Talanta 81 (2010) 1203–1208

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A rotating microfluidic array chip for staining assays

Hong Chen, XiuJun Li, Lin Wang, Paul C.H. Li*

Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

ARTICLE INFO

Article history:
Received 5 November 2009
Received in revised form 4 February 2010
Accepted 4 February 2010
Available online 11 February 2010

Keywords:
Microfluidic chip
Spiral microchannel
Centrifugal pumping
Staining assay
Cell array
Cell encapsulation

ABSTRACT

We have developed a microfluidic method to construct an array on a circular disk for staining assays. In this method, convenient centrifugal liquid pumping has been achieved within the spiral microchannels by disk rotation or spinning. Moreover, the liquids flowing in spiral channels effectively interact with the along-channel intercepted cell trapping holes. Live cells were encapsulated in wet low-melting point agarose along radial strips on the disk. When embedded in agarose, the cells remained viable to interact with, and respond to, test reagents. This method illustrates the potential use of the circular microfluidic chip to construct the cell array, intended for multi-cell multi-reagent tests.

1. Introduction

There is great interest in generating cell patterns or arrays for many life science applications such as drug testing. Currently, there are numerous ways to generate cell arrays, e.g. pin spotting [1,2], encapsulation in PEG hydrogels [3–6]. In addition, cell array construction has been accomplished in microfluidic channels, by using cell adhesion on patterned protein layers [7–10]. To date, laminar flow has been used to deliver two streams of reagents along the same sample channel containing patterned cells [11,12]. Nevertheless, in the use of cell arrays, only one sample or test reagent was applied per array. Therefore, the tests on multiple reagents require many cell arrays, resulting in high cost in both reagents and cell arrays.

Accordingly, it will be attractive to develop a method in which different drug compounds can be applied to multiple sample channels that intersect with different patterned rows of cells. To achieve parallel delivery of several reagents, we take advantage of using centrifugal pumping in parallel spiral channels on a rotating circular disk, as previously developed in our laboratory [13,14].

As shown in Fig. 1, the microfluidic array chip consists of 16 spiral channels. Along an imaginary radial row, adjacent holes on each spiral channel are punched to form reservoirs. These reservoirs are to accommodate cell samples that are embedded in agarose. There are 16 imaginary rows of holes on the entire chip. The inset shows greater details about how the holes are spaced apart in an alternate manner on each imaginary radial row. Upon the flow of a reagent along the spiral channel, the reagent will consecutively interact with the cells embedded in agarose in holes intercepting the channel. On this chip, up to 16 reagents could interact with up to 16 cell samples upon one single disk rotation. The radial arrangement of the cell trapping holes makes the sample application easier because the operator can run the applicator containing the first cell sample along one radial row, and the second cell sample along another radial row, and so on. In order to embed live cells, especially mammalian cells, in gel-like materials, we adopted to use low-melting point agarose (LMPA). With a lower working temperature of ~37 °C, this material has been widely used to embed the cells in single-cell electrophoresis or comet assay [15], at a reasonably low temperature. Since the cells were applied in physically separated holes punched along the spiral microchannels, no diffusion problem occurred to a great extent during experiments.

This approach is more convenient than a previously developed microfluidic cell array in which there are cell channels and reagent channels, and a semiporous polyester membrane is needed to physically separate them [16], and, in addition, careful alignment is required [17]. We have demonstrated the feasibility of this method using both the colored dyed solution and fluorescent vital stain as the

* Corresponding author. Tel.: +1 778 782 5956; fax: +1 778 782 3765.
E-mail address: paulli@sfu.ca (P.C.H. Li).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.02.011

