Analytical Biochemistry 436 (2013) 160–164

Contents lists available at SciVerse ScienceDirect

Analytical Biochemistry

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

A lateral flow biosensor for the detection of human pluripotent stem cells

Wei Wu [1], Luxin Yu [1], Zhiyuan Fang, Puchang Lie, Lingwen Zeng *

Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China

ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form 27 December 2012
Accepted 29 January 2013
Available online 12 February 2013

Keywords:
Lateral flow biosensor
Human pluripotent stem cells
SSEA-3
SSEA-4

ABSTRACT

A lateral flow biosensor based on immunoassay has been developed for the detection of human stem cells for the first time. Antibody specific for a human stem cell surface antigen, SSEA-4, is coated onto gold nanoparticles, whereas antibody against another human pluripotent stem cell surface antigen, SSEA-3, is immobilized on the test zone of the NC membrane. Target cells bind to the antibody coated on the gold nanoparticles to form nanoparticles–stem cell complexes, and the complexes are then captured by another antibody immobilized on the test zone to form a red line for visual detection. This biosensor has been successfully applied to human embryonic stem cells and induced pluripotent stem cells. It is capable of detecting a minimum of 10,000 human embryonic stem cells by the naked eye and 7000 cells with a portable strip reader within 20 min. This approach has also shown excellent specificity to distinguish other types of cells. The biosensor shows great promise for specific and handy detection of human pluripotent stem cells.

© 2013 Elsevier Inc. All rights reserved.

Recently, stem cell research has elicited continued scientific, commercial, and public interest. Stem cells, such as embryonic stem (ES)[2] cells and induced pluripotent stem (iPS) cells, are pluripotent and can differentiate into any of the hundreds of cell types. The biological properties of stem cells have raised exciting new prospects of generating an ideal source of healer cells that has no adverse effect and low risk of immune rejection [1]. These unique capabilities make stem cells a renewable source of a wide range of cell types for potential use in drug screening and therapies for many diseases [2–4]. Discrimination of stem cells from other cells is very important in two aspects: induction of pluripotent stem cells from somatic cells and direct differentiation of stem cells to somatic cells. An efficient approach for the detection of stem cells is critical in research and clinics. Therefore, it is important to develop a convenient method to discriminate stem cells from other cells using specific genes or protein markers.

Most of the methods, except the flow cytometric method and immunofluorescence [5], for stem cell identification are based on the analyzing of cell lysate such as reverse transcription–polymerase chain reaction (RT–PCR) [6], Western blot [7], and Northern blot [8]. They use specific expressed proteins [5.7] and messenger RNAs (mRNAs) [8] in stem cells as markers. These methods are time-consuming and cumbersome to perform.

Detection of biological entities using cell-based assays has already found its niche in biomedical research as well as in clinics [9]. Various biosensors for whole cell detection have been reported to detect cancer cells [10,11] and immunocytes [12–14], such as microfluidic cell-based biosensor (mfCBB) [15], light-addressable potentiometric sensor (LAPS) [16], and localized surface plasmon resonance biosensor (LSPRB) [17], whereas no stem cell biosensor has been reported.

Gold nanoparticles (AuNPs) are better than other signal transducers by the virtue of their facile synthesis, large specific surface area, high chemical stability, favorable biocompatibility, good conductivity, and high affinity of binding to amine/thiol-containing molecules [18]. Previously, we developed different kinds of biosensors based on AuNPs to detect transcriptional factors [19], copper [20], nucleic acid [21], and cancer cells [10], which offer a promising approach to realize handy detection of whole stem cells.

In this article, we show the development and working principle of a lateral flow biosensor (LFB) for stem cell detection. The surface proteins, SSEA-3 and SSEA-4 [22], are used to mark human pluripotent stem cells (hPSCs). AuNPs–SSEA-4 antibody–stem cells complexes captured by the antibody of SSEA-3 accumulate on the test line and produce a characteristic red band that can be used for either qualitative (i.e., visual evaluation) or quantitative detection of cells by a portable strip reader. Antibodies of human immunoglobulin G (IgG) are immobilized on the control line to capture the excess of AuNPs–SSEA-4 antibody, resulting in a second red line. The LFB has the following features: (i) short detection time

* Corresponding author.
E-mail address: zeng_lingwen@gibh.ac.cn (L. Zeng).

