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MICROFLUIDIC RNA INTERFERENCE SCREENING PLATFORM FOR HOST FACTORS REQUIRED FOR RIFT VALLEY FEVER VIRUS INFECTION

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High-throughput genome-wide RNA interference (RNAi) screening is a powerful systems biology approach for the discovery of novel genes and signaling pathways involved with normal cellular processes and pathogenic states [1]. Application of this systems approach to the study of cellular pathogen-host interactions will, in principle, allow for the identification of all genes in a given host cell that either promote or inhibit infection. In this context, RNAi has enormous potential for the discovery of both new therapeutic targets, and a fundamental understanding of pathogen-host interactions [2]. However, the accessibility of genome-wide RNAi technology is limited by capital-expense, reagent costs and portability of high-throughput equipment into high-level biocontainment facilities necessary for studies involving highly pathogenic infections. ***In order to advance the current technology of RNA interference screening targeted specifically to viral pathogen studies, a portable, low cost and miniaturized high-throughput microfluidic RNAi screening platform is being developed.***

To this end, the microfluidic device design is based on cellular microarray technology [3-4], created on microscope slides that use chemical means to introduce small interfering RNA (siRNA) in mammalian cells. Additionally, we have circumvented cross-contamination issues by using PDMS-based devices with microchambers in a close system thus preventing contamination issues observed in open systems [5-6]. We are also concurrently validating siRNA hits from a genome-wide Rift Valley Fever virus screen performed, an emerging pathogen that causes serious morbidity and mortality in both humans and livestock. [7].

We have developed one device with side-chambers capable of culturing HeLa cells in batches of 300-500 cells per well (Figure 1). This design focuses on separation of cells within the device, while allowing for media transfer via diffusion within the microfluidic device. After culture, cells are subjected to reverse transfection with an array of siRNA via spotting located within each side-chamber in the device. Red and green labeled RNA has been transfected into mammalian cells using this spotting technique (Figure 2). Lastly, Rift Valley Fever Virus is introduced to the cells and subsequently analyzed for degree of infection (Figure 3). Current results demonstrate the effectiveness of this device, which has been integrated to a spotted array of scrambled siRNA (control), GFP-knockdown siRNA, and no siRNA present within each of 96 wells. Each well was then incubated with HeLa cells undergoing reverse transfection for 24 hours, followed by infection with RVFV-MP12-GFP, which turns cells green upon infection. Cells transfected with control or no siRNA should infect normally, while cells transfected with GFP-kd will eliminate GFP before production happens within the cell.

In our results, we have shown 75% knockdown using the GFP-kd siRNA (Figure 4), which mirrors results we have observed at the benchtop screen we have performed. Future results will focus on additional fabrication steps to isolate library delivery within the chip, as well as standardize cell culture within the wells for tighter distribution of infected and non-infected cell counts.

Word Count: 477

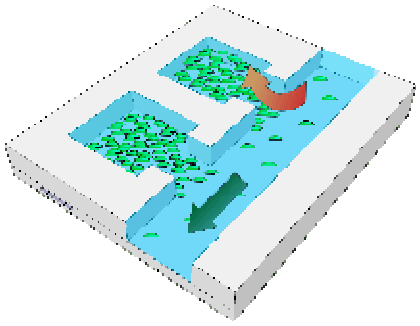

Figure 1: Schematic of the diffusion-based cell culture process. The device is placed under vacuum to remove air from the device to prevent future bubbles. Afterward, a single cell suspension is fed into the device, with cells filling the full volume of the chip. This design can culture cells up to four days.

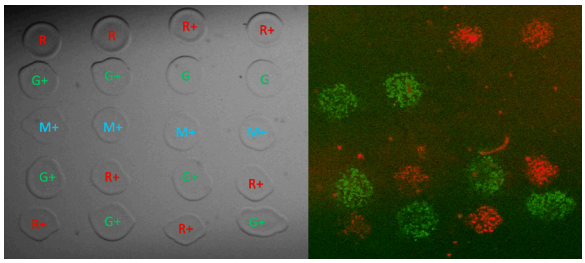

Figure 2. (left) Spots of Lipofectamine mixed with red- or green-labeled siRNA are manually spotted across a glass microscope slide. (right) Only spots with both Lipofectamine (+) and labeled RNA (R/G) demonstrate fluorescence in 293T cells that are cultured over the entire open microscope slide.

