Efficient on-chip isolation of HIV subtypes

ShuQi Wang,† Matin Esfahani,† Umut A. Gurkan, Fatih Inci, Daniel R. Kuritzkes and Utkan Demirci*

Received 1st April 2011, Accepted 6th January 2012
DOI: 10.1039/c2lc20706k

HIV has caused a global pandemic over the last three decades. There is an unmet need to develop point-of-care (POC) viral load diagnostics to initiate and monitor antiretroviral treatment in resource-constrained settings. Particularly, geographical distribution of HIV subtypes poses significant challenges for POC immunoassays. Here, we demonstrated a microfluidic device that can effectively capture various subtypes of HIV particles through anti-gp120 antibodies, which were immobilized on the microchannel surface. We first optimized an antibody immobilization process using fluorescent antibodies, quantum dot staining and AFM studies. The results showed that anti-gp120 antibodies were immobilized on the microchannel surface with an elevated antibody density and uniform antibody orientation using a Protein G-based surface chemistry. Further, RT-qPCR analysis showed that HIV particles of subtypes A, B and C were captured repeatably with high efficiencies of 77.2 ± 13.2%, 82.1 ± 18.8, and 80.9 ± 14.0% from culture supernatant, and 73.2 ± 13.6, 74.4 ± 14.6 and 78.3 ± 13.3% from spiked whole blood at a viral load of 1000 copies per mL, respectively. HIV particles of subtypes A, B and C were captured with high efficiencies of 81.8 ± 9.4%, 72.5 ± 18.7, and 87.8 ± 3.2% from culture supernatant, and 74.6 ± 12.9, 75.5 ± 6.7 and 69.7 ± 9.5% from spiked whole blood at a viral load of 10 000 copies per mL, respectively. The presented immuno-sensing device enables the development of POC on-chip technologies to monitor viral load and guide antiretroviral treatment (ART) in resource-constrained settings.

Introduction

33.3 million people are living with HIV-1 worldwide, with Sub-Saharan Africa accounting for 67% of the infected population.[1] To curb this pandemic, the World Health Organization (WHO) is rapidly expanding the number of AIDS patients receiving antiretroviral therapy (ART) in resource-constrained settings. These efforts, however, are significantly restricted by the prohibitive cost to implement ART monitoring tools, . ., CD4 cell counts by flow cytometry, and HIV viral load monitoring by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). To address this challenge, various portable CD4 cell counting methods have been developed, including electrical sensing,[2] microfluidic lensless imaging,[3–6] fluorescence staining,[7,8] microscopy counting[9,10] and flow cytometry.[11] Although CD4 cell count in combination with WHO disease staging is widely used to monitor patients, studies have shown that CD4 monitoring cannot detect early treatment failure that may result in

25 C for 5 minutes, 45 C for 60 minutes and 95 C for 3 minutes. In qPCR, 50 μL of the master mixture consisted of 1× core PCR buffer, 0.4 μM of forward primer LTR-F (5′-TAAAGCTTGCCTTGAGTGCT-3′) and reverse primer LTR-R2, 0.2 μM of TaqMan probe LTR-P (5′-AGTAGTGT GTGCCCGTCTGTTGTGTG-3′, JOE as the fluorophore and TAMRA as the quencher), 2.5 U of SureStart Taq polymerase, and 10 μL of cDNA template. The amplification reaction was performed on the 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA) with a protocol of 25 C for 5 minutes and 95 C for 10 minutes, which was followed by 50 cycles of 60 C for 1 minute and 95 C for 30 seconds.

Results and discussion

1. Optimization of Protein G-based antibody immobilization

To find the optimal concentration of Protein G for antibody immobilization, varying concentrations of Protein G were incubated in functionalized channels prior to fluorescent antibody (FITC conjugated anti-gp120 antibody) incubation. As shown in Fig. 2a, the average channel fluorescence intensity increased with Protein G concentrations (0–10 mg mL^{-1}), indicating that more antibodies were immobilized on the surface at higher concentrations of Protein G. We observed that the increase of antibody density did not linearly correlate with the increase of Protein G concentration (Fig. 2a). Once the Protein G concentration was more than 3 mg mL^{-1}, the fluorescence intensity only increased by 16.6% compared to the fluorescence intensity at 3 mg mL^{-1} of Protein G. In comparison, the fluorescence intensity increased by 83.3% when the Protein G concentration increased from 0 to 3 mg mL^{-1}. Thus, 3 mg mL^{-1} of Protein G was used to immobilize antibodies on the microchannel surface for the rest of the experiments.

