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Microfluidic Platforms for RNA Interference Screening

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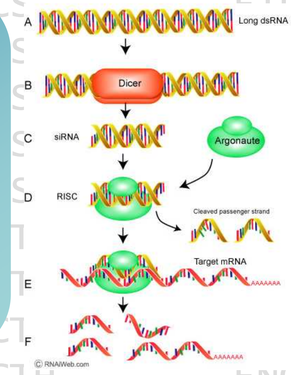
Rift Valley Fever Virus

Challenge: Rift Valley Fever Virus (RVFV) is a potential bioterrorism threat, due to its impact on livestock and animal-to-human transmission and lack of commercially available vaccine. Recent outbreaks have occurred in the sub-Saharan South Africa. RVFV is particularly lethal to livestock, infecting cattle, sheep, camels, and goats. Lethality rates in livestock strongly correlate to age: 10% of older livestock such as sheep, 90% of lambs, and 100% of fetuses die during the course of infection. Due to the nature of this threat it is important to develop a deeper understanding of how human genes participate in infection, which can be done on a genomic level using RNA Interference (RNAi).

Outbreaks of RVF Virus 2010, Center for Biosecurity of UPMC

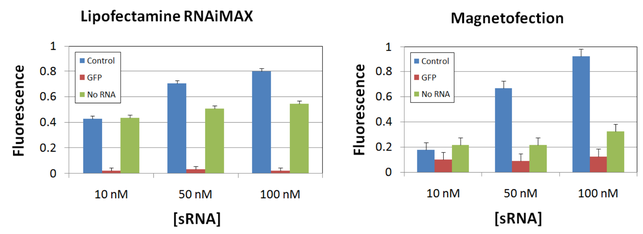
RNA Interference

Method: RNA interference is a biological technique used to knock down gene expression within the cell by using corresponding small RNA. This technique can be used to individually analyze the participation of a gene in an infection process. Given the tens of thousands of human genes, a high throughput process is required to examine the impact of each gene.



384-Plate washer from BioTek

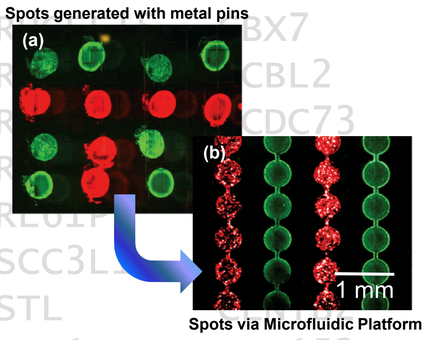
Transfection Techniques



Technique: Reverse transfection is performed on cells in a microtiter plate, using two different methods of transfection. Scrambled sRNA (Control), GFP-knockdown sRNA (GFP) and no RNA are introduced to each well at 10, 50 or 100 nM in concentration. Transfection protocol is followed for each well. 24 hours later, the cells are exposed to RVFV-MP12-GFP, which produces GFP upon infection in the cells. Cells are lysed, and then inserted into a plate reader to be scanned for GFP. The graphs above demonstrate the normalized fluorescence results.

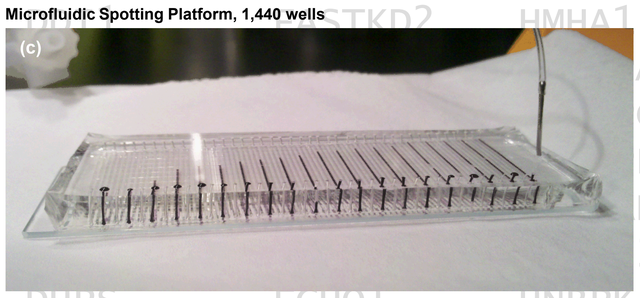
RNAi Library Indexing

Spots generated with metal pins



Spots via Microfluidic Platform

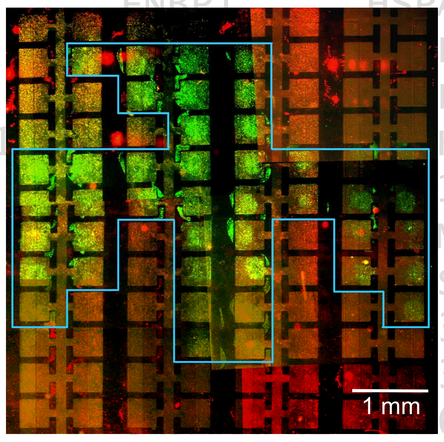
Microfluidic Spotting Platform, 1,440 wells