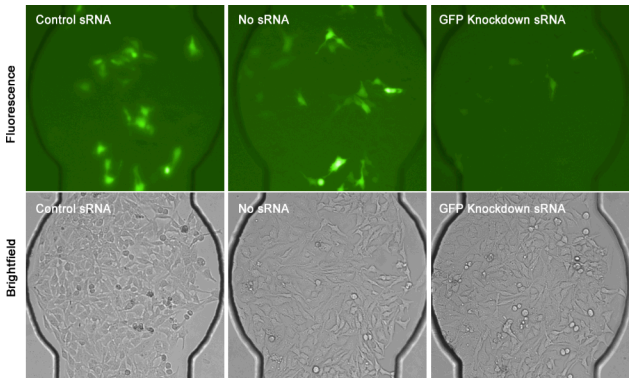

Figure 3. (top) Colorized fluorescence images of HeLa cells cultured in the microfluidic RNAi screening chip. (bottom) Brightfield images of ~400 HeLa cells cultured within wells 600 µm wide. For the control or no siRNA wells, the number of cells that demonstrate infection by RVFV-MP12-GFP are ~23. Cells transfected with GFP-kd siRNA only demonstrate infection of ~5 cells.

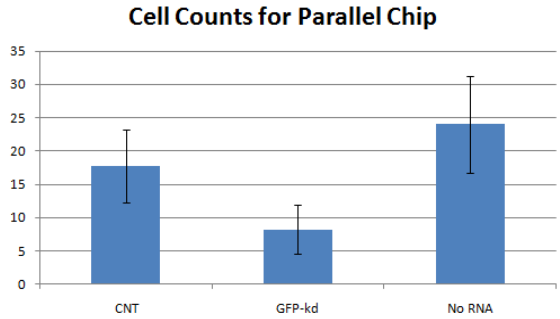

Figure 4. Graph of current results of 96 wells of the RNAi screening chip. Averaged cell counts of infected cells show approximately 75% from untreated cells.

REFERENCES:

1. "Genome-wide RNAi screening in *Caenorhabditis elegans*," R.S. Kamath, J. Ahringer, Methods, **30(4)**, 313-321, (2003).
2. "Genome-Wide RNAi Screen for Host Factors Required for Intracellular Bacterial Infection," H. Agaisse, L.A. Burrack, J.A. Philips, E.J. Rubin, N. Perrimon, D.E. Higgins, Science, **309(5738)**, 1248-1251, (2005).
3. "Microarrays of cells expressing defined cDNAs," J. Ziauddin, D.M. Sabatini, Nature, **411**, 107 (2001).
4. "RNAi living-cell microarrays for loss-of-function screens in *Drosophila melanogaster* cells," D.B. Wheeler, S.N. Bailey, D.A. Guertin, A.E. Carpenter, C.O. Higgins, D.M. Sabatini, Nature Methods, **1(2)**, 1, (2004).
5. "A microfluidic device for monitoring siRNA delivery under fluid flow," A.D. van der Meer, M.M.J. Kamphuis, A.A. Poot, J. Feijen, I. Vermes, Journal of Controlled Release, **132(3)**, e42-e44 (2008).
6. "Automated MEMS-based *Drosophila* embryo injection system for high-throughput RNAi screens," S. Zappe, M. Fish, M.P. Scott, O. Solgaard, Lab on a Chip, **6**, 1012-1019, (2006).
7. "Genome-wide RNA Interference screen for host factors required for rift valley fever virus infection," B. Harmon, O. Negrete, A.K. Singh, Chemical and Biological Defense Science and Technology Conference, Orlando, FL, *Poster*, (2010).