[1] Joint first authors; these two authors contributed equally to this work.

[2] *Abbreviations used:* ES, embryonic stem; iPS, induced pluripotent stem; AuNP, gold nanoparticle; LFB, lateral flow biosensor; hPSC, human pluripotent stem cell; IgG, immunoglobulin G; BSA, bovine serum albumin; NC, nitrocellulose; PBS, phosphate-buffered saline; S/N, signal/noise; SSC, saline–sodium citrate; TBS, Tris-buffered saline.

0003-2697/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2013.01.034

(within 20 min), (ii) tested cells without lysis, and (iii) no need for complex and expensive instrumentation.

Materials and methods

Materials

Human SSEA-3 and SSEA-4 antibodies were purchased from R&D Systems (Minneapolis, MN, USA). Bovine serum albumin (BSA) and $HAuCl_4$ were purchased from Sigma–Aldrich (Steinheim, Germany). Nitrocellulose (NC) membranes were purchased from Shantou Ealon (Shantou, China). All other reagents were purchased from standard commercial sources and were of analytical grade. All buffer solutions used in this study were prepared in our laboratory using ultrapure water (resistance of 18 MΩ).

Preparation of AuNPs–SSEA-4 antibody conjugates

AuNPs (average of 25 nm) were prepared according to the literature with slight modification [10]. Briefly, in a 250-ml round-bottom flask, 4 ml of 1% trisodium citrate was added to 100 ml of rapidly stirred and boiling $HAuCl_4$. The color of the solution turned deep blue in 20 s and then turned wine red in 60 s. The solution was kept boiling for approximately 10 min and then was returned to room temperature by natural cooling to form AuNPs. The resulting AuNPs solution was stored at 4 °C and was used for the preparation of the AuNPs–SSEA-4 antibody conjugates.

The pH of AuNPs solution was adjusted to 8.0 with 100 mM K_2CO_3. Then 9 μg of human SSEA-4 antibody was added to the 100 μl of AuNPs solution. After shaking at 200 rpm for 1 h at room temperature, 10% (w/v) BSA stock solution was added to the AuNPs solution to a final concentration of 1% (w/v). Next, 1 h later, the resulting AuNPs solution was concentrated by centrifugation. After centrifugation for 30 min at 12,000 rpm, the supernatant was removed without disturbing the pellet. The loose part of the pellet was resuspended with resuspension solution (20 nM Na_3PO_4, 10% BSA, and 0.05% NaN_3) in a small volume (~100 μl).

Preparation of LFB for stem cell detection

The LFB for stem cell detection is fabricated as the schematic diagram shown in Fig. 1. The biosensor consists of three components: sample pad, NC membrane, and absorbent pad. The sample pad is prepared by soaking glass fiber (17 mm × 30 cm) in the sample pad buffer (1% Triton, 1% BSA, 2% glucose, and 50 nM boric acid, pH 8.0). Then it was dried and stored in a low-humidity chamber at room temperature.

Human SSEA-3 monoclonal antibody (30 μl of 1 mg/ml) and IgG antibody (30 μl, 1 mg/ml) were dispensed onto the NC membrane (25 mm × 30 cm) to form a test line and control line with a lateral flow dispenser (Shanghai Kinbio, Shanghai, China), respectively. The distance between the test line and the control line was approximately 5 mm. The membrane was then dried at room temperature for 12 h and stored at 4 °C. Finally, the three components of the LFB were assembled on a plastic adhesive backing (60 mm × 30 cm). Each part overlapped 2 mm to ensure the migration of sample solution through the biosensor during the assay. Strips with a 4 mm width were cut by using a paper cutter (programmed high-speed cutter, Shanghai Kinbio).

Detection of stem cells by LFB

The experiments were performed in 80 μl of sample solution containing different amounts of stem cells and 2 μl of AuNPs–SSEA-4 antibody conjugates in 10 mM phosphate-buffered saline (PBS). After reaction for 10 min at room temperature, the solution was applied to the sample application well and the test line and control line were visualized within 10 min. For quantitative detection, the biosensor was scanned using a handheld strip reader (Shanghai Kinbio). The optical intensity of the test line and control line could be recorded simultaneously by using the Shanghai Kinbio strip reader software. To define a positive result, we used the signal/noise (S/N) ratio ($S/N \geqslant 3$) as reference. Signal is the optical intensity of the test line with detected sample, and noise is the intensity of the test line with PBS.