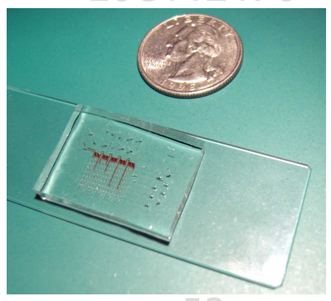
Procedure: Gelatin-stabilized siRNA is spotted with high positional accuracy using a microfluidic spotting platform. Spots 500µm in diameter are dotted using a microarray, creating a library that is stable for up to a year. Above, red and green labeled siRNA is spotted with Lipofectamine RNAiMAX in gelatin solution. (a) Spots produced using a manual pin system, similar to robotic handling capabilities. (b) Spots produced using a microfluidic spotting platform. (c) The 1440-well microfluidic spotting platform from a side view. Since actuation and filling is performed via vacuum lines in a multilayer device, little precious reagent is wasted at the inlets.

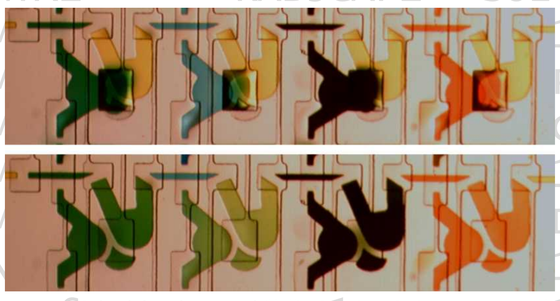
On-Chip Transfection

RNAi Transfection: Here, the Sandia thunderbird design is shown with 293T cells uptaking red and green labeled RNA patterned across 96 wells 1 nL in volume. Under media flow, these wells demonstrate a discrete isolation of the spots within the device, which show a lack of cross-contamination despite open wells. This technique is applied to library spotting in the full screen.



Screening at the Microscale

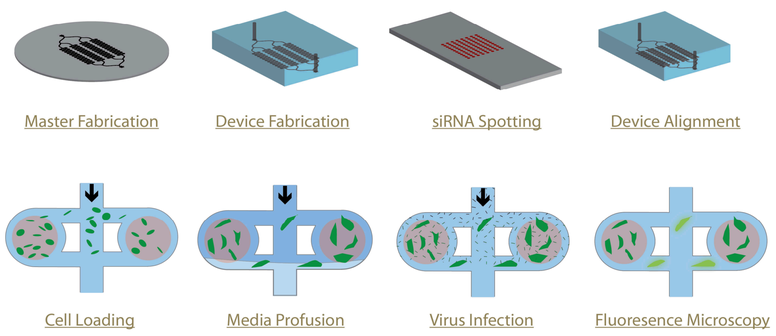




Schudel, Lab on a Chip, **2009,** 9, *1676*.

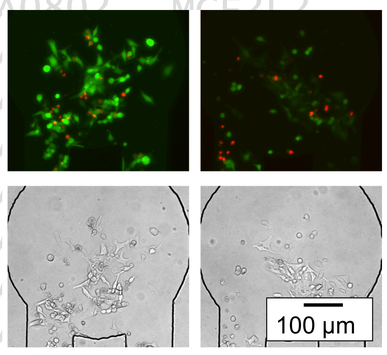
Advantages: One way to achieve higher experiment densities than current high throughput systems involves using microfluidic devices, to achieve volumes below that of well plates (nanoliters vs. microliters). Microfluidic systems, with nanoliter or sub-nanoliter volumes, use less time and reagent with results equal to that of larger scale screens. As an example, the above pictures show a combinatorial chemistry chip with reactor volumes of 200 pL.

