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White Paper

Vena8 Fluoro+™ Biochip

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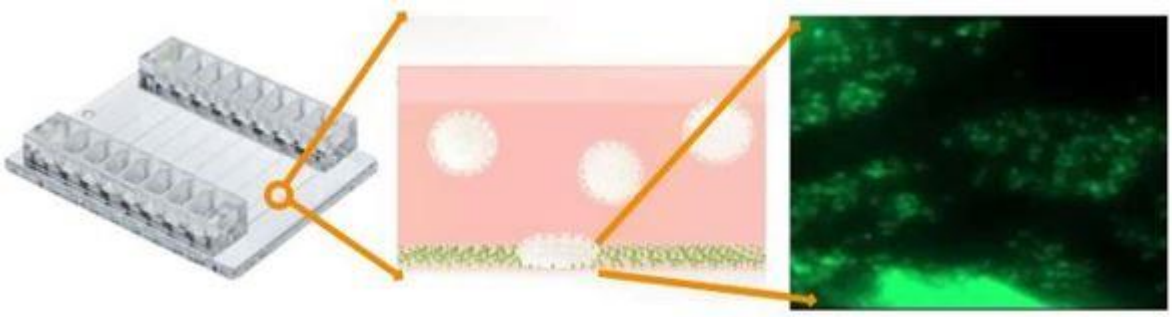
Validation of Vena8 Fluoro+ Biochip

Abstract
To infuse THP-1 monocytic leukemia cells on rhVCAM-1 coated channels of six Vena8 Fluoro+ biochips at a shear stress of 0.5 dynes/cm^2. Specifically, to validate the biochip by estimating channel to channel and biochip to biochip variation.

Validation of Vena8 Fluoro+ Biochip by Monocyte (THP-1) rhVCAM-1 Adhesion Assay using Cellix VenaFlux Platform

Introduction
Cellix Ltd. has developed a novel microfluidic platform consisting of a PC-controlled nanopump with microfluidic biochips and Image Pro Premier analysis software. The nanopump enables very accurate flow rates to be achieved which are more reproducible and consistent compared to anything currently available. Importantly, flow rates are extremely low (5 pL/min to 10 µL/min) and the shear stress levels that the pump can mimic (up to 450 dynes/cm^2) are equivalent to those found in blood vessels in vivo.
Vena8 Fluoro+ biochips contain 8 parallel enclosed microcapillaries for continuous flow cell-based assays. Each microcapillary may be coated with a different adhesion molecule. Cell suspensions may then be injected using the Mirus Evo nanopump which supports a range of shear stresses for dynamic flow-based assays. Vena8 Fluoro+ biochips are particularly suited for applications requiring fluorescent immunostaining or confocal microscopy observation combined with flow-based experiments.

Objective
To infuse THP-1 monocytic leukaemia cells on rhVCAM-1 coated channels of six Vena8 Fluoro+ biochips at a shear stress of 0.5 dynes/cm^2. Specifically, to validate the biochip by estimating channel to channel and biochip to biochip variation.



Vena8 Fluoro+ biochip

Illustration: cell adhesion inside Vena8 Fluoro+ biochip

Adhesion of platelets to collagen-coated channel at shear stress of 60 dyne/cm2

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Cellix VenaFlux platform

Technical specifications	
Material	Topas
Number of channels per biochip	8
Volume of each channel	1.12 µL
Dimensions of each channel	400 µm (W) x 100 µm (D) x 28 mm (L)
Dead volume at input port	0.1 µL
Thickness of bottom substrate	0.17 mm

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Materials	
Adhesion protein	rhVCAM-1 (cat. no. ADP5, R&D systems)
Cell line information	
Primary cells	HUVEC (human umbilical vein endothelial cells)
Growth properties	Suspension
Organ	Peripheral blood
Cell line	THP-1
Growth properties	Suspension
Organism	Homo sapiens (human) organ
Organ	Peripheral blood
Disease	Acute monocytic leukaemia
Cell maintenance	RPMI 1640 (Gibco 31870
	FBS-10%
	2 mM L-glutamine, 100 µg/ml Penicillin/Streptomycin (Sigma G6784)

Methods

- Coating Vena8 Fluoro+ biochips
  All the channels were coated with 20 µg/ml of rhVCAM-1 overnight in a humidified chamber at 4°C. The channels were blocked by 0.1% BSA after incubation.
- Preparation of cells
  THP-1 cells were maintained in the recommended growth media. Cell suspension of 5 x 10^6/mL cells were prepared for the experiment.
- Adhesion assay
  VenaFlux assay software was used to perform experiments. THP-1 cells were infused into the channels at a shear stress of 0.5 dynes/cm^2 using Mirus Evo nanopump. The cells were perfused for 5 mins per channel. Images were acquired at positions 3, 4 and 5 after 4 mins of perfusion of every channel. Ten images per position were captured using high definition QImaging camera with the aid of the Marzhauser IM series motorized stage. Six Vena8 Fluoro+ biochips were used for the assay. All the experiments were performed at 37°C using Oko-lab's microscope cage incubator.
- Image analysis
  The adhered cells were counted using Image Pro Premier application software and exported to Excel for calculations and interpretations.

