Active Sealing for Soft Polymer Microchips

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ABSTRACT

This paper presents a new sealing method for soft polymer (elastomer) microchips. A robust and reversible sealing method, which allows various materials to be bonded and sealed tightly with each other even in aqueous solutions, is developed. A poly (dimethylsiloxane) (PDMS) microchip system, which can actively generate bonding and sealing forces by itself, is invented. By inducing negative pressure into additional closed areas, an instant sucking disk is made. This disk is used to tighten up the conformal contact of soft polymers. Other functionalities of active sealing such as making reusable microchips, patterning cells, and performing cellular assays in a single dish have also been examined and will be discussed hereunder. This technique gives a robust and universal solution for microchip sealing issues by sealing soft polymers with diverse materials in various conditions. Active sealing will simplify numerous assays in lab-on-a-chip industry and will open a new era for cellular microchip assays.

Keywords: active sealing, reversible sealing, soft polymer, cell patterning, cytotoxicity test

1 INTRODUCTION

In the lab-on-a-chip industry, various kinds of materials are being used for making microchips such as polymers, glass, quartz, silicon wafer, metal substrates, and so on. In particular, soft polymers (elastomers) have many desirable properties when used in microfluidic devices [1]. However, sealing these elastomers with each other is too case sensitive and time-consuming. Moreover there are combinations of materials that no feasible bonding protocols can ever be found, and even if such bonding protocols are developed, they are in many cases not suitable for mass production.

Until recently many protocols have been developed to resolve this problem [2]. But, they still greatly depend case by case, and so, researchers are still restricted from freely choosing the materials to be used for devices. Assays also restrict researchers in choosing materials, which further complicates the problem.

We invented a microchip that can actively generate bonding and sealing forces. This is called 'active sealing'. The main idea of active sealing is that active vacuum sealing helps tighten up the sealing of microchannels by producing an instant sucking disk. Basically, elastomers have an additional advantage over glass, silicon, and hard plastics in that it makes reversible van der Waals contact (conformal contact) to smooth surfaces [1]. By inducing negative pressure into additional closed areas, we can tighten up the conformal contact of soft polymers. This is the basic mechanism of sucking disk.

The concept has been verified using a poly (dimethylsiloxane) (PDMS) microchip by inspecting sealing performance. The additional functionalities of active sealing such as making reusable microchips, patterning cells, and performing various cellular assays also have been examined and will be discussed hereunder.

2 MATERIAL AND METHODS

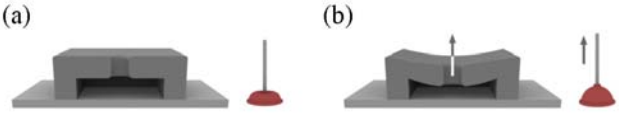
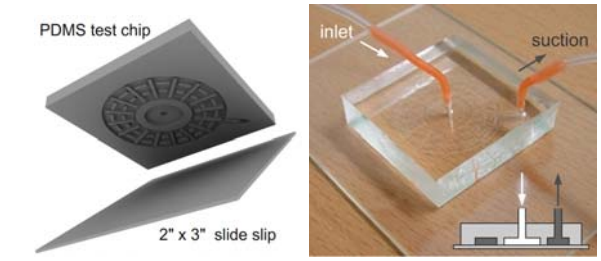


Figure 1: the active sealing scheme (a) Conformal contact only (b) Conformal contact + active sealing

The active sealing scheme is illustrated in Figure 1. To test this scheme, a PDMS microchips was fabricated by soft lithography using elastomeric polymer molding, a method that allows rapid prototyping of microfluidic devices [3]. Briefly, CAD file drawing was printed onto a transparency film with a high-resolution image setter. The transparency was used in 1:1 contact photolithography with SU-8 photoresist to generate a negative master mold, consisting of patterned photoresist on a Si wafer. Positive replicas with embossed channels were fabricated by molding PDMS against the master. Channel inlets and outlets were then drilled into the PDMS using a borer (a hand-held puncher), and finally, the channels were sealed by conformal contact on a slide slip.