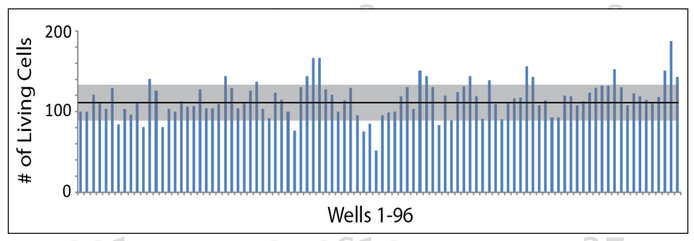
Fabrication Method



Fabrication: The RNAi microfluidic screening platform is assembled using a two-part process. First, two platforms are fabricated using soft lithography techniques. A microfluidic spotting platform is developed, which is used to spot siRNA across a prepared microscope slide. Second, a microfluidic siRNA screening platform is aligned to the spots, providing inlets and outlets for cell loading, culture and transfection. After transfection (1-3 days of incubation), virus is administered to the microfluidic screening platform and fluorescence is measured.

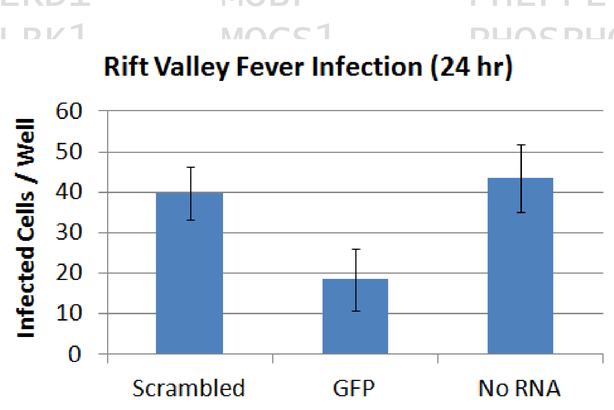
On-Chip Cell Viability

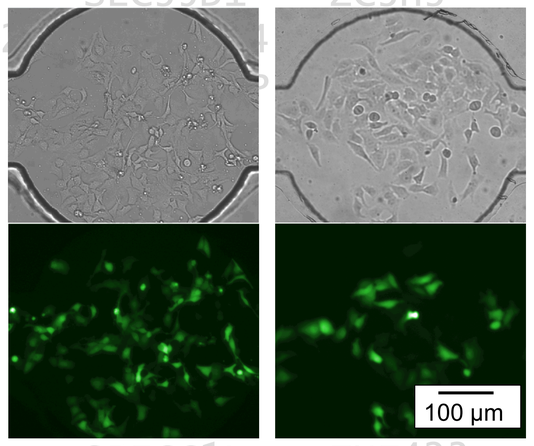




Design: The RNAi microfluidic screening chip is designed with an array of 96 isolated wells that can be treated with a library of RNA compounds spotted directly onto a glass slide. At left, fluorescent images are taken of HeLa cells that have cultured for 48 hours and stained for LIVE/DEAD analysis. All wells are located in series, such that every well receives the same cell loading, incubation time, media replenishment and virus incubation. The graph here demonstrates the reliability of the device, with 96 wells of cell culture incubating 126 +/- 19 live cells per well.

On-Chip Infection and Imaging





Current Results: The microfluidic screen utilizes the modified virus strain used in the benchtop screen (RVFV-MP12-GFP). Upon infection, the virus will produce GFP, which correlates with infection rate. Above, wells are cultured with HeLa cells for 24 hours while being reverse transfected with a GFP siRNA (positive control), a scrambled siRNA (negative control) and no siRNA. After 24 hours of transfection, virus is introduced. The GFP siRNA knocks down the expression of GFP within the cells, causing fewer green cells (approximately 75% knockdown). Error bars are standard deviation.

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