Cellix Ltd
Unit 1, Longmile Business Park,
Longmile Rd., Dublin 12, Ireland
Tel: +353-1-4500-155
Fax: +353-1-4500-158
Email: info@cellixltd.com
Web: www.cellixltd.com

White Paper

Validation of Vena8 Fluoro+™ Biochip

Validation of Vena8 Fluoro+™ biochip by Monocyte (THP-1) rhVCAM-1 adhesion assay using Cellix VenaFlux™ platform

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.

14th Oct 2010

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 DucoCell™ 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*.

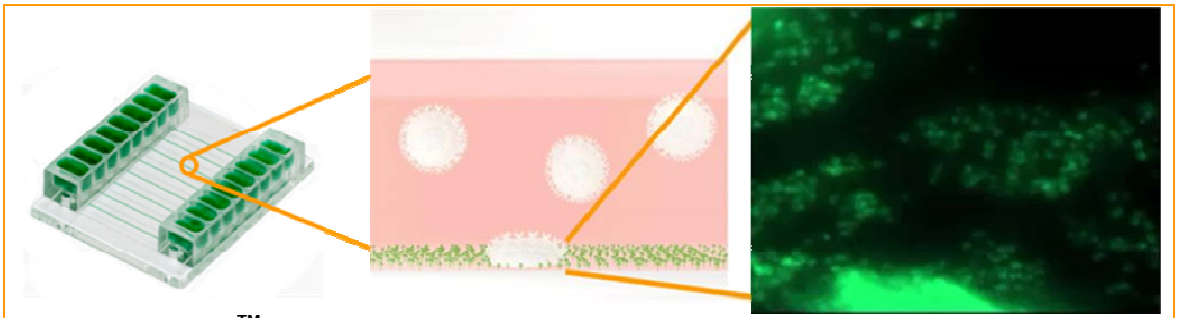
Vena8 Fluoro+™ Biochips contain 8 parallel enclosed microcapillaries for continuous flow cell based assays. Each microcapillary may be coated with a different adhesion molecule. Cell suspensions may then be injected using the Mirus™ Nanopump which supports a range of shear stresses for dynamic flow based assays. **Vena8 Fluoro+™** Biochips are particularly suited for applications requiring fluorescent immunostaning or confocal microscopy observation combined with flow based experiments.

OBJECTIVE

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.

Fig.1 Cellix VenaFlux™ Platform

Vena8 Fluoro+^{TM} Biochip



Vena8 Fluoro+^{TM} Biochip

Illustration: cell adhesion inside Vena8 Fluoro+^{TM} Biochip

Adhesion of platelets to collagen-coated channel at shear stress of 60 dyne/cm^2

Fig. 2 Vena8 Fluoro+^{TM} Biochip

Technical Specifications	
Number of channels per biochip	8
Volume of each channel	1.12 µL
Depth of channel	100 µm / 0.1mm
Width of channel	400 µm / 0.4mm
Length of channel	28mm
Dead volume at input port	0.1 µL
Vena8 biochip dimensions	400 µm (W) x 100 µm (D) x 28 mm (L)
Thickness of bottom substrate	0.17 mm

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	• Peripheral blood
Disease	• Acute monocytic leukemia
Cell maintenance	• RPMI 1640 (Gibco 31870)
	• FBS-10%
	• 2mM L-glutamine, 100µg/ml Penicillin/Streptomycin (Sigma G6784)

METHODS

i) 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.

ii) 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.

iii) 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 **Mirus1.0™ 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. 10 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.**

iv) Image analysis

The adhered cells were counted using the **Ducocell™** application software and exported to Excel for calculations and interpretations.

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%**.