(Fig. 3a). The fluorescence intensity in both channels was approximately 25 AU in the absence of BSA blocking due to non-specific binding of QDs. When BSA (1%) was applied after the antibody immobilization, the fluorescence intensity significantly reduced, indicating that BSA blocked the binding sites on

methods enhanced the fluorescence to 15 AU compared to the

supernatant and spiked whole blood. For culture supernatant samples, the capture efficiency of subtype A was 77.2 ± 13.2, 81.9 ± 9.4 and 83.9 ±

22 J. Hemelaar, E. Gouws, P. D. Ghys, S. Osmanov and W.-U.N.H.I. C, , 2011, 25, 679–689.
23 B. S. Taylor, M. E. Sobieszczyk, F. E. McCutchan and S. M. Hammer, . ., 2008, 358, 1590–1602.
24 R. Dhumpa, M. Bu, K. J. Handberg, A. Wolff and D. D. Bang, . , 2010, 169, 228–231.
25 B. C. Kim, M. K. Ju, A. Dan-Chin-Yu and P. Sommer, ., 2009, 81, 2388–2393.
26 Y. F. Lee, K. Y. Lien, H. Y. Lei and G. B. Lee, ., 2009, 25, 745–752.
27 Y. Li, S. A. Migueles, B. Welcher, K. Svehla, A. Phogat, M. K. Louder, X. Wu, G. M. Shaw, M. Connors, R. T. Wyatt and J. R. Mascola, ., 2007, 13, 1032–1034.
28 R. Wyatt, P. D. Kwong, E. Desjardins, R. W. Sweet, J. Robinson, W. A. Hendrickson and J. G. Sodroski, , 1998, 393, 705–711.
29 T. Zhou, L. Xu, B. Dey, A. J. Hessell, D. Van Ryk, S. H. Xiang, X. Yang, M. Y. Zhang, M. B. Zwick, J. Arthos, D. R. Burton, D. S. Dimitrov, J. Sodroski, R. Wyatt, G. J. Nabel and P. D. Kwong, , 2007, 445, 732–737.
30 Y. G. Kim, S. Moon, D. R. Kuritzkes and U. Demirci, ., 2009, 25, 253–258.
31 S. Wang, X. Zhao, I. Khimji, R. Akbas, W. Qiu, D. Edwards, D. W. Cramer, B. Ye and U. Demirci, , 2011, 11, 3411–3418.
32 D. Candotti, J. Temple, S. Owusu-Ofori and J. P. Allain, ., 2004, 118, 39–47.
33 N. Dinauer, S. Balthasar, C. Weber, J. Kreuter, K. Langer and H. von Briesen, , 2005, 26, 5898–5906.
34 B. M. Lingerfelt, H. Mattoussi, E. R. Goldman, J. M. Mauro and G. P. Anderson, ., 2003, 75, 4043–4049.
35 X. Wang, Y. Wang, H. Xu, H. Shan and J. R. Lu, ., 2008, 323, 18–25.
36 K. Kato, L. Y. Lian, I. L. Barsukov, J. P. Derrick, H. Kim, R. Tanaka, A. Yoshino, M. Shiraishi, I. Shimada and Y. Arata, ., , 1995, 3, 79–85.
37 I.-H. Cho, E.-H. Paek, H. Lee, J. Y. Kang, T. S. Kim and S.-H. Paek, ., 2007, 365, 14–23.
38 J. Pietzsch, J. F. Scheid, H. Mouquet, F. Klein, M. S. Seaman, M. Jankovic, D. Corti, A. Lanzavecchia and M. C. Nussenzweig, . ., 2010, 207, 1995–2002.
39 X. Wu, Z. Y. Yang, Y. Li, C. M. Hogerkorp, W. R. Schief, M. S. Seaman, T. Zhou, S. D. Schmidt, L. Wu, L. Xu, N. S. Longo, K. McKee, S. O'Dell, M. K. Louder, D. L. Wycuff, Y. Feng, M. Nason, N. Doria-Rose, M. Connors, P. D. Kwong, M. Roederer, R. T. Wyatt, G. J. Nabel and J. R. Mascola, , 2010, 329, 856–861.
40 B. Chackerian, L. M. Rudensey and J. Overbaugh, ., 1997,