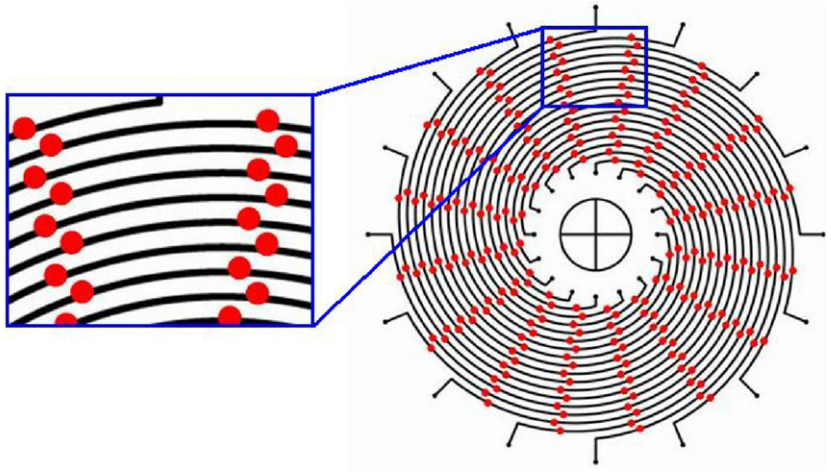

Fig. 1. The rotating microfluidic array chip fabricated in PDMS. The schematic diagram showing 16 spiral channels fabricated on the chip, with 256 (i.e. 16 × 16) cell trapping holes punched on the chip. In the left inset, the holes (red dots) and the channels (black line) are shown in greater detail. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

reagents.

2. Experimental

2.1. Materials

Circular glass disks (1 mm thick, 4″ in diameter with a center hole of 0.59″ in diameter) were obtained from Precision Glass & Optics (Santa Ana, CA, USA). Photoresist SU-8 and its developer were purchased from MicroChem Corporation (Newton, MA, USA). Sylgard® 184 silicone elastomer base and its curing agent were obtained from Dow Corning Corporation (Midland, MI, USA).

Low-melting point agarose (LMPA) was purchased from Invitrogen. Sodium dodecyl sulphate (SDS), fluorescein diacetate (FDA) were obtained from Sigma–Aldrich. The red and blue food dyes were obtained from Scott-Bathgate (Vancouver, BC).

2.2. Procedures

2.2.1. Fabrication of PDMS channel chips

The PDMS channel chips were fabricated according to the procedure described previously [13,18]. Briefly, the photomask with the microchannel pattern was printed on a transparency film. The spiral channel design has previously been described [19], and a total channel length is 18.4 cm. Using this photomask, an SU-8 molding master consisting of 25-μm high positive relief structures was fabricated on a silicon wafer. PDMS prepolymer was cast against the molding master to yield the polymeric channel chip. As shown in Fig. 2a, the PDMS chip (4″ in diameter) consists of 16 spiral channels, spaced 2 mm apart. Each spiral channel is 1 mm wide and is 25 μm deep. At the starting point (near the centre) and ending point (near the perimeter) of each spiral channel, 2 mm diameter holes were punched to serve as the solution inlet and outlet reservoirs, respectively. The PDMS material was punched using a flat-tip syringe needle (15-gauge). On each spiral channel, 16 cell trapping holes (2 mm in diameter) were punched. These holes were constructed along 16 imaginary radial rows, thus resulting in a total of 256 holes. Fig. 2b shows the closeup of one such radial row of cell trapping holes. The holes in adjacent spiral channels are slightly displaced to increase their separation distances.

2.2.2. Staining experiments

Low-melting point agarose (LMPA) was dissolved in PBS, and put in a boiling water bath for 5 min. The melted agarose was kept in a