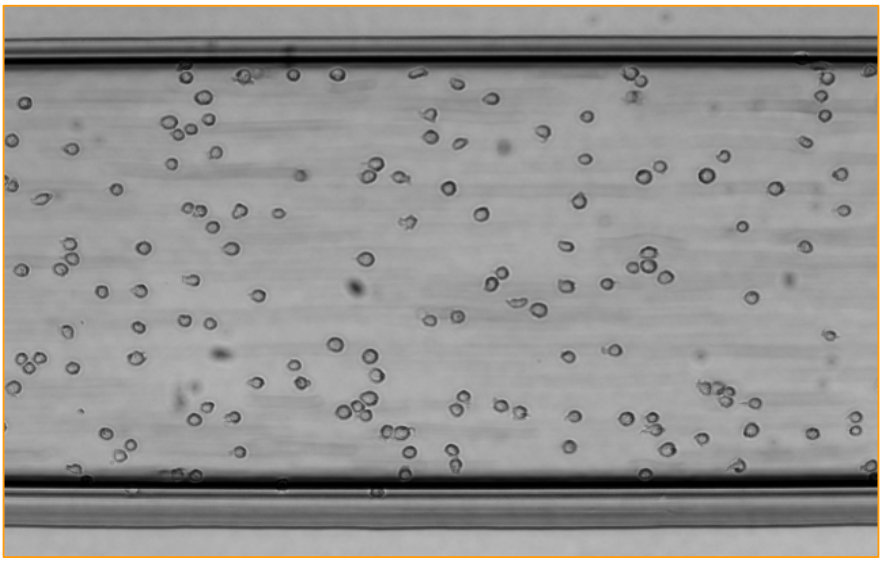


Fig.3 THP-1 cell adhesion to rhVCAM-1 coated channel at a shear stress of 0.5 dynes/cm^2 in a **Vena8 Fluoro+™** biochip.

RESULTS

Channel to Channel analysis					Chip to Chip analysis			
Chip No	Av. No. of Cells (THP1) / 8 channels	STDEV	%CV	SEM	Av. No. of Cells	STDEV	%CV	SEM
1	121	5.69	4.7	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				

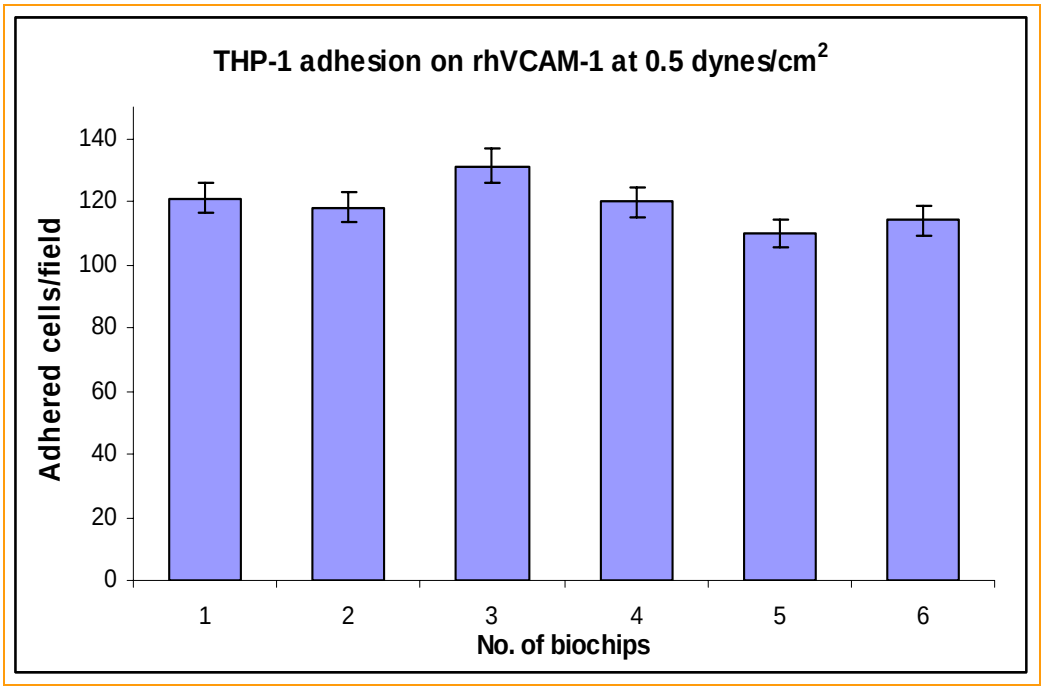


Fig.4 THP-1 cell adhesion to rhVCAM-1 coated channel at a constant shear stress of 0.5 dynes/cm^2 in a **Vena8 Fluoro+™** biochip

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

Cellix Ltd.,
Unit 1, Longmile Business Park,
Longmile Road,
Dublin 12,
Ireland

For more information please contact us:

Web: www.cellixltd.com
Email: info@cellixltd.com
Phone: Ireland: +353-1-4500-155
USA: +1-212-231-8274