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White Paper

Validation of Vena8 Endothelial+™ Biochip

Primary HUVEC - Monocyte (THP-1) adhesion assay using Cellix VenaFlux™ platform

Abstract

Culture Primary HUVEC in **Vena8 Endothelial+™** biochips and infuse THP-1 monocytic leukemia cells over primary HUVEC cultured in the channels of five **Vena8 Endothelial+™** biochips at a shear stress of 0.5 dynes/cm^2.

1st Nov 2010

Validation of Vena8 Endothelial+™ biochip by Primary HUVEC - Monocyte (THP-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^{-1} to 10 µL min^{-1}) 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 Endothelial+™ Biochip contains 8 parallel enclosed microcapillaries for culturing primary endothelial cells and continuous flow cell based assays. Primary cell monolayer is obtained in less than 3 hrs in the channels which permits the user to perform quick experiments in a single day. Wide range of primary cells can be cultured in **Vena8 Endothelial+ ™** biochip. For example HUVEC, HAEC, HMVEC etc. Primary endothelial cells are cultured and cell suspensions may then be injected using the **Mirus™ Nanopump** which supports a range of shear stresses / shear flow rates for dynamic flow based assays.

OBJECTIVE

To culture Primary HUVEC in **Vena8 Endothelial+™** biochips and infuse THP-1 monocytic leukemia cells on primary HUVEC cultured in the channels of five **Vena8 Endothelial+™** 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 by estimating number of THP-1 cells adhered to stimulated Primary HUVEC.

Fig.1 Cellix VenaFlux™ Platform

Vena8 Endothelial+^{TM} Biochip

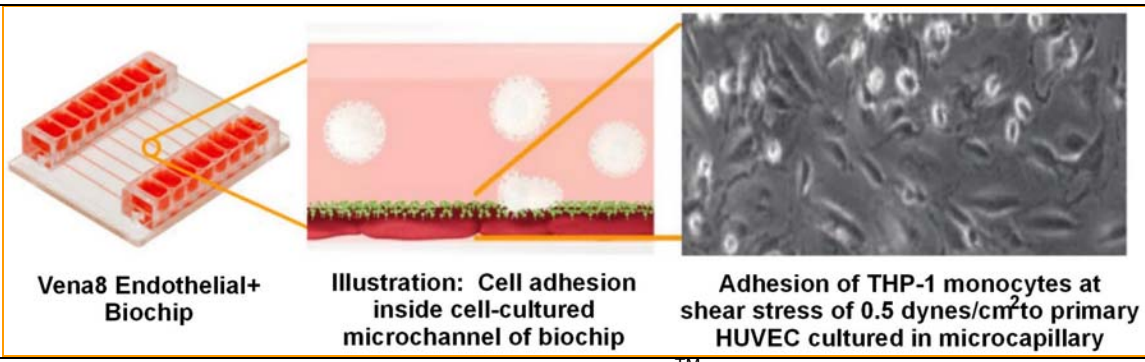

Fig. 2 Vena8 Endothelial+^{TM} Biochip

Technical Specifications	
Number of channels per biochip	8
Volume of each channel	2.69 µL
Depth of channel	120 µm / 0.12mm
Width of channel	800 µm / 0.8mm
Length of channel	28mm
Dead volume at input port	0.1 µL
Vena8 biochip dimensions	800 µm (W) x 120 µm (D) x 28 mm (L)
Thickness of bottom substrate	0.5mm

Materials	
Adhesion protein	• Laminin (Sigma L6274)
Stimulation protein	• rhTNF-α (Cat.No. 210-TA, R&D Systems)
Cell line information:	
Primary Cells	• HUVEC (Human Umbilical Vein Endothelial Cells)
Growth Properties	• Adherent
Organ	• Human Umbilical Vein
Cell maintenance	• Endothelial Medium Kit (Promocell C-22110)
	• Detach Kit -30 (Promocell C-41200)
	• Accutase Solution (Promocell C-41310)
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)

Method

i) Stimulation of Primary HUVEC

Primary HUVEC (90% confluent) were stimulated with 10ng/mL rhTNF-α overnight (16-18 hrs) in T75 cm^2 flask.

ii) Coating Vena8 Endothelial+™ biochips

Vena8 Endothelial+™ biochip was kept under UV for 20-30mins and all the channels were coated with 12µl of 100µg/mL Laminin (Sigma, Cat.no L-6274). The biochip was then kept in an opened humidified chamber in CO_2 incubator for 1-1.5 hrs.

iii) Preparation of primary cells

Primary HUVEC were maintained in the recommended growth medium. Cell suspension of 1.5 x 10^6 per 100µL was prepared for seeding in the channels.