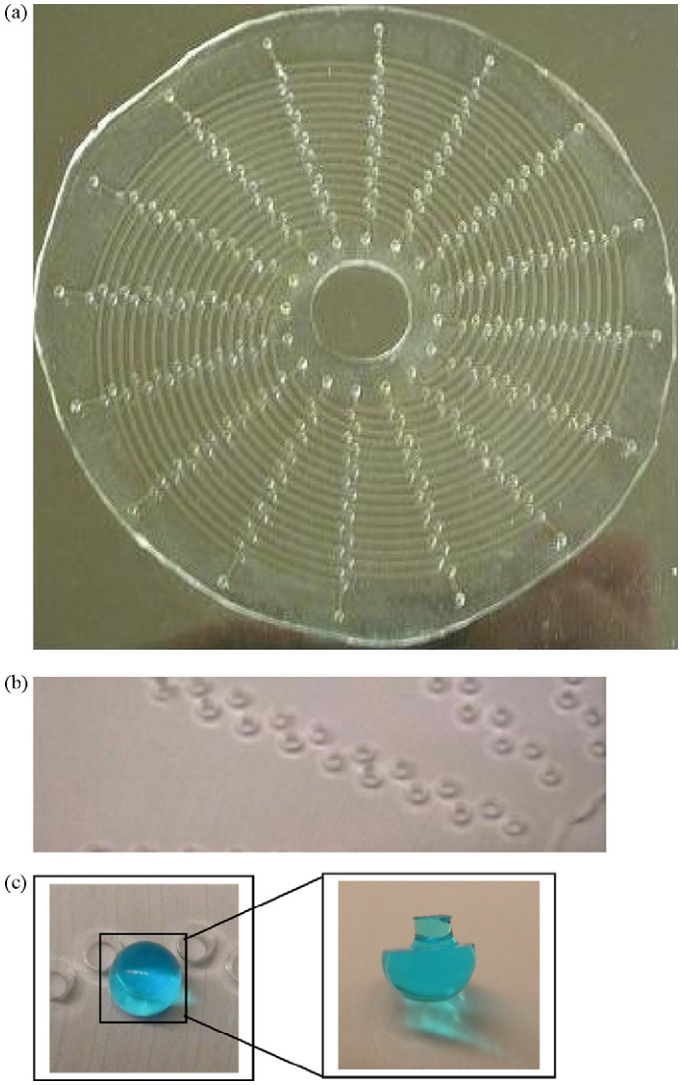

Fig. 2. Cell trapping holes on the PDMS chip. (a) The image of the microfluidic array chip (4″ in diameter) consisting of 16 punched holes intercepting each of the 16 spiral channels. (b) Closeup view of one radial row of punched holes (2 mm in diameter). (c) LMPA solutions stained with blue dye was added on one hole and gelled. The inset shows the mushroom shape of the gelled agarose after it was pulled out and inverted upside down.

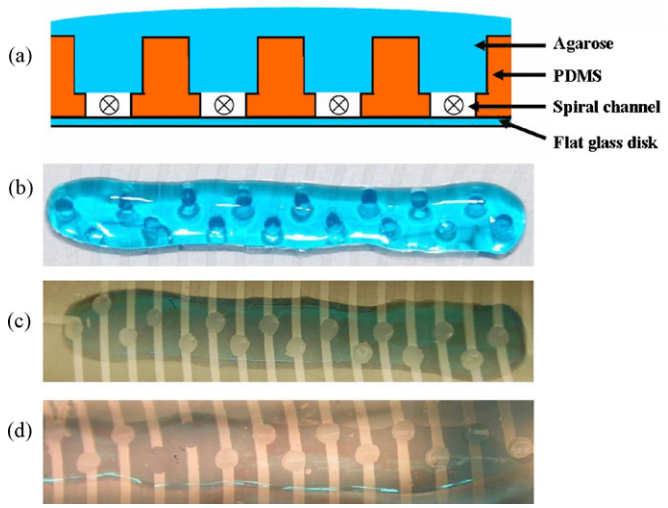

Fig. 3. (a) Schematic diagram showing LMPA formed as a radial row covering several holes. (b) Front image of the 3% LMPA (pre-stained with the blue dye) added along the radial rows of 16 holes. (c) Back image showing no leakage of 3% LMPA into spiral channels (1 mm wide). (d) Back image showing some leakage of 2% LMPA into two spiral channels, resulting in blockage.

37 °C water bath until use. The LMPA solution was introduced to the trapping reservoirs on the microfluidic array chip. The agarose was introduced as a radial strip by running a dispensing pipette along the imaginary row of holes. A volume of ~100 μL was usually used for 1 radial strip. The agarose composition was optimized to be 3% so that it would only flow and gel inside the reservoirs, but not leak into the spiral channels and block them. To aid visualization of the gel formed in the reservoirs, the gel was pre-stained with a blue dye. After 1 min of agarose gelling, the PDMS chip was reversibly sealed with a plain circular glass disk to enclose the spiral channels. However, the PDMS chip can also be presealed to the glass disk before applying LMPA into the holes.

To introduce reagent solutions into the spiral channels, the solutions (3 μL) were placed at the inlet reservoirs near the centre. To aid visualization, blue or red dyed solutions have been used. Then the chip was put on a rotating platform, and was spun at 1000 rpm. The liquid flow inside the channels was examined by a stroboscope light, as previously described [13,20]. The liquid movement inside the spiral channel takes about half a minute, as shown in the video clip (see Electronic Supplementary Information). Simulation study for liquid diffusion was performed using a computational fluid dynamics (CFD) software (ESI, CFD-ACE+, Farmington Hills, MI). For the simulations, the temperature was 300 K, and diffusion coefficient for the dye (in gel) was 4.25×10^{-6} cm^2 s^{-1}.