Statistical analysis

SPSS 10.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. The results are expressed as means ± standard deviations of three independent experiments. Individual comparisons were made by Student's t test for paired data, and P values less than 0.05 were considered to be significant.

Results and discussion

Principle of LFB for stem cell detection

In this study, an LFB for stem cell detection based on the double antibody sandwich method is proposed, as depicted in Fig. 1. The human SSEA-4 antibody is conjugated with AuNPs to form AuNPs–SSEA-4 antibody conjugates. The human SSEA-3 antibody is immobilized on the test line of the LFB. In a typical assay, sample solution containing stem cells and AuNPs–anti-SSEA-4 conjugates is premixed and applied on the LFB sample pad. The solution containing the AuNPs–SSEA-4 antibody–stem cell complex migrates by capillary action until it is captured on the test line by SSEA-3 antibody immobilized on the membrane. The accumulation of AuNPs on the test line is visualized as a characteristic red band (test line). The excess of AuNPs–anti-SSEA-4 conjugates continues to migrate to the control line in which the anti-human IgG antibody is immobilized. The excess of AuNPs–anti-SSEA-4 conjugates is then captured by the anti-mouse IgG antibody to form a second red band (control line). In the absence of stem cells, no red band is observed in the test line. In this case, a red control band shows that the LFB is working properly. Qualitative analysis is performed by observing the color change caused by the accumulation of AuNPs on the test line, and quantitative detection can be realized by recording the optical density of the test line with the strip reader.

Optimization of experimental conditions

To improve sensitivity and specificity for the detection of stem cells, the experimental conditions were optimized systematically. We studied the effect of the amount of AuNPs–anti-SSEA-4 conjugates and the reaction buffer on the S/N ratio of the assay. The amount of AuNPs–anti-SSEA-4 conjugates is one of the most important factors to affect the sensitivity of the LFB. Different volumes of the AuNPs–anti-SSEA-4 conjugates, ranging from 1 to 3 μl, were used to conjugate with 1×10^5 stem cells. The highest S/N ratio was obtained with a volume of 2 μl of AuNPs–anti-SSEA-4 conjugate (Fig. 2A). Therefore, 2 μl of AuNPs–anti-SSEA-4 conjugate was selected in the following experiments. Sample buffer is another important factor affecting the sensitivity and specificity of the LFB. In this study, three types of reaction buffer—10 mM PBS, 10× saline–sodium citrate (SSC), and 10 mM Tris-buffered saline (TBS)—were used to test the assay performance. The best S/N ratio was obtained when 10 mM PBS was used in the assay. Accordingly, 10 mM PBS was used throughout the experiments to obtain the best signal (Fig. 2B).