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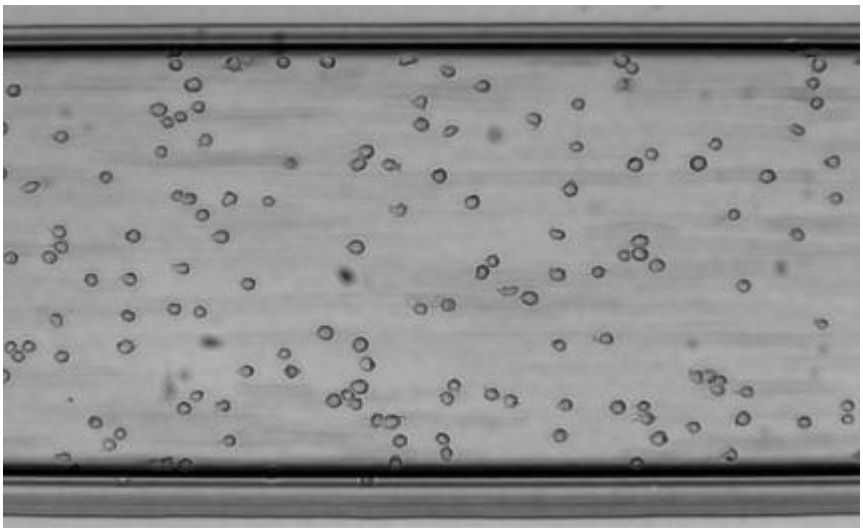
Results

An average of 119 cells (THP-1) adhered per frame of the channels. Channel to channel variation was less than 8% and chip to chip variation was 6%.



THP-1 cell adhesion to rhVCAM-1 coated channel at a shear stress of 0.5 dynes/cm² in a Vena8 Fluoro+ biochip

Channel to channel analysis					Chip to chip analysis			
No	Av. no. of cells (THP1)/8 channels	STDEV	%CV	SEM	Av. no. of cells	STDEV	%CV	SEM
1	121	5.69	4.4	1.2	119	6.92	6	0.7
2	118	9.09	7.7	2.2				
3	131	5.93	4.5	1.3				
4	120	7.38	6.2	1.6				
5	110	7.42	6.7	1.5				
6	114	5.88	5.2	1.4				

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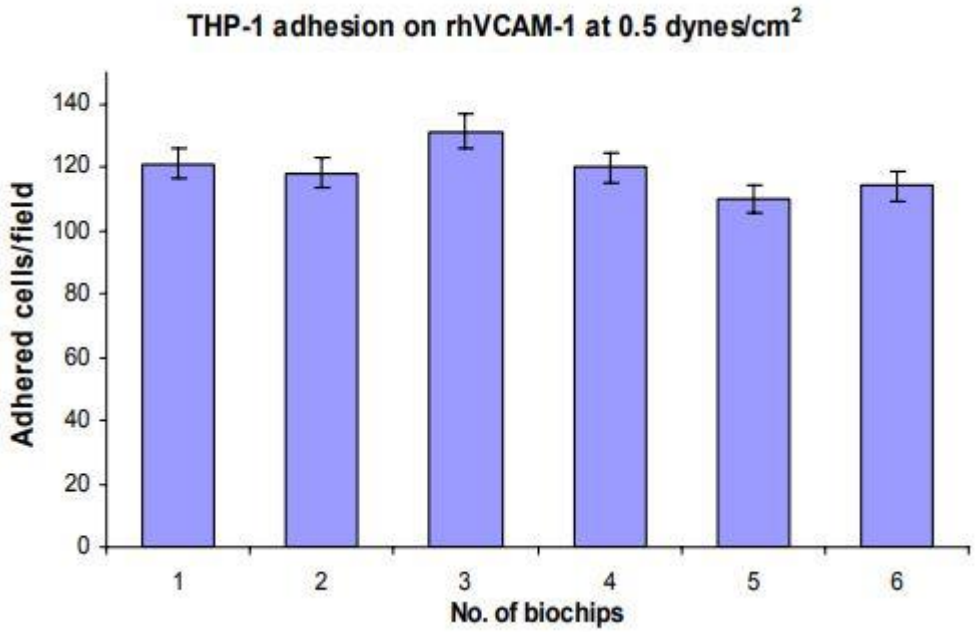

THP-1 cell adhesion to rhVCAM-1 coated channel at a constant shear stress of 0.5 dynes/cm² in a Vena8 Fluoro+ biochip

Acknowledgement

This work was supported by a grant from the European Commission:

"Target-Melanoma" is an EU FP7 Fellowship Industry Academia Partnership grant focusing on the molecular dissection of melanoma progression.

Contract number: 230614