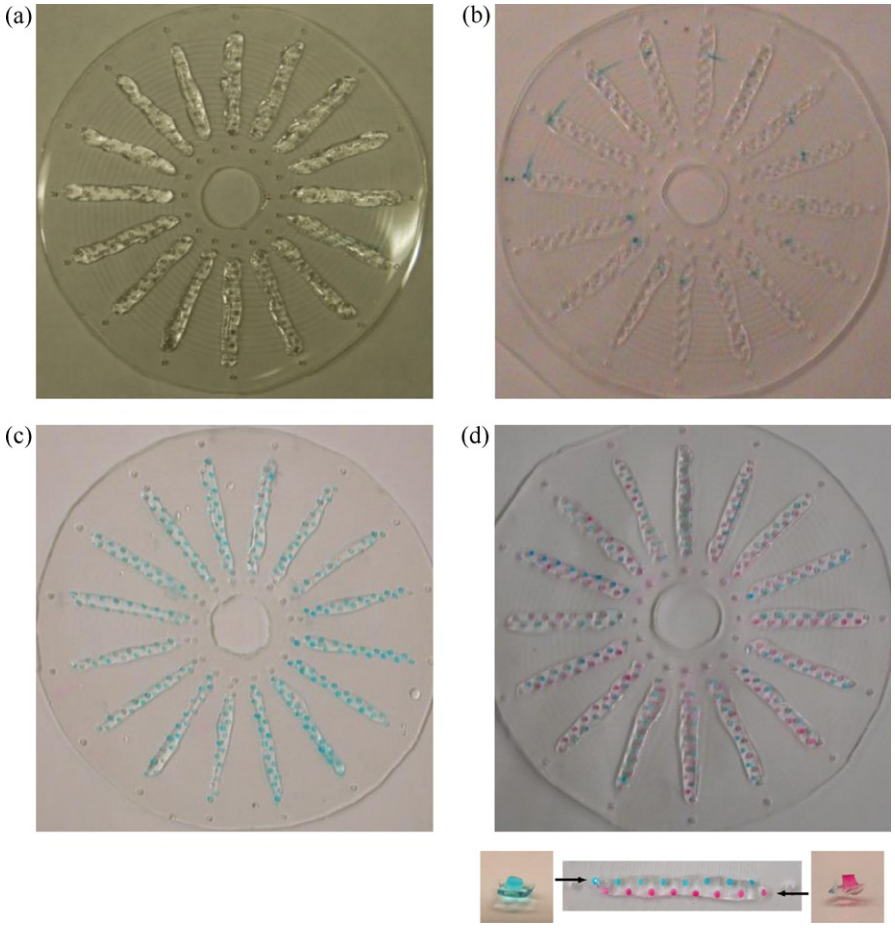

Fig. 4. Color staining of the agarose at the trapping holes by blue dye solutions delivered by centrifugal flow in spiral channels. (a) 3% LMPA was added in 16 rows of holes on the PDMS chip; (b) centrifugal flow of dyed solutions through one spiral channel showed the blue staining at the trapping holes; (c) staining of all trapping holes by blue dyed solutions; (d) staining by alternate red and blue food dyes, with a closeup view (bottom) of a row of the cell trapping agarose stained by alternate dye solutions. The insets show the staining of the "mushroom stem" (2 mm long), after removal from the holes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