iii) Cell Seeding in Vena8 Endothelial+™ biochip

After the incubation period, 5 µl of 1.5 x 10^6 per 100µL of Primary endothelial cells were gently added into each channel of **Vena8 Endothelial+™** biochip. Biochip was kept in the CO_2 incubator for 15 – 20 minutes. The biochip was observed under the microscope and all the reservoirs were topped up with 40µL of media. The biochip was kept for a further 1.5 to 2 hrs in the CO_2 incubator to obtain a Primary cell monolayer inside the channels of the **Vena8 Endothelial+™** biochip.

iv) Preparation of Cell line

THP1 cells were maintained in the recommended growth media. Cell suspension of 5x10^6/mL cells was prepared for the experiment.

v) Adhesion Assay

VenaFlux™ assay software was used to perform experiments. After the incubation period, THP-1 cells were infused over the HUVEC monolayer inside the channels at a shear stress of 0.5 dynes/cm^2 using the **Mirus Nanopump 2.0**. The THP-1 cells were perfused for 3 mins per channel. Images were acquired at positions 3, 4 and 5 after 2mins 40secs of perfusion in every channel. 10 images per position were captured using a high definition **QImaging** camera with the help of the **Marzhauser IM** series motorized stage. Five **Vena8 Endothelial+™** biochips were used for the assay. All the experiments were performed at 37^0C using **Oko-lab's Microscope Cage incubator.**

vi) Image Analysis

The adhered THP-1 cells on Primary HUVEC were counted using the **Ducocell** application software and **Image Pro Analyser 7.0**. The data was exported to Excel for calculations and interpretations

RESULTS

An average of **100** cells (THP-1) adhered on Primary HUVEC monolayer per frame. The channel to channel variation was less than **10%** and chip to chip variation was **4%**.

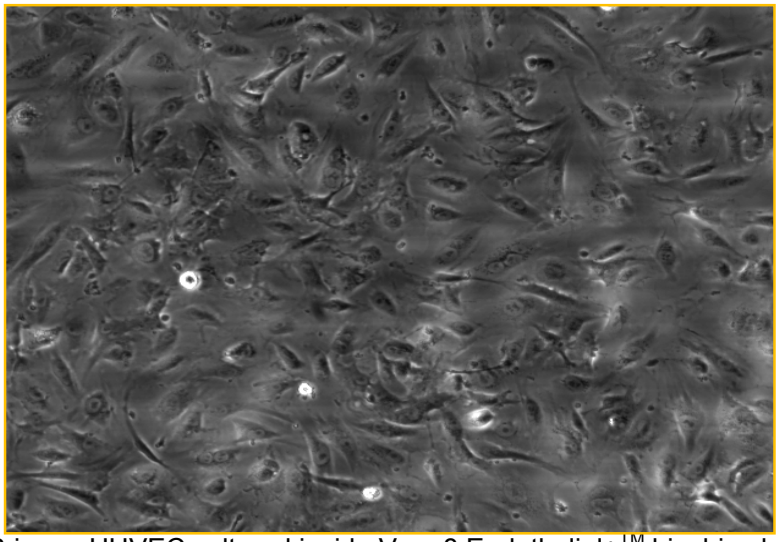

Fig.3 Primary HUVEC cultured inside Vena8 Endothelial+™ biochip channels.

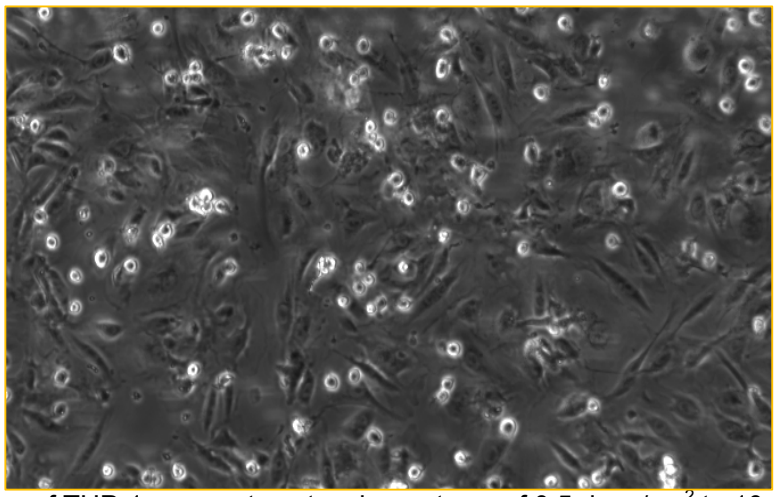

Fig.4 Adhesion of THP-1 monocytes at a shear stress of 0.5 dyne/cm^2 to 10ng/ml rhTNF-α stimulated Primary HUVEC cultured in channel.

