate (PMMA) [27] and polyimide [28] have become an appropriate material for the disposable microfluidics device due to straightforward fabrication processes and low cost. Especially, PDMS has a unique flexibility (the shear modulus vary between 100 kPa and 3 MPa and the

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Young's modulus of 360-870 kPa. c.f. the Young's modulus of PMMA is 1800-3100 MPa [29]) and gas permeability, which suit well for certain applications. In order to realize nanofluidic systems in a PDMS microfluidics format, various methods, including reversible bonding between glass and PDMS [30], the junction gap breakdown of PDMS at high electrical voltages [31] and integration of nano-capillary arrays [21], have been reported. Cracking of oxidized PDMS has also been utilized for various nanofluidic applications [32]. However, most of those nanostructures could suffer from reproducibility issues (mainly due to shifting chemical nature of PDMS surfaces) or complex 3-dimensional microfabrication process [21, 26]. Moreover, because aforementioned techniques can create nanostructures only at the interface between PDMS and cover materials, nanostructures at low aspect ratio (a.k.a. planar type) was inevitably fabricated [30-32]. The planar type junctions typically have the dimension of nanometer scale depth and micrometer scale width. This led to relatively poor coupling between the microchannels and the nanojunctions. On the other hand, nanostructures at high aspect ratio (a.k.a. vertical type) have the dimension of micrometer scale depth and nanometer scale width, therefore the fluid coupling between microchannels can be made over the entire microchannel height.

In this paper, we present a novel fabrication method for polymeric nanoporous-junctions integrated inside PDMS microchips. This method takes advantage of the flexibility of PDMS material to provide robust, reliable seal between PDMS and any heterogeneous polymer materials in the junction. As a result, the method does not require any complicated microfabrication techniques or complex chemical treatments on the PDMS-polymer surfaces. This practically resolves the main weakness of polymeric nanostructues (system integratability) and allows one to utilize the benefit (diverse chemical functionality) in standard PDMS microchannels. While any types of polymer materials can be used in this method, we used Nafion resin in order to realize the perm-selective nanoporous-junction. Nafion is a porous polymer with anionic surface groups that make it permeable to cations and polar compounds [33]. Reproducibility check was also done by measuring initial ion current through the nanostructures. As a practical example, we demonstrated ion/protein preconcentration which directly utilizes the concentration polarization phenomena. Finally, the high throughput nanofluidic preconcentrator in a large microchannel (1000μm×100μm cross section), was successfully demonstrated, which was enabled by the efficient vertical integration between nanoporous-junctions and microchannels.

EXPERIMENTAL SECTION

Fabrication of self-sealed polymeric nanoporous structures

Schematics of fabrication processes were shown in Figure 1. Desired PDMS microchannels can be obtained from the standard PDMS chip fabrication processes (Figure 1(a)) [23-25]. We fabricated various PDMS microchannels with dimensions of 50 μm (width) × 5 μm (depth), 100 μm × 10 μm and 1000 μm × 100 μm. The center microchannel was connected to side microchannels through one side wall in single gate device (SG) and both side walls in dual gate device (DG). The electrokinetic behaviors in DG device are symmetric and more stable than in the SG device, thus DG device is practically preferred, while SG device can give easier theoretical interpretations of the electrokinetic flow [3,4,12]. Since the nanofluidic applications usually consisted of the microchannels connected by nano-structures, we mechanically cut across the microchannels using conventional razor blades for guiding Nafion infiltration after punching sample loading holes (Figure 1(b)). The depth of the cutting should be deep enough to reach over the microchannel depth (typically 500 μm~1000 μm). Once the gap was created, PDMS tended to restore its inherent geometric structure due to its flexibility. By bending the chip, the gap was opened and a drop of 1.5 μL Nafion 117 solution (Fluka) was put on the edge of the gap (Figure 1(c)). Then the Nafion solution can immediately fill both the gap and a

portion of microchannels by capillary forces. The Nafion is a sulfonated tetrafluorethylene copolymer, widely used as a proton conductor for proton exchange membrane [34]. While there has never been any direct measurement of the pore size of Nafion, X-ray experiments and theoretical modeling predicted that the pore size should be around 5nm, subject to change due to ionic strength conditions. After 10 minutes of curing at 95C, solvents in Nafion resin would evaporate and the gap was bonded by Nafion resin with the adhesive-assisting role of Nafion resin (Figure 1(d)). The elastic nature of PDMS seals the Nafion junction rather tightly between the PDMS walls in the gap. Any remaining Nafion resins on the top of the PDMS surface and inside microchannel can be removed at once by taping because the residuals at both locations were usually connected to each other. Vertical type nanoporous-junctions are created between PDMS as shown in the box of Figure 1(d). Finally, another glass plate can be bonded on top of the device using plasma treatment (Figure 1(e)).