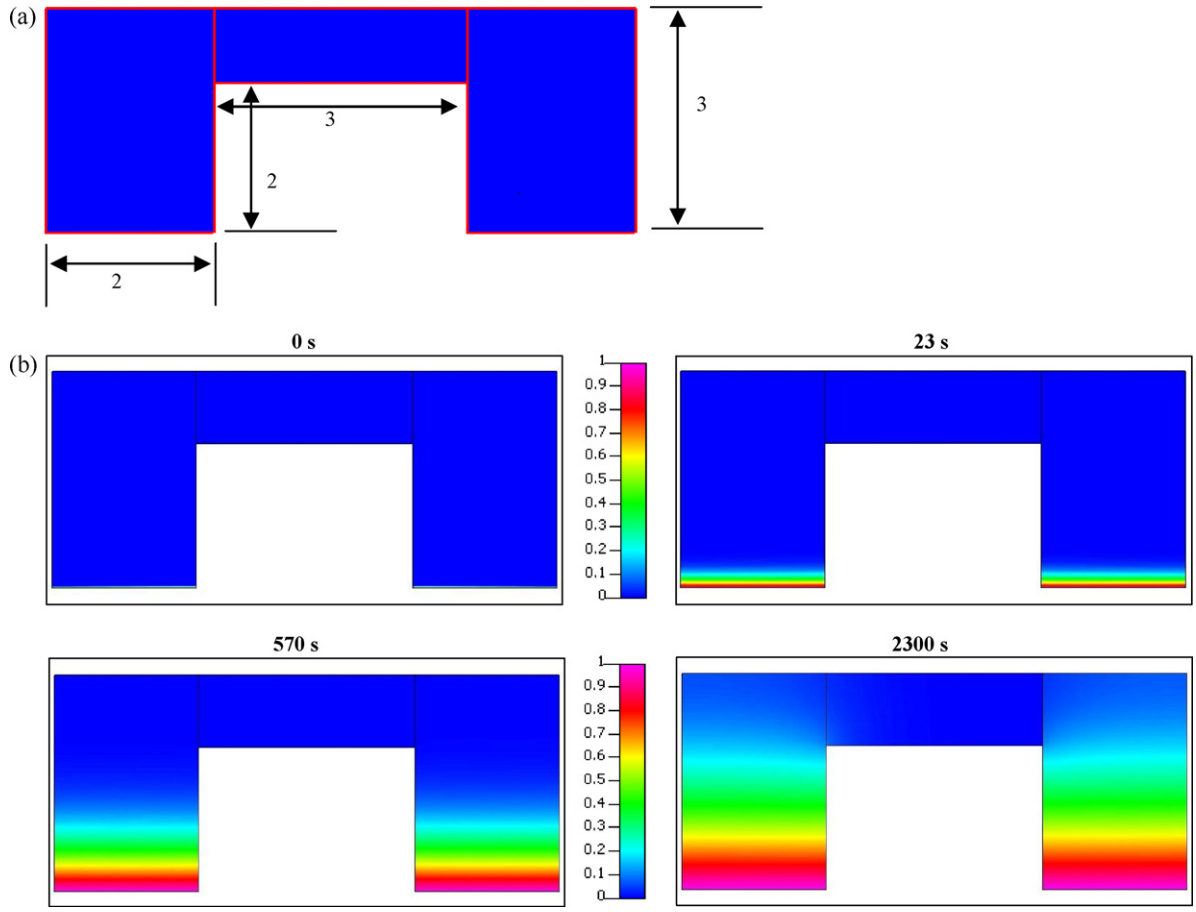

Fig. 5. Simulation of diffusion of reagents in the agarose within the trapping hole. (a) Dimensions are shown in millimeter, i.e. the mushroom stems are 2 mm wide at the bottom and 2 mm high; the centre-to-centre distance between the two mushroom stems is 5 mm. (b) Diffusion at various times (0, 23, 570, and 2300 s) between two adjacent agarose-containing trapping holes.

2.2.3. Cell staining assay

HL-60 cells were sub-cultured every 3 days using RPMI medium in a CO_2 incubator (at 5% CO_2, 95% RH, and 37 °C). The cells were washed with PBS solution for 3 times and re-suspended in fresh medium before experiment. The cells were added to warm clear LMPA (3%, at 37 °C) at a final cell density of 5×10^5/ml. The cell suspension was pipetted up and down several times to produce a uniformly turbid mixture. Then the agarose was introduced as a radial strip by running a dispensing pipette along the imaginary row of holes. A volume of ~100 μL was usually used for 1 radial strip. After 1 min, the chip was reversibly sealed with a circular glass disk. Thereafter, reagents (e.g. 10 μM FDA and trypan blue, 3 μL) were added to the inlet reservoirs in adjacent spiral channels. Centrifugal liquid delivery was achieved by spinning the disk at 1000 rpm. After 3 min of spinning, fluorescent or bright-field imaging of stained cells was achieved using an inverted microscope (Nikon TE300) as previously described [21].

As for the cell viability test, the chip was put back to the CO_2 incubator after trypan blue staining. In the case of staining cells not in a gel (as a control), a small aliquot of cell suspension was mixed with trypan blue, and then the cells were counted using a hemocytometer.