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	104	7.76	7.4	1.6	100	4.41	4	0.4
2	95	7.98	8.4	1.6				
3	106	7.59	7.2	1.5				
4	98	8.81	9.0	1.9				
5	98	5.57	5.7	1.2				

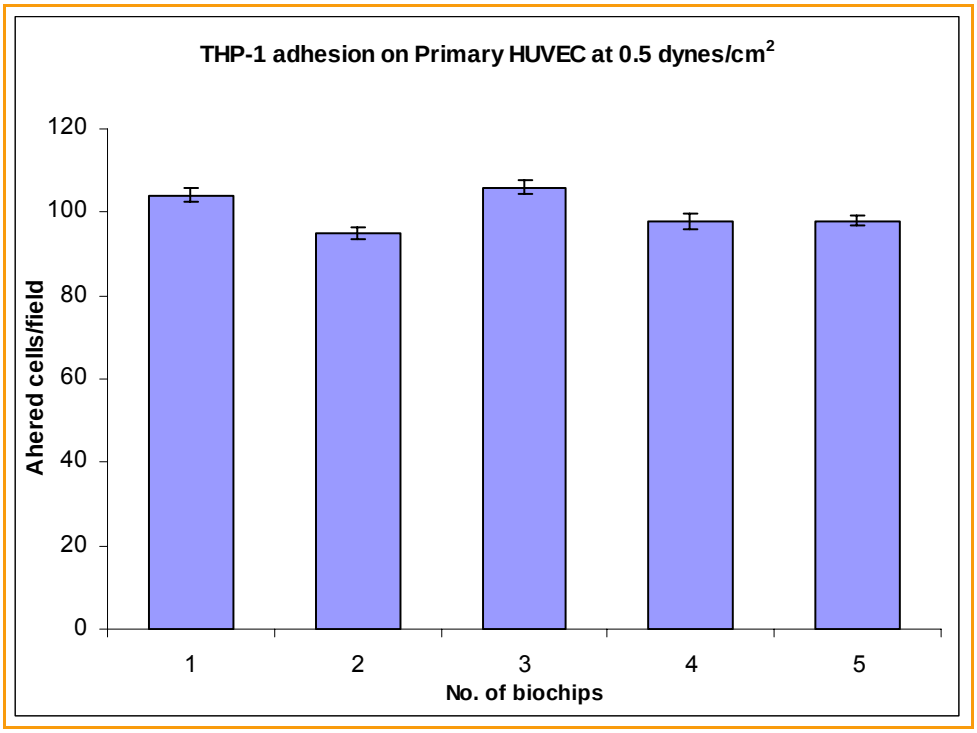

Fig.5 THP-1 cell adhesion to 10ng/mL rhTNF-α stimulated primary HUVEC at a constant shear stress of 0.5 dynes/cm^2 on **Vena8 Endothelial+^{TM}** biochip.

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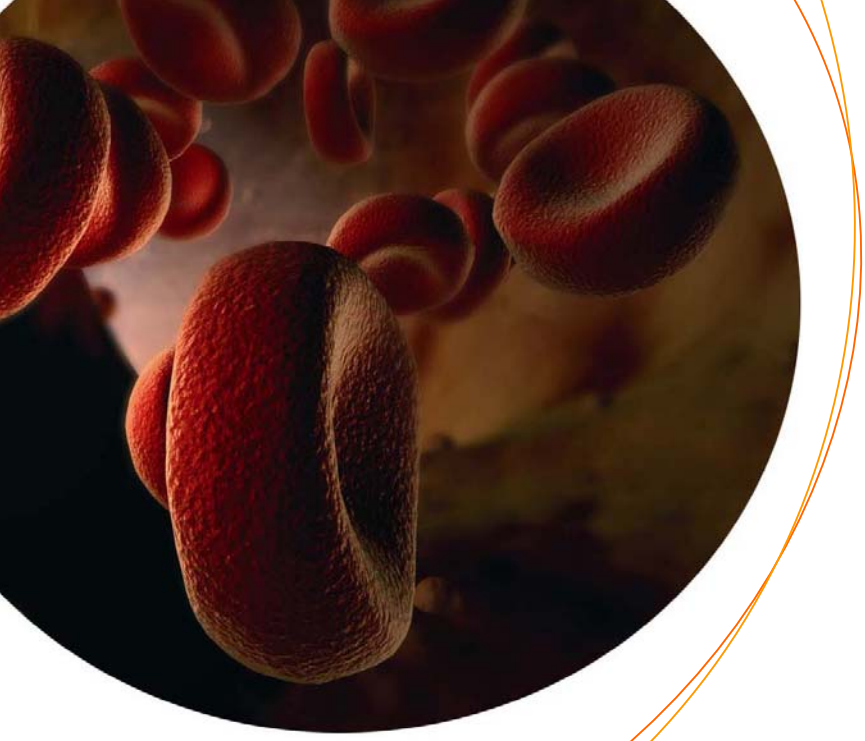

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