The microscope image of fabricated nanoporous-junctions and cooperating microchannels of DG device were shown in Figure 2(a). Three microchannels were connected each other by self-sealed nanostructures which lay across the microchannels. Since the Nafion resin reached over 100 μm in height (See supplementary information), it can act as a "vertical type nanoporous-junctions" and there were no void in the junction, which could cause unwanted leakage along the junction. This vertical type Nafion nanoporous-junction had the dimension of O(100) μm ~ O(1) mm (height) × O(1) μm (width) while the planar type junctions usually have the dimension of O(10) nm (height) × O(1) μm (width). Thus, the cross-sectional area of nanostructure, which is critical for high ion current, can be theoretically at least 10^4 times larger in case of vertical type structures than planar type.

Repeatability test

The DC ion current through the polymeric nanoporous-junction can be an excellent indicator for testing reliability and repeatability. After applying electric potential through the nanoporous-junction, the concentration polarization was initiated and the ionic concentration at anodic side started to decreased (ion depletion) [3,4,17]. Thus, the ion current dropped immediately after applying electric potentials and only the initial ion current could be measured and compared reliably. The measurement was done using Keithley 236 Current/Voltage Source-Measure Unit (Keithley Instruments, Inc.) which was connected to the SG device (microchannels has the dimension of 50 μm (width) × 5 μm (depth)) as shown in Figure 2(b). The microchannel was filled with 1 mM phosphate buffer. Four devices were fabricated and the tests were done three times for each device. After an I-V point was measured, the buffer inside the microchannel should be flushed, otherwise, it shows the typical limiting / over-limiting current behaviors [3,4,17]. The current was proportional to the applied voltage and showed an excellent linearity. At higher electrical potential, they tended to diverge but the usual electrical voltages used in the nanofluidic application have the electrical potential less than 50 V. Thus we can conclude that all of these devices were practically repeatable and reliable. Compared to these devices, a device without Nafion resin (only mechanical cutting) was fabricated. After the plasma treatment, we checked that the PDMS/PDMS gap can be opened again when we bent it. Measured current through the gap was less than 10 nA, which indicates that there is not enough ionic current to initiate the concentration polarization (ion depletion). From these comparison results between PDMS/Nafion/PDMS and PDMS/PDMS gap, one can conclude that the nanoporous Nafion junction plays an important role in transporting the bulk of ionic current.

RESULTS AND DISCUSSION

Ion/protein preconcentration test

To demonstrate the functionality of self-sealed nanoporous junction, we conducted the ion/protein preconcentration using charged dye molecules and proteins. With the aid of the tangential electric field through ion depletion zone created near nano structure, charged species including ions and proteins can be concentrated up to 10^3~10^6 folds [3,11,12,30,31,35]. The preconcentration factors of BODIPY disulfonate (Invitrogen) for three different concentrations (0.1 nM, 1 nM and 10 nM) in 1 mM phosphate buffer solution using DG device (microchannels has the dimension of 100 μm (width) × 10 μm (depth)) were shown in Figure 3(a) with the average tangential electric field of 50 V/cm. The fluorescent intensities were measured and analyzed with an inverted fluorescence microscope (IX-51) which has CCD camera (SensiCam, Cooke corp.) and Image Pro Plus 5.0 (Media Cybernetics inc.). Compared with its standard signal intensities (0.1 μM, 1 μM and 10 μM), the results showed that the preconcentration factors up to 10^4 have been achieved within 15 minutes. However, the concentration factors largely depended on the operating conditions such as applied electrical voltage, the charges of target species, buffer concentration and the dimension of microchannels. For example, we tested the preconcentration of β-phycoerythrin (β-PE) protein in the same device as shown in Figure 3(b) for two initial concentrations (1.67 nM (4×10^{-4} mg/ml) and 16.7 pM (4×10^{-6} mg/ml)). In this case, the concentration factors up to 10^4 can be accomplished within 22 minutes. Accordingly, the performance of the self sealed devices is at least comparable to the previous polymeric nano structure's.