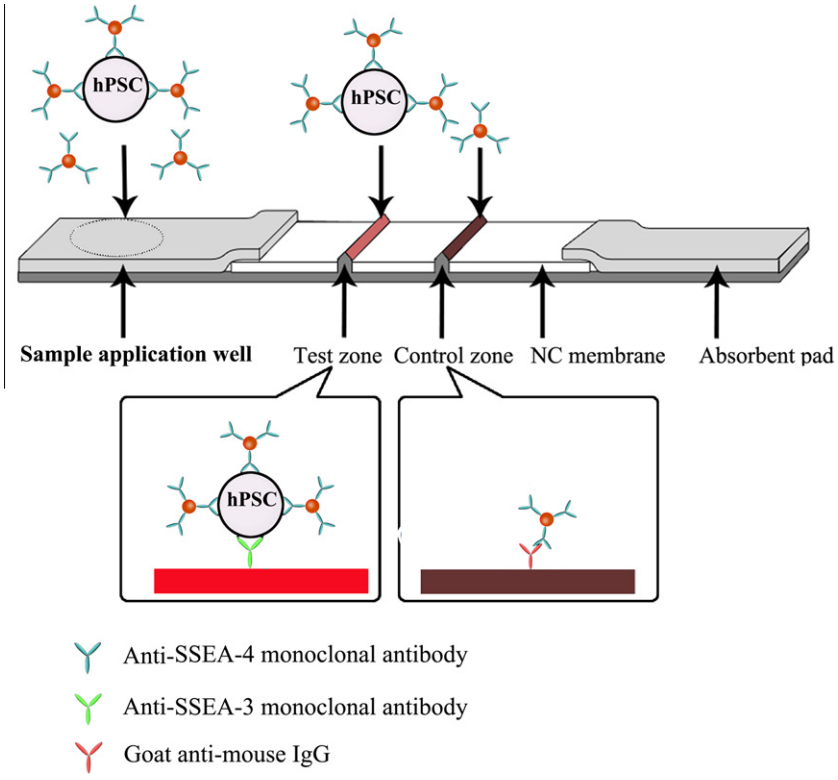

Fig.1. Schematic illustration of the LFB for the detection of human pluripotent stem cells (hPSCs).

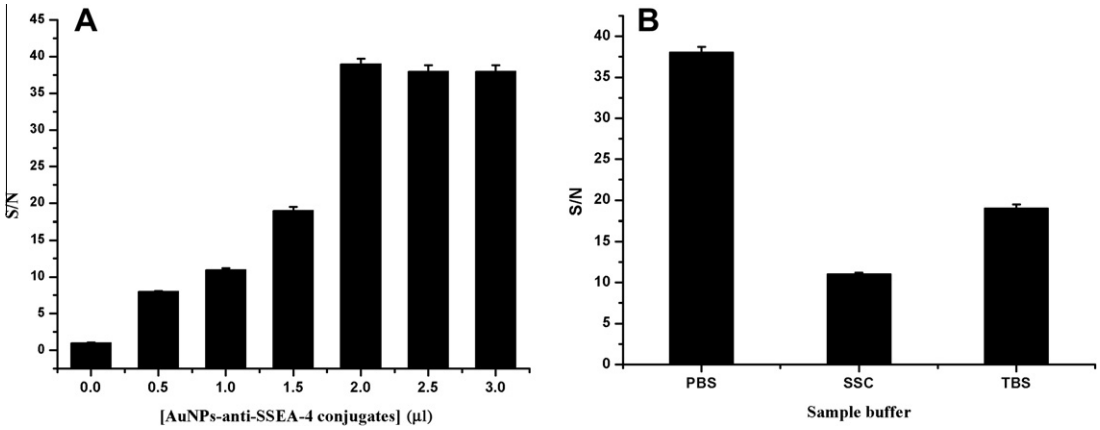

Fig.2. (A) Effect of the amount of AuNPs–anti-SSEA-4 conjugate on the S/N ratio of 1×10^5 stem cells assay. (B) Effect of different sample buffers on the S/N ratio of LFB. Amount of stem cells: 1×10^5; volume of sample solution: 80 μl ($n = 3$).

Performance of stem cell sensor

At the optimized conditions, the stem cell sensor was used to detect different amounts of stem cells in the sample solution. First, stem cells were mixed in sample buffer at seven dilutions as follows: 0, 1×10^4, 2×10^4, 4×10^4, 8×10^4, 1×10^5, and 2×10^5. The visual judgment of the test line could be used for the qualitative detection of stem cells. Fig. 3A shows the typical photo images (left) and the corresponding optical responses (right; intensities of test line and control line) of the LFB with the sample buffer containing different amounts of stem cells. There is no red band observed on the test line of the LFB in the absence of stem cells (negative control). The red bands on the test line are observed with as little as 1×10^4 stem cells. The red bands appearing on the control line LFB indicate that the LFB was working properly. Quantitative detections were performed by recording the intensities of the test lines with a strip reader. The peak areas increased with the increase of the target cell amounts (Fig. 3A, curves a–g). The resulting calibration plot of the peak areas versus the logarithm of stem cell number is linear over the 1×10^4 to 2×10^5 range. The detection limit of 7000 stem cells (based on S/N = 3) was estimated (Fig. 3B).