3 EXPERIMENTS AND RESULTS

(a) (b)



(c)

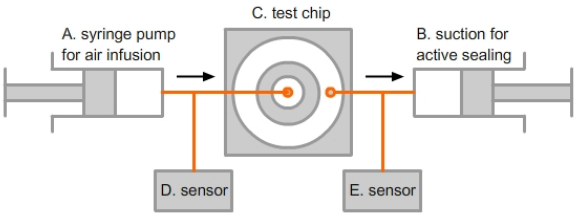


Figure 2: (a) a 3D geometry of test chip. (b) Fabricated PDMS microchip (c) Connections for syringes and pressure sensors and its pneumatic diagram

The sealing performance of active sealing was examined by measuring the maximum pressure endurance. As in Figure 2(b), a test chip was contacted with a slide slip. The 3-dimensional structure of the test chip was designed in a labyrinthine manner to avoid the effect of elastic deflections (see Figure 2(a)). A syringe pump was connected to inlet port to gradually increase inlet pressure. A vacuum syringe was connected to the suction port to activate active sealing, and pressure sensors were connected to each port. The pneumatic diagram for this test apparatus is illustrated in Figure 2(c). We measured the maximum inlet pressure without leakage with decreasing negative suction (sealing) pressure. If sealing fails by leakage at some pressure level, the pressure on the sensors would suddenly change. We checked the pressure level at that moment.

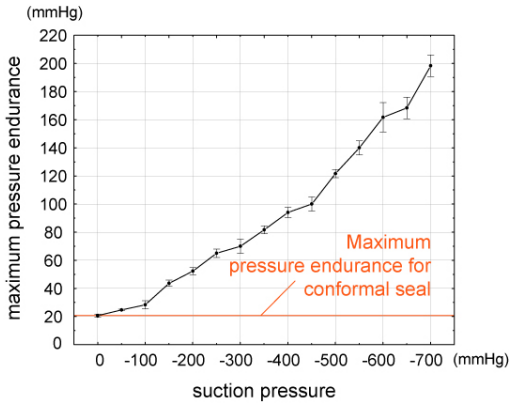


Figure 4: Max. pressure endurance of active sealing

In Figure 4, maximum pressure endurance of the test chip with respect to applied suction pressure is plotted. As seen in the figure, conformal contact alone (with zero suction pressure) cannot hold up more than 20 mmHg inlet pressure. So, we can say that microchips sealed with conformal contact alone should be used with small pressure input or with negative suction drive. But with active sealing, chips were found to endure far beyond the pressure level that conformal contact alone is incapable of upholding. With decreasing suction pressure, test chips endured more than 150mmHg which is sufficient for laboratory microchip applications.

Successively, we replaced the slide slip for other common materials such as polymethyl methacrylate (PMMA) plates, polypropylene (PP) transparency films, polycarbonate (PC) petri dishes, metal plates and quartz plates. We even used wet materials such as petri dishes containing liquid sample (Figure 5). This would not have been possible if not for active sealing, which proves that this method can be applied universally for all materials.

4 PRACTICAL APPLICATIONS OF ACTIVE SEALING

There were several reasons for the development of this method. First, we wanted to study the influence of surface characteristics of substrates in microchannels. We considered flow focusing with air sheath using various materials for flow cytometry applications. We assumed that if it was possible to focus liquid sample using air sheath, this would remove all the bulky part of flow cytometer related with massive sheath buffer. Focusing liquid sample with air sheath is very complicated because its stability strongly depends on the surface characteristics [4]. Figure 5 shows a successful operation of flow focusing with air sheath using PDMS microchannel and PMMA substrate. Without active sealing, it is not easy to bond PDMS and PMMA together.

