Microfluidic Chips for RNA Interference Screening

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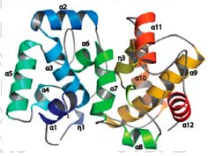
Rift Valley Fever Virus

Challenge: Rift Valley Fever Virus (RVFV) has been identified as a potential bioterrorism threat, due to its impact on livestock and animal-to-human transmission. No vaccine is available commercially at this time. Due to the nature of this threat, it is important to develop a deeper understanding of how human genes participate in infection.



Structure of RVFV (Smith, *PNAS*, **2010**)

Outbreaks of RVF Virus, CBC-ED (www.bepast.org)

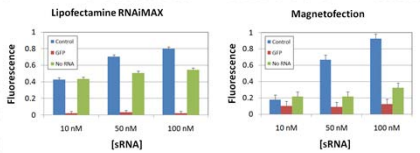
Occurrences: Recent outbreaks have occurred in the sub-Saharan South Africa. RVFV is particularly lethal to livestock, infecting cattle, sheep, camels, and goats. Lethality rates in livestock strongly correlate to age: 10% of older livestock such as sheep, 90% of lambs, and 100% of fetuses die during the course of infection.

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.

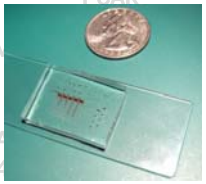
384-Plate washer from BioTek

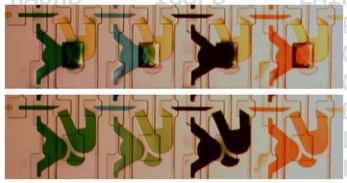
RNA Interference



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.

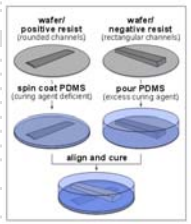
Screening at the Microscale

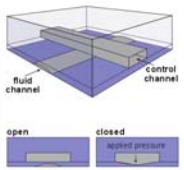




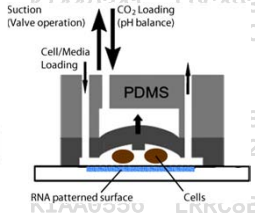
Advantages: One way to achieve higher experiment densities than current high throughput systems involves using microfluidic devices, to achieve volumes below that of well plates (nanoliters vs. microliters). Microfluidic systems, with nanoliter or sub-nanoliter volumes, can perform similar screening experiments, but 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.

RNAi Screening



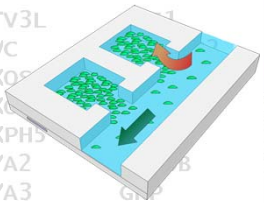


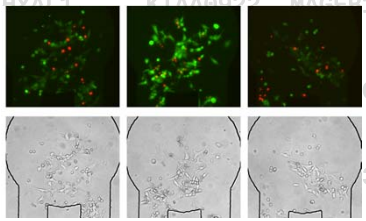
Schudel, Lab on a Chip, **2009,** 9, *1676.*



Methods: The creation and control of nanoliter and sub-nanoliter fluidic reagent-containing plugs for the chip above was performed using multilayer soft lithography (MSL) in PDMS. An elastic polymer, PDMS is used to develop a device with a control layer and a thin, easily deformed fluid layer. By permanently sealing these water-tight layers together, a device is crafted that can selectively bend portions of a chip, forming fluidic pockets of fluid that can be mixed, separated and stored for subsequent analysis.

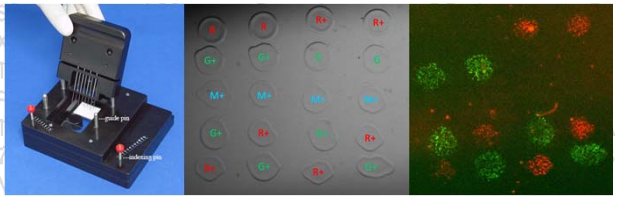
Microfluidic Cell Culture





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, a schematic of the device demonstrates separation of patterned cells in a microfluidic device, with media exchanged operated mainly by diffusion. At top right, three wells are loaded with HeLa cells and cultured for 48 hours. A live/dead stain performed at 48 hours demonstrates a significant number of cells remain viable at the 2-day point.

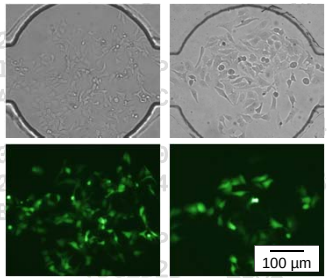
RNAi Library Delivery



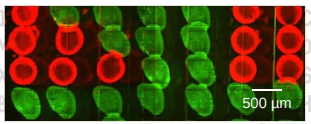
Procedure: RNA is spotted directly onto a glass slide pretreated with poly-L-lysine. Spots 500µm are dotted using a microarray, creating a library that is stable for up to a year. Above, red and green labeled RNA are spotted with and without Lipofectamine 2000 (+). 293T cells are seeded across the glass slide within a cell culture dish. Only the spots with Lipofectamine and labeled RNA fluoresce after 24 hours of incubation, demonstrating transfection of RNA into cells culture directly onto a glass slide. Some cross contamination can be seen in the spots, which can be remedied with the use of microfluidic devices.

Cell Infection On Chip

Capability: The microfluidic screen utilizes the modified virus strain used in the benchtop screen (RVFV-MP12-GFP). Upon infection, the virus will produce GFP, which can be quantified as a function of infection rate. At right, two wells are cultured with HeLa cells for 24 hours. After exposure to the virus, a fraction of the cells will turn green within the device. Variations from well to well will need to be accounted for in the final chip using intra-well controls.

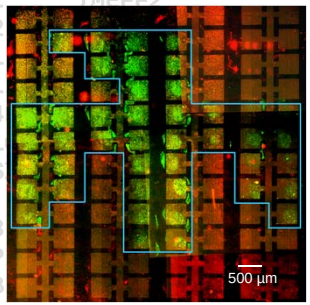


Results



Library Patterning: Red and green-labeled RNA has been printed and aligned to a 96-well device with spots 450 µm wide.

RNA 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. In subsequent tests, a full library of gene-specific RNA will be printed followed by infection with GFP producing RVFV virus.



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