For the specificity assay, many kinds of cell lines without the expression of SSEA-3 and SSEA-4 were tested. Fig. 4 displays that high peak area was observed on the test line of the biosensor when the human embryonic stem cells were tested, but low peak areas on test bands were observed when mouse embryonic stem cells, SP20 cell line, 293T cell line, human fibroblast cells, and HeLa cells were tested. These results demonstrate that the biosensor is specific for human pluripotent stem cells, not for stem cells from other species or human somatic cells.

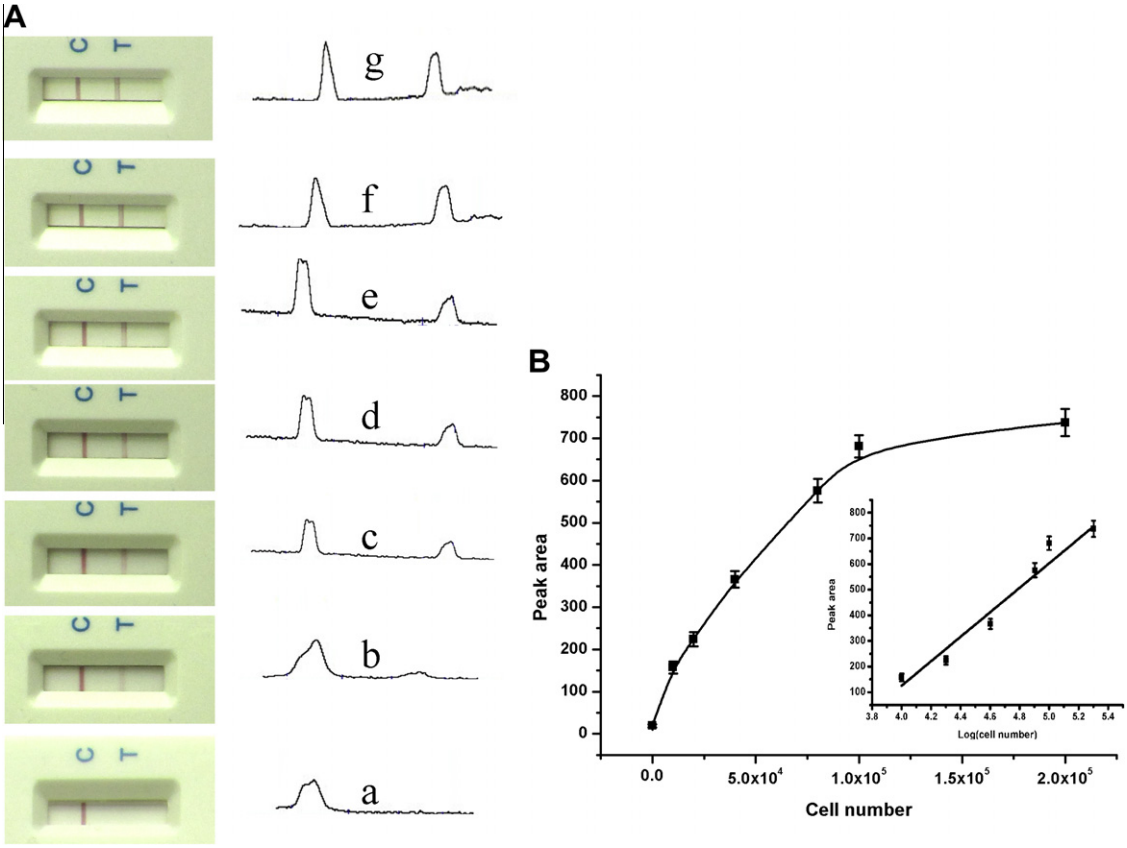

Fig.3. (A) Typical responses of LFB with increasing amounts of stem cells. The amounts of stem cells were 0 (a), 1×10^4 (b), 2×10^4 (c), 4×10^4 (d), 8×10^4 (e), 1×10^5 (f), and 2×10^5 (g). (B) Calibration curve of the assay with different amounts of stem cells. The peak areas were averages for three measurements.