(a) (b)

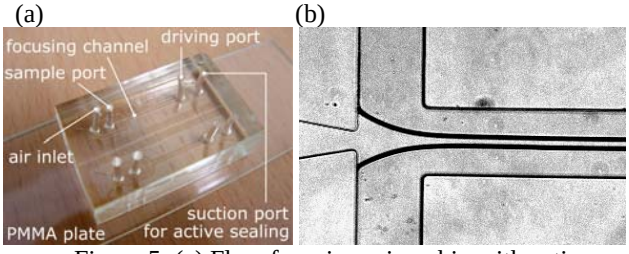


Figure 5: (a) Flow focusing microchip with active sealing (b) Focusing ethanol sample with air sheath on a PMMA substrate

Second, we wanted to thoroughly clean up clogged microchannels so that they can be reused many times thereafter. Microchannels with micro-scale geometries entail relatively more work to be made but become more easily clogged when used in experiments. So, a kind of 'peel off, clean up and re-stick' type device was needed. The soft elastomer material even tolerates small contaminations, such as dust particles between the chip and cover glass without seriously interfering with the functioning of the device [5].

Third, we wanted to seal microchips firmly on substrates which are patterned with bio materials (cells) and flooded with aqueous solutions. By doing so, it would make it easy to selectively infuse drugs or nutrients to cells already in culture. As can be seen in Figure 7, we mounted a microchip on cell-patterned petri dish and sealed it by active sealing. Then, we cultured cells in microchannels by infusing culture media only through microchannels.

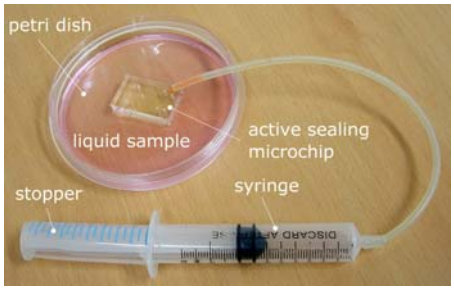


Figure 7: Simple setup of active sealing

As shown in Figure 8, cells in microchannel stayed viable for several weeks but cells in other areas showed no viability. This means that this method can be widely applied for cell based microchannel applications.

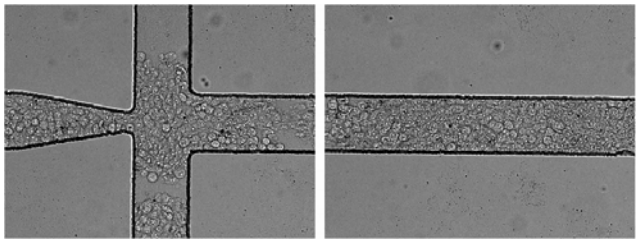


Figure 8: Culturing cells in microchannel

Culturing cells in microchannels is an important topic in tissue engineering [6]. Using microchannels, continuous nutrition and oxygen supply and waste removal through the culture medium can easily be ensured [7]. And PDMS is a favorable material because of its biocompatibility and high gas permeability [8]. In conventional cell culture formats such as dishes and macroscale bioreactors, it is quite difficult to realize the delivery of a sufficient amount of those substances throughout the cultured tissue [9]. This is due to the difficulty in designing and fabricating large complex bioreactors in which the cells are fed by a spatially homogeneous distribution of the fluid flow [10].

Moreover, active sealing itself can selectively control viability over cell cultured area. By squeezing cells with active sealing pattern, we can control viability by areas with no additional drug treatment. We can see the spatial distribution of cell viability controlled by active sealing pattern in Figure 9. The microchip was soaked in culture media but it was still tightly sealed with cell cultured surface. The whole system for this experiment was very small in size as shown in Fig. 7. So, the equipments could be easily put into a CO_2 incubator.

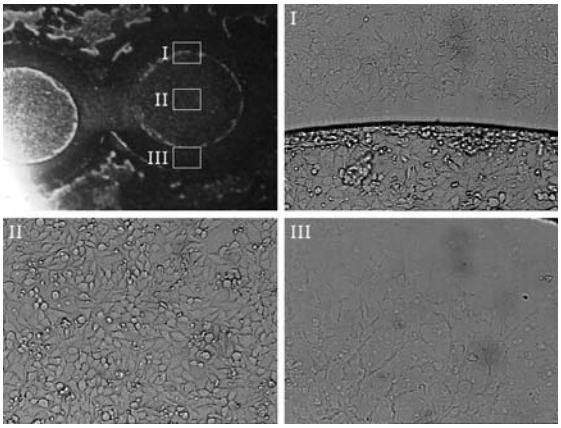


Figure 9: Cell lysis occurs only in squeezed areas.