Pressure driven ion/protein preconcentration operation

Pressure driven preconcentration operations are challenging for high throughput sample preparation with both conventional silicon/glass and polymeric devices. Since it utilized the external high pressure field from one reservoir instead of the tangential electric field, (1) high ion current (for strong concentration polarization and ion depletion), (2) high electrical tolerance of materials (for enduring high voltage operation) and (3) no leakage through the nanostructure are required for creating the preconcentrating plug under the pressure field. In order to meet those requirements, the nanojunctions should be vertical, the device should be made of polymeric material (or glass) and they should be bonded tightly. In these senses, polymeric self-sealed vertical nanoporous-junction can meet all of the requirements. Figure 4 shows the preconcentration operation using external pressure fields. The operation voltages of 120 V were applied at the both reservoir (i.e. depletion voltage condition) and the external pressure flow was added by a syringe pump (Harvard) at 35nl/min from right to left. (See supplementary video.) The pressure field and the electrical voltage can be simultaneously applied using a conductive MicroTight union (Upchurch, M-572). This tangential pressure flows (35nl/min: corresponding to 2.6 mm/sec in linear fluid velocity in this microchannel dimension) was at least 20 times faster than the electrokinetic velocity that can be obtained by the tangential field of 50 V/cm used in Figure 3. The speed of preconcentration was enhanced approximately 2X greater than the one from Figure 3 (10^4 fold within 7 minutes from Figure 4). The pressure fields can accelerate the supply of charged species into the preconcentration plug, but the preconcentrating plug slowly loses its additional samples by fast vortical convective flows inside the depletion zone [4]. A systematic optimization is strongly required for obtaining fast preconcentration without the loss of samples.

Ion depletion and preconcentration in semi-macrochannel

While handling nL~pL scale volume can provide us the minimizing consumption of expensive reagent and fast reaction, collecting the volume up to several μL could take hours to even a day. Parallelization of microchannel can be an indirect method to increase sample volume [36]. In addition to the parallelization, increasing the size of microchannels can directly

enhance the sample throughput volume. However, larger microchannel in the nanofluidic preconcentration system can lead to a fast, nonlinear electrokinetic flow [4], which could significantly deteriorate the ion concentration polarization and preconcentration efficiency. Reliable ion preconcentration can only be achieved with much higher ion current through the nanostructure. In order to demonstrate the efficiency of our vertical Nafion nanoporous-junctions, we fabricated a larger microchannel which has the dimension of 1000 μm (width) × 100 μm (depth). Since the deeper microchannel (larger cross-sectional area of nanoporous junction) gives higher ion current in the vertical type of nanostructure, ion current through the nano structure was approximately 10 times greater than the previous one (100 μm (width) × 10 μm (depth) in Figure 2(b)) as shown in Figure 5(a). Due to this high ion current through the perm-selective Nafion junction, we successfully demonstrated the ion depletion in SG device shown in Figure 5(b) and the ion preconcentration in DG device shown in Figure 5(c) (See supplementary videos). The volume of preconcentration plug was nearly 10 nL which has never been demonstrated before and suitable for connecting with the commercial analytical systems such as mass spectrometry (MS) and matrix-assisted laser desorption/ionization (MALDI).

CONCLUSION

This study provides several important implications in nanofluidic research. The fabrication method described in this paper allows one to integrate any polymeric nanoporous materials in a standard PDMS microfluidic channels, without the need of complicated fabrication processes or complex chemical treatment of PDMS surface. DC current measurements show that this fabrication technique has a good reproducibility. As an example, nanofluidic preconcentration system was realized using this fabrication method. This nanoporous-junction enables us to operate a pressure-driven ion/protein preconcentration and the higher preconcentration factor than one from any other polymeric nanostructure was obtained due to its vertical structure and tight sealing. In addition to this, the enhancement of preconcentrating sample volume for high throughput nanofluidic systems in semi-macrochannel was demonstrated for the first time using the vertical type nano structure. With the aid of the presenting method, we can lower the barrier that has blocked novel nanofluidic researches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was mainly supported by NIH (EB005743, CA119402).