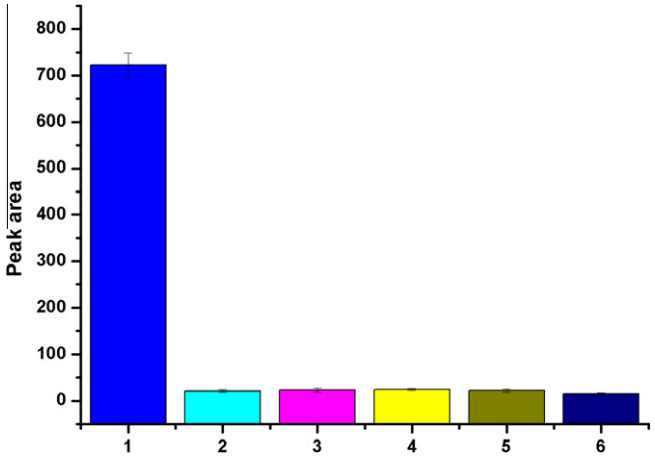

Fig.4. Photo images of LFB for specificity analysis with stem cells. Column 1: human embryonic stem cells; column 2: mouse embryonic stem cells; column 3: SP20 cell line; column 4: 293T cell line; column 5: human fibroblast cells; column 6: HeLa cells.

Conclusion

In the current work, we have developed a rapid, sensitive, and highly specific biosensor for the detection of human pluripotent stem cells. The LFB is capable of detecting a minimum of 10,000 stem cells within 20 min by the naked eye and 7000 stem cells with a portable strip reader. The response of the optimized LFB is highly linear over a range of 1×10^4 to 2×10^5 human stem cells. The LFB can have important applications in the field of regenerative medicine such as to determine the efficiency of iPS cells from somatic cells and to determine the efficiency of direct differentiation from ES or iPS cells to somatic cells, including hematopoietic cells, cartilage cells, and adipose cells. By analogy, this approach can be extended to detect different types of stem cells. For example, a pair of antibodies to mouse stem cell surface markers, SSEA-1 and Thy-1, can be used in the LFB for the detection of mouse stem cells. Furthermore, it is possible to develop a stem cell biosensor for the simultaneous detection of two kinds of stem cells. Such an LFB does not require any specialized equipment or skills. It is inexpensive and rapid and, thus, holds great potential for wide applications, especially in stem cell clinical therapy.

Acknowledgments

This work was financially supported by the Ministry of Science and Technology 973 Project (2013CB967100). We thank Duanqing Pei and Donghai Wu for sharing the ES cell line and iPS cells.

References

[1] M. Politis, O. Lindvall, Clinical application of stem cell therapy in Parkinson's disease, BMC Med. 10 (2012) 1.
[2] T. Jadczyk, A. Faulkner, P. Madeddu, Stem cell therapy for cardiovascular disease: the demise of alchemy and rise of pharmacology, Br. J. Pharmacol. (in press), http://dx.doi.org/10.1111/j.1476-5381.2012.01965.x.
[3] H.P. Kiem, K.R. Jerome, S.G. Deeks, J.M. McCune, Hematopoietic-stem-cell-based gene therapy for HIV disease, Cell Stem Cell 10 (2012) 137–147.
[4] T. Takami, S. Terai, I. Sakaida, Stem cell therapy in chronic liver disease, Curr. Opin. Gastroenterol. 28 (2012) 203–208.
[5] H. Suila, V. Pitkanen, T. Hirvonen, A. Heiskanen, H. Anderson, A. Laitinen, S. Natunen, H. Miller-Podraza, T. Satomaa, J. Natunen, S. Laitinen, L. Valmu, Are globoseries glycosphingolipids SSEA-3 and -4 markers for stem cells derived from human umbilical cord blood?, J Mol. Cell. Biol. 3 (2011) 99–107.