The ability to pattern mammalian cells in specific areas on a surface has become a very important topic of research because of its applications in tissue engineering, cell arrays, and biosensors [11]. Usually, selective cell attachment is achieved indirectly by microfabricating a template to which cells adhere preferentially [12]. The template may be made of metals [13] self-assembled monolayers [14] polymers [15] extracellular matrix proteins [16], cell adhesive peptides [17] or a combination thereof. Or cell suspensions and others can be delivered onto selected regions of a substrate by means of microfluidic channels [18]. Another alternative consists of using a stencil (i.e., a thin sheet containing holes of specified shapes and sizes) [19]. But there are limitations to these systems including long-term cell resistance and substrate choice [11]. Basically these systems are too case sensitive, and are often entangled with the drawbacks made by micro structures.

We controlled the spatial distribution of cells by selectively removing cultured cells. By adopting conventional cell culture protocols and controlling spatial viability over cell cultured areas later, we ignored all the undesirable side effects generated by modifying cell culture procedures for cell patterning. Figure 9 shows successful control of spatial distribution of cells on a petri dish. It can be seen that only the cells in squeezed areas have undergone cell lysis and cells in culture zones are still viable.

Currently, we are experimenting on multilayered microchip integrated with active sealing and serial dilution module with the aim of achieving cytotoxicity assay using only one petri dish. Through active sealing, a cell cultured domain can be divided into multiple well chambers instantly. By integrating serial dilution module [20], cytotoxic drug solutions are automatically and accurately infused into individual well chambers at serially increasing concentrations [21]. With that, the cytotoxicity can be tested with only one petri dish. Testing the cytotoxicity of a drug in a single dish means that cells with exactly the same condition are used. This in turn means a far more reliable

data will be obtained. Figure 10 shows the integrated cytotoxicity test device.

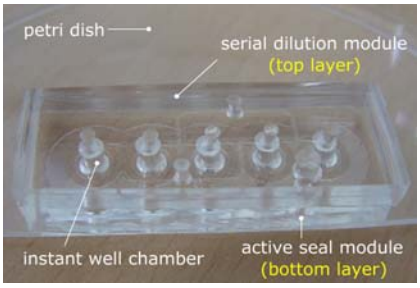


Figure 10: Multi-layered cytotoxicity assay chip

5 CONCLUSIONS

The newly developed active sealing method is a robust and universal solution to microchip sealing issues. It will also simplify numerous assays in lab-on-a-chip industry. We believe that this will give a universal method while sealing soft polymers with diverse materials in various conditions, and thus open a new era for cellular microchip assays.

REFERENCES

[1] M. K. J. Ng, I. Gitlin, A. D. Stroock and G. M. Whitesides, "Components for integrated poly(dimethylsiloxane) microfluidic systems", Electrophoresis, 23, 3461-3473, 2002

[2] J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu and G. M. Whitesides, "Fabrication of microfluidic systems in poly(dimethylsiloxane)", Electrophoresis, 21, 27-40, 2000

[3] Y. Xia and G. M. Whitesides, "Soft Lithography", Annu. Rev. Mater. Sci., 28, 153-184, 1998

[4] D. Huh, A. H. Tkaczyk, J. H. Bahng, Y. Chang, H. H. Wei, J. B. Grotberg, C. J. Kim, K. Kurabayashi and S. Takayama, "Reversible switching of high-speed air-liquid two-phase flows using electrowetting-assisted flow-pattern change", J. Am. Chem. Soc., 125, 14678-14679, 2003