REFERENCES

1. Han J, Craighead HG. Science 2000;288:1026–1029. [PubMed: 10807568]
2. Fu J, Schoch RB, Stevens AL, Tannenbaum SR, Han J. Nature Nanotech 2007;2:121–128.
3. Wang YC, Stevens AL, Han J. Anal. Chem 2005;77:4293–4299. [PubMed: 16013838]
4. Kim SJ, Wang YC, Lee JH, Jang H, Han J. Phys. Rev. Lett 2007;99:044501.1–4. [PubMed: 17678369]
5. Stone HA, Stroock AD, Ajdari A. Annu. Rev. Fluid Mech 2004;36:381–411.
6. Squires TM, Quake SR. Rev. Mod. Phys 2005;77:977–1026.
7. Mao P, Han J. Lab Chip 2005;5:837–844. [PubMed: 16027934]
8. Lee C, Yang EH, Myung NV, George T. Nano Lett 2003;3:1339–1340.
9. Stern MB, Geis MW, Curtin JE. J. Vac. Sci. Technol. B 1997;15:2887–2891.
10. Gao LJ, Cheng X, Chou CF. Nano Lett 2004;4:69–73.
11. Pu Q, Yun J, Temkin H, Liu S. Nano Lett 2004;4:1099–1103.

12. Plecis A, Schoch RB, Renaud P. Nano Lett 2005;5:1147–1155. [PubMed: 15943459]
13. Daiguji H, Oka Y, Shirono K. Nano Lett 2005;5:2274–2280. [PubMed: 16277467]
14. Cheng LJ, Guo LJ. Nano Lett 2007;7:3165–3171. [PubMed: 17894519]
15. Holt JK, Park HG, Wang Y, Stadermann M, Artyukhin AB, Grigoropoulos CP, Noy A, Bakajin O. Science 2006;312:1034–1037. [PubMed: 16709781]
16. Majumder RS, Choudhury N, Ghosh SK. J. Chem. Phys 2007;127:054706. [PubMed: 17688356]
17. Holtzel A, Tallarek U. J. Sep. Sci 2007;30:1398–1419. [PubMed: 17623420]
18. Leinweber FC, Tallarek U. Langmuir 2004;20:11637–11648. [PubMed: 15595793]
19. Zeng CC, Wang SN, Lee L. Materials Letters 2005;59:3095–3098.
20. Khandurina J, Jacobson SC, Waters LC, Foote RS, Ramsey JM. Anal. Chem 1999;71:1815–1819. [PubMed: 10330910]
21. Zhang Y, Timperman AT. Analyst 2003;128:537–542. [PubMed: 12866863]
22. Dhopeshwarkar R, Crooks RM, Hlushkou D, Tallarek U. Anal. Chem 2008;80:1039–1048. [PubMed: 18197694]
23. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. Anal. Chem 1998;70:4974–4984.
24. Quake SR, Scherer A. Science 2000;290:1536–1540. [PubMed: 11090344]
25. Lotters JC, Olthuis W, Veltink PH, Bergveld P. J. Micromech. Microeng 1997;7:145–147.
26. Kuo TC, Cannon DM, Chen YN, Tulock JJ, Shannon MA, Sweedler JV, Bohn PW. Anal. Chem 2003;75:1861–1867. [PubMed: 12713044]
27. Galloway M, Stryjewski W, Henry A, Ford SM, Llopis S, McCarley RL, Soper SA. Anal. Chem 2002;74:2407–2415. [PubMed: 12038768]
28. Metz S, Trautmann C, Bertsch A, Renaud P. J. Micromech. Microeng 2004;14:324–331.
29. Mark, J. Polymer Data Handbook. Oxford Uni. Press; New York: 1999.
30. Kim SM, Burns MA, Hasselbrink EF. Anal. Chem 2006;78:4779–4785. [PubMed: 16841895]
31. Lee JH, Chung S, Kim SJ, Han J. Anal. Chem 2007;79:6868–6873. [PubMed: 17628080]
32. Huh D, Mills KL, Zhu X, Burns MA, Thouless MD, Takayama S. Nature Mater 2007;6:424–428. [PubMed: 17486084]
33. Gao L, Seliskar CJ. Chem. Mater 1998;10:2481–2489.
34. Mauritz KA, Moore RB. Chem. Rev 2004;104:4535–4585. [PubMed: 15669162]
35. Breadmore MC. Electrophoresis 2007;28:254–281. [PubMed: 17149782]
36. Lee JH, Song YA, Han J. Lab Chip 2008;8:596–601. [PubMed: 18369515]
37. Kim SJ, Song Y-A, Skipper PL, Han J. Anal. Chem 2006;78:8011–8019. [PubMed: 17134134]
38. Koster S, Verpoorte E. Lab Chip 2007;7:1394–1412. [PubMed: 17960264]