[6] J. Vermeulen, S. Ballet, O. Oberlin, M. Peter, G. Pierron, E. Longavenne, V. Laurence, J. Kanold, P. Chastagner, O. Lejars, J.Y. Blay, P. Marec-Berard, J. Michon, O. Delattre, G. Schleiermacher, Incidence and prognostic value of tumour cells detected by RT–PCR in peripheral blood stem cell collections from patients with Ewing tumour, Br. J. Cancer 95 (2006) 1326–1333.
[7] Y. Guo, C. Mantel, R.A. Hromas, H.E. Broxmeyer, Oct-4 is critical for survival/antiapoptosis of murine embryonic stem cells subjected to stress: effects associated with Stat3/survivin, Stem Cells 26 (2008) 30–34.
[8] E. Luzi, F. Marini, S.C. Sala, I. Tognarini, G. Galli, M.L. Brandi, Osteogenic differentiation of human adipose tissue-derived stem cells is modulated by the miR-26a targeting of the SMAD1 transcription factor, J. Bone Miner. Res. 23 (2008) 287–295.
[9] E.R. Fairey, M.Y. Bottein Dechraoui, M.F. Sheets, J. Ramsdell, Modification of the cell based assay for brevetoxins using human cardiac voltage dependent sodium channels expressed in HEK-293 cells, Biosens. Bioelectron. 16 (2001) 579–586.
[10] G. Liu, X. Mao, J.A. Phillips, H. Xu, W. Tan, L. Zeng, Aptamer–nanoparticle strip biosensor for sensitive detection of cancer cells, Anal. Chem. 81 (2009) 10013–10018.
[11] T. Norikura, K. Fujiwara, T. Narita, S. Yamaguchi, Y. Morinaga, K. Iwai, H. Matsue, Anticancer activities of thelephantin O and vialinin A isolated from *Thelephora aurantiotincta*, J. Agric. Food Chem. 59 (2011) 6974–6979.
[12] P. Banerjee, D. Lenz, J.P. Robinson, J.L. Rickus, A.K. Bhunia, A novel and simple cell-based detection system with a collagen-encapsulated B-lymphocyte cell line as a biosensor for rapid detection of pathogens and toxins, Lab. Invest. 88 (2008) 196–206.
[13] T. Curtis, R.M. Naal, C. Batt, J. Tabb, D. Holowka, Development of a mast cell-based biosensor, Biosens. Bioelectron. 23 (2008) 1024–1031.
[14] S.B. Kim, H. Bae, J.M. Cha, S.J. Moon, M.R. Dokmeci, D.M. Cropek, A. Khademhosseini, A cell-based biosensor for real-time detection of cardiotoxicity using lens-free imaging, Lab Chip 11 (2011) 1801–1807.
[15] J. Sun, P. Chen, X. Feng, W. Du, B.F. Liu, Development of a microfluidic cell-based biosensor integrating a millisecond chemical pulse generator, Biosens. Bioelectron. 26 (2011) 3413–3419.
[16] Q. Liu, N. Hu, F. Zhang, H. Wang, W. Ye, P. Wang, Neurosecretory cell-based biosensor: monitoring secretion of adrenal chromaffin cells by local extracellular acidification using light-addressable potentiometric sensor, Biosens. Bioelectron. 35 (2012) 421–424.
[17] T. Endo, S. Yamamura, K. Kerman, E. Tamiya, Label-free cell-based assay using localized surface plasmon resonance biosensor, Anal. Chim. Acta 614 (2008) 182–189.
[18] Y. Song, K. Cui, L. Wang, S. Chen, The electrodeposition of Ag nanoparticles on a type I collagen-modified glassy carbon electrode and their applications as a hydrogen peroxide sensor, Nanotechnology 20 (2009) 105501.
[19] Z. Fang, C. Ge, W. Zhang, P. Lie, L. Zeng, A lateral flow biosensor for rapid detection of DNA-binding protein c-jun, Biosens. Bioelectron. 27 (2011) 192–196.
[20] Z. Fang, J. Huang, P. Lie, Z. Xiao, C. Ouyang, Q. Wu, Y. Wu, G. Liu, L. Zeng, Lateral flow nucleic acid biosensor for Cu^{2+} detection in aqueous solution with high sensitivity and selectivity, Chem. Commun. 46 (2010) 9043–9045.
[21] P. Lie, J. Liu, Z. Fang, B. Dun, L. Zeng, A lateral flow biosensor for detection of nucleic acids with high sensitivity and selectivity, Chem. Commun. 48 (2012) 236–238.
[22] T. Enver, M. Pera, C. Peterson, P.W. Andrews, Stem cell states, fates, and the rules of attraction, Cell Stem Cell 4 (2009) 387–397.