[5] P. S. Dittrich and P. Schwille, "An integrated microfluidic system for reaction, high sensitivity detection and sorting of fluorescent cells and particles", Anal. Chem., 75, 5767-5774, 2003

[6] H. Andersson and A. Berga, "Microfabrication and microfluidics for tissue engineering: state of the art and future opportunities [Review]", Lab Chip, 4, 98-103, 2004

[7] J. R. Anderson, D. T. Chiu, J. C. McDonald,, R. J. Jackman, O. Cherniavskaya, H. Wu, S. Whitesides and G. M. Whitesides, "Fabrication of topologically complex three-dimensional microfluidic systems in PDMS by rapid prototyping", Anal. Chem., 72, 3158-3164, 2000

[8] N. Szita, A. Zanzotto, P. Boccazzi, A. Sinskey, M. Schmidt and K. Jensen, "Monitoring of cell growth, oxygen and pH in microfermentors", Micro Total Analysis System conference Nara Japan, 79, 2002

[9] E. Leclerc, Y. Sakai and T. Fujii, "A microfluidic PDMS (polydimethylsiloxane) bioreactor for large-scale culture of hepatocytes", Biotechnol. Prog., 20, 750-755, 2004

[10] J. W. Allen, T. Hassanein and S. N. Bathia, "Advances in bioartificial liver devices Hepatology", 34 (3), 447-455, 2001

[11] M. C. Berg, S. Y. Yang, P. T. Hammond and M. F. Rubner, "Controlling mammalian cell interactions on patterned polyelectrolyte multilayer surfaces", Langmuir, 20, 1362-1368, 2004

[12] A. Folch, B. H. Jo, O. Hurtado, D. J. Beebe and M. Toner, "Microfabricated elastomeric stencils for micropatterning cell cultures", Journal of Biomedical Materials Research, 52 (2), 346-353, 2000

[13] L. C. O'Neil, P. Jordan, P. Riddle and G. J. Ireland, "Narrow linear strips of adhesive substratum are powerful inducers of both growth and total focal contact area", Cell Sci., 95, 577, 1990

[14] R. Singhvi, A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. Wang, G. M. Whitesides and D. E. Ingber, "Engineering cell shape and function", Science, 264, 696, 1994

[15] C. H. Thomas, J. B. Lhoest, D. G. Castner, C. D. McFarland and K. E. Healy, "Surfaces designed to control the projected area and shape of individual cells", J. Biomech. Eng., 121, 40, 1999

[16] A. Folch and M. Toner, "Cellular micropatterns on biocompatible materials", Biotechnol. Prog., 14, 388, 1998

[17] C. B. Herbert, T. L. McLernon, C. L. Hypolite, D. N. Adams, L. Pikus, C. C. Huang, G. B. Fields, P. C. Letourneau, M. D. Distefano and W. S. Hu, "Micropatterning gradients and controlling surface densities of photoactivatable biomolecules on self-assembled monolayers of oligo(ethylene glycol) alkanethiolates", Chem. Biol., 4, 731, 1997

[18] S. Takayama,, J. C. McDonald,, E. Ostuni, M. N. Liang, P. J. A. Kenis, R. F. Ismagilov and G. M. Whitesides, "Patterning cells and their environments using multiple laminar fluid flows in capillary networks", Proc. Natl. Acad. Sci. USA, 96, 5545, 1999

[19] K. Atsuta, H. Nojia and S. Takeuchia, "Micro patterning of active proteins with perforated PDMS sheets (PDMS sieve)", Lab Chip, 4, 333-336, 2004

[20] J. K. Chang, H. Bang, S. J. Park, S. Chung, C. Chung and D. C. Han, "Fabrication of the PDMS microchip for serially diluting sample with buffer", Microsystem Technologies, 9, 555-558, 2003

[21] H. Bang, S. H. Lim, Y. K. Lee, S. Chung, C. Chung, D. C. Han, and J. K. Chang, "Serial dilution microchip for cytotoxicity test", J. Micromech. Microeng., 14, 1165-1170, 2004