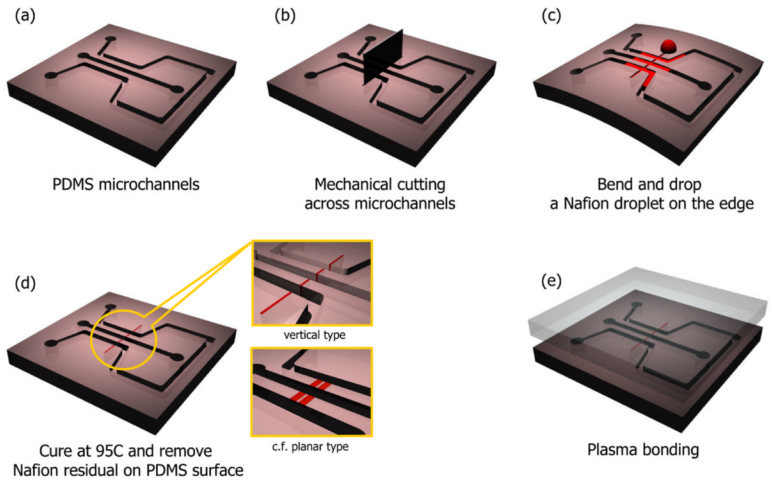

Figure 1.
Schematics of fabrication processes.

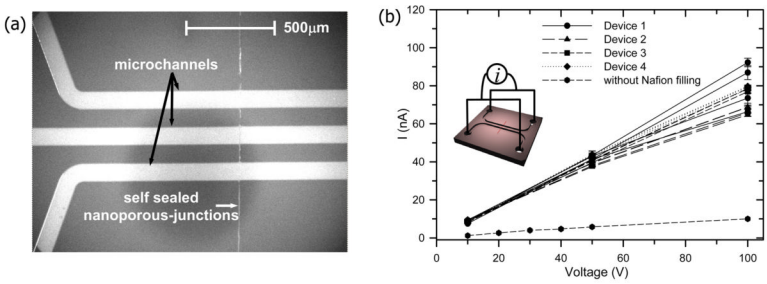

Figure 2.
(a) Microscope image of fabricated PDMS microchannels and self sealed nanoporous-junctions and (b) I-V plot for confirming fabrication repeatability.