3. Results and discussion

3.1. The low-melting point agarose

Warm LMPA solution, which was premixed with a blue dye, was introduced in the cell trapping hole for gelling, see Fig. 2c. The inset shows the gel after it was pulled out from the hole and turned upside down. The mushroom stem shows the part of the gel that was in the cell trapping hole.

The LMPA solution should be viscous enough to stay within the cell trapping hole, but not to enter the spiral channel underneath and block them, see Fig. 3a. To examine if this is the case, the warm LMPA solution was put as a strip over individual holes and several images were taken from above (Fig. 3b) and below (Fig. 3c and d). In Fig. 3c, the holes looked bright and the shade of its color was the same as that of the channels, suggesting that no LMPA has leaked into the channels and they were not blocked. This was achieved after we have optimized the LMPA concentrations and found that

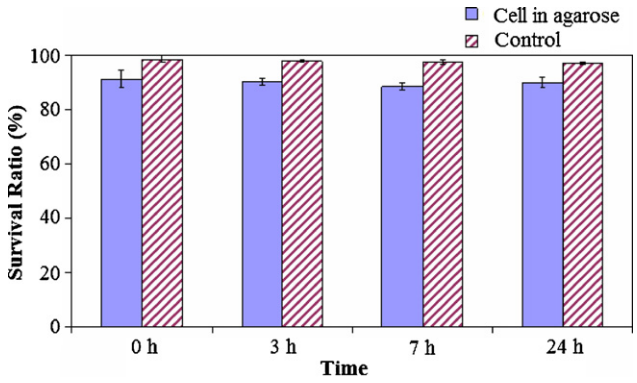

Fig. 6. Cell survival ratio after trapped inside agarose, as compared to agarose-free controls.

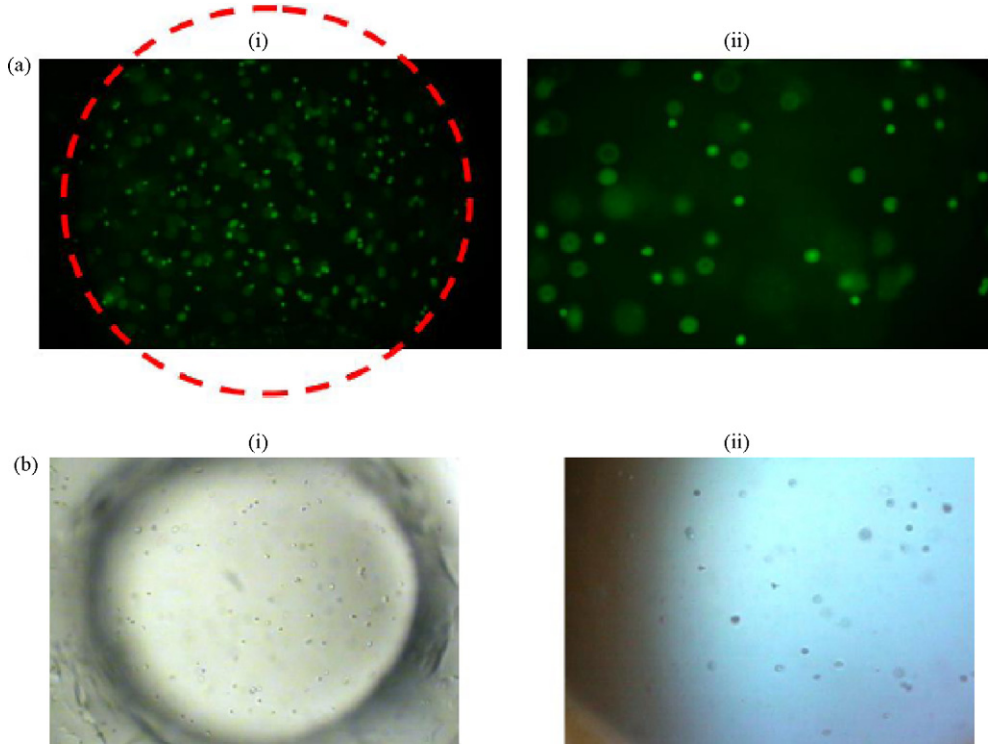

Fig. 7. Images of HL-60 cells in the cell trapping agarose. (a) Cells stained by fluorescein after the delivery of fluorescein diacetate (FDA) (i) at 10× magnification, with the red circle showing the location of the hole, and (ii) at 20× magnification. (b) Cells stained by trypan blue (i) at 10× magnification, and (ii) at 20× magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3% LMPA solutions are viscous enough not to leak into the spiral channels. For comparison, Fig. 3d depicts the situation in which the holes at two spiral channels have the same shade of color as the LMPA gel, showing that blockage has occurred in this case of using 2% LMPA. On the other hand, gel formed from 4% LMPA was too rigid after gelling, and this would damage the entrapped cells or slow down reagent diffusion. Therefore, 3% LMPA was selected for use in subsequent experiments.