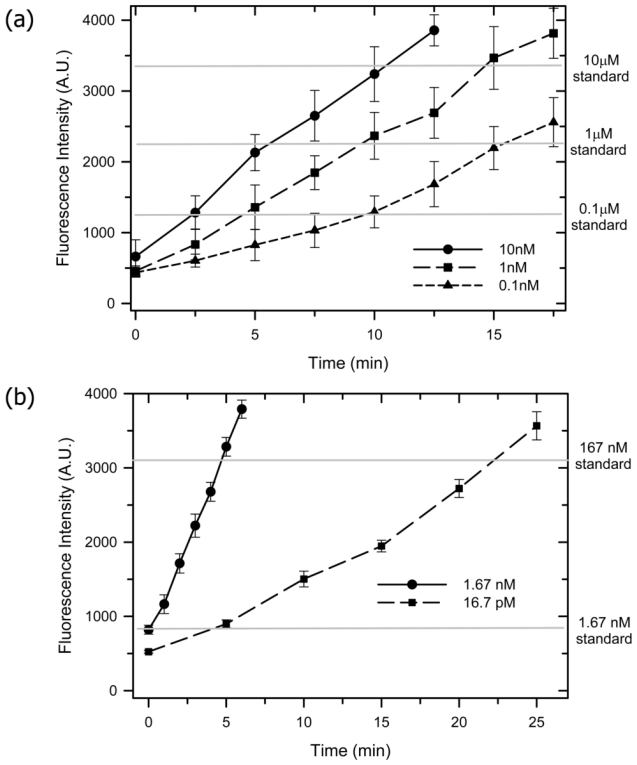

Figure 3.
Preconcentration factors of (a) BODIPY dye molecules and (b) β-PE proteins.

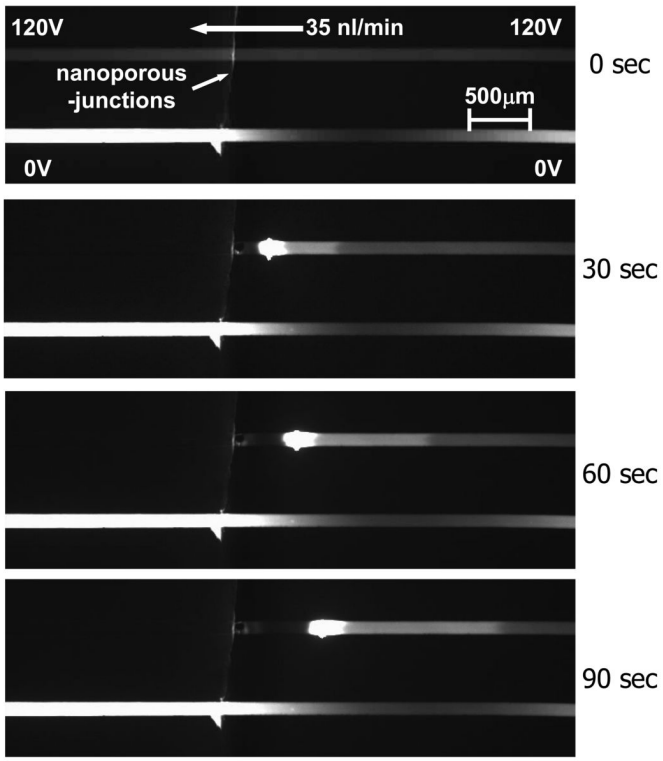

Figure 4.
Pressure-driven preconcentration operation of BODIPY in SG device (50 μm (width) × 4.5 μm (depth)). The external flow was applied by syringe pump at 35 nl/min. The unbounded signals near the concentrated plug were due to the saturation of over-exposed signal, not a leakage of the sample.

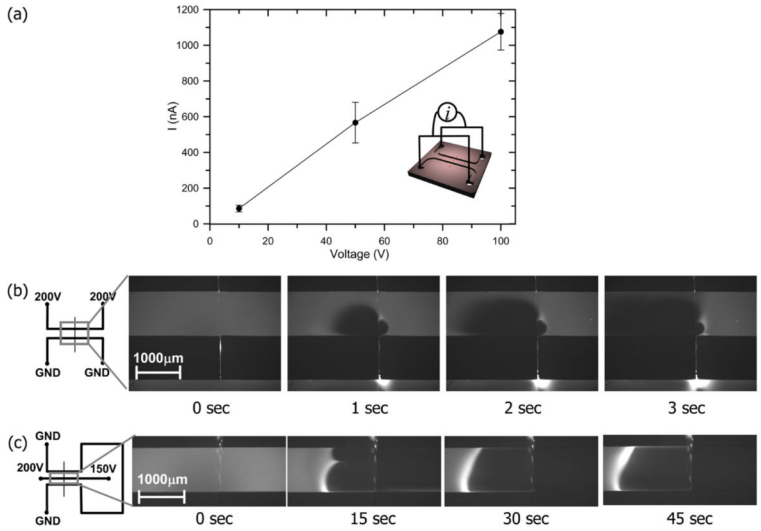

Figure 5.
(a) I-V plot from semi-macrochannel and (b) ion depletion in SG device and (c) ion preconcentration in DG device. All microchannels have the dimension of 1000 μm (width) × 100 μm (depth).