3.2. The staining experiments via the spiral channels

Then, we tested the staining of the LMPA by the reagent delivered using centrifugal pumping within the spiral channels. As shown in Fig. 4a, 16 radial rows of LMPA were applied on the chip at the location of the trapping holes. A blue dyed solution was put at the inlet reservoir and delivered via one spiral channel to show immediate staining of the agarose. This is seen as a discontinuous anti-clockwise blue spiral track in Fig. 4b, spiraling out from the centre to the perimeter of the chip. When the dyed solution flowed past each of the trapping holes, the solution was accumulated inside the hole, and reached the bottom of the agarose to stain it. This observation was also shown in the video clip in Electronic Supplementary Information using red dyed solutions.

Subsequently, the food dyes were applied into all of the 16 inlet reservoirs to flow through the 16 spiral channels. Fig. 4c shows the result of all of the 256 holes being stained in blue. Thereafter, two food dyes (blue and red) were used to stain alternate spiral channels. Fig. 4d shows the chip after the delivery process was completed, and it was observed that the agarose inside the holes has been stained in blue and red colors alternately.

Fig. 4d (bottom) depicts an enlarged region showing one radial row of agarose that has been stained alternately in red and blue, and it is conceivable that all the holes can be stained in 16 different colors. After the mushroom-like LMPA was pulled out from the hole, it was confirmed that only the bottom of the mushroom stem, but not the mushroom head, was stained by the food dye.

Since the distance between the holes was 5 mm (from center to center), they were far enough not to induce cross contamination from diffusion of reagents over adjacent holes, as evidenced by the clear regions between individual red and blue dots.

The diffusion of reagents from the spiral microchannel up to the cell trapping reservoir has been reasonably fast. A simulation study has been launched, see the dimension of the geometry of gel in two adjacent cell trapping reservoirs with 5 mm apart (Fig. 5a). For the diffusion of reagent to the bottom 100 μm layer in the gel only takes ~23 s, as determined in a simulation study, see Fig. 5b. However, it takes another 570 s to rise up to 1 mm on the stem. This is consistent with time frame of obtaining the stained mushroom stem results shown in Fig. 4d inset. On the other hand, it takes 2300 s for the dye to diffuse up to 2 mm, indicating that diffusion of the reagent up one hole and then transfer to another hole takes much longer time. Since the centre-to-centre distance between holes is 5 mm apart, this indicates that even after 2300 s, the solutions from two adjacent cell trapping holes have not met. Such a situation has been depicted in Fig. 4d. Therefore, our design has provided the physical barrier needed to resolve the contamination problem encountered in multi-channel cell array studies [16,17].

3.3. Cell staining assay

The cells remained viable after being treated at the gelling temperature of LMPA at ~37 °C. The survival ratio of HL-60 cells, which is given by the ratio of the number of non-trypan blue stained cells to the total number of cells, is depicted in Fig. 6 over a duration of 0–24 h. It is found that the cells embedded in LMPA remained

high for 0–24 h. The survival ratio is only slightly lower than that obtained in the non-gel solution control, indicating LMPA is not detrimental to embed cells.

In cell viability tests, fluorescein diacetate (FDA) and trypan blue have been used to distinguish between live and dead cells, respectively, embedded in LMPA on the circular microfluidic array chip. It is because only live cells, but not dead cells, contain the enzymes to hydrolyze FDA to produce fluorescein. On the other hand, trypan blue only stains the cells with compromised cell membranes, but not live cells.

Fig. 7a(i) shows the fluorescent image (10× magnification) obtained at the cell trapping hole. The observation of green fluorescent cells indicates the presence of viable cells embedded in this location (the red circle indicate the outline of cell trapping reservoir). A magnified fluorescent image (20×) of the live cells was depicted in Fig. 7a(ii), showing a closeup image of the stained viable cells.

To mimic the situation involving dead cells, HL-60 cells were preheated to 90 °C in the LMPA solution. Fig. 7b (i) shows the 10× bright-field images of the dead cells inside the trapping hole before trypan blue staining. While in Fig. 7b(ii), the cells have been treated by the delivery of trypan blue in the spiral channels, showing all the dead cells were stained in blue.

So far, the cell staining experiments have been shown using two reagents, but it is conceivable that all the cell trapping holes along one radial strip can be tested with 16 different reagents.

4. Conclusion

The microfluidic array chip has been demonstrated in numerous staining experiments. One application is in cell viability assay using HL-60 cells. The advantages of the chip include flexibility in cell array generation (16 radial agarose strips), low reagent consumption (3 μL), simultaneous in-channel liquid delivery (16 channels), and multi-cell multi-reagent capability. The method may also be useful in a non-biological context to reveal the color change obtainable at the trapping holes only upon disk rotation.

Acknowledgement

We thank Natural Sciences and Engineering Research Council (NSERC) of Canada for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.02.011.

References

[1] C.W. Xu, Genome Res. 12 (2002) 482–486.
[2] J. Korbelik, M. Cardeno, J.P. Matisic, A.C. Carraro, C. MacAulay, Cell. Oncol. 29 (2007) 435–442.
[3] D.R. Albrecht, V.L. Tsang, R.L. Sah, S.N. Bhatia, Lab Chip 5 (2005) 111–118.
[4] T. Peterbauer, J. Heitz, M. Olbrichb, S. Hering, Lab Chip 6 (2006) 857–863.
[5] G.T. Franzesi, B. Ni, Y. Ling, A. Khademhosseini, J. Am. Chem. Soc. 128 (2006) 15064–15065.
[6] W.G. Koh, L.J. Itle, M.V. Pishko, Anal. Chem. 75 (2003) 5783–5789.
[7] S.N. Bhatia, U.J. Balis, M.L. Yarmush, M. Toner, Biotechnol. Prog. 14 (1998) 378–387.
[8] A. Khademhosseini, K.Y. Suh, S. Jon, G. Eng, J. Yeh, G.-J. Chen, R. Langer, Anal. Chem. 7 (2004) 3675–3681.
[9] M. Mrksich, C.S. Chen, Y. Xia, L.E. Dike, D.E. Ingber, G.M. Whitesides, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 10775–10778.
[10] H. Tokano, J. Sul, M.L. Mazzanti, R.T. Doyle, P.G. Haydon, M.D. Porter, Anal. Chem. 74 (2002) 4640–4646.
[11] H. Kaji, M. Nishizawa, T. Matsue, Lab Chip 3 (2003) 208–211.
[12] S. Takayama, E. Ostuni, P. LeDuc, K. Naruse, D.E. Ingber, G.M. Whitesides, Nature 411 (2001) 1016.
[13] L. Wang, P.C.H. Li, H.Z. Yu, A.M. Parameswaran, Anal. Chim. Acta 610 (2008) 97–104.
[14] X.Y. Peng, P.C.H. Li, H.Z. Yu, A.M. Parameswaran, W.L. Chou, Sens. Actuators B 128 (2007) 64–69.
[15] P.C.H. Li, E. Lam, W.P. Roos, M.Z. Zdzienicka, B. Kaina, T. Efferth, Cancer Res. 68 (2008) 4347–4351.
[16] B. Chueh, D. Huh, C.R. Kystsos, T. Houssin, N. Futai, S. Takayama, Anal. Chem. 79 (2007) 3504–3508.
[17] A. Khademhosseini, J. Yeh, G. Eng, J. Karp, H. Kaji, J. Borenstein, O.C. Farokhzad, R. Langer, Lab Chip 5 (2005) 1380–1386.
[18] L. Wang, P.C.H. Li, Anal. Biochem. (2010), doi:10.1016/j.ab.2010.01.017.
[19] X.Y. Peng, P.C.H. Li, Can. J. Pure Appl. Sci. 2 (2008) 551–556.
[20] H. Chen, L. Wang, P.C.H. Li, Lab Chip 8 (2008) 826–829.
[21] X.Y. Peng, P.C.H. Li, Lab Chip 5 (2005) 1298–1302.