we are cellix

White Paper

Vena8 Endothelial+™ Biochip

Cellix Ltd.
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

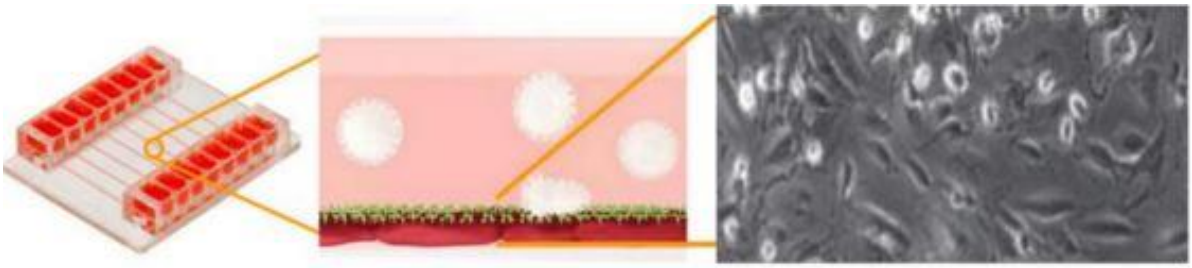
Validation of Vena8 Endothelial+ Biochip

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

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 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^{-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 Evo 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 leukaemia 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.



Vena8 Endothelial+ biochip

Illustration: cell adhesion inside cell-cultured microchannel of biochip

Adhesion of THP-1 monocytes at shear stress of 0.5 dyne/cm^2 to primary HUVEC cultured in microcapillary

Cellix Ltd.
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

Cellix VenaFlux platform

Technical specifications	
Material	Topas
Number of channels per biochip	8
Volume of each channel	2.69 µL
Dimensions of each channel	800 µm (W) x 120 µm (D) x 28 mm (L)
Dead volume at input port	0.1 µL
Thickness of bottom substrate	0.17 mm

Cellix Ltd.
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

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 leukaemia
Cell maintenance	RPMI 1640 (Gibco 31870
	FBS-10%
	2 mM L-glutamine, 100 µg/ml Penicillin/Streptomycin (Sigma G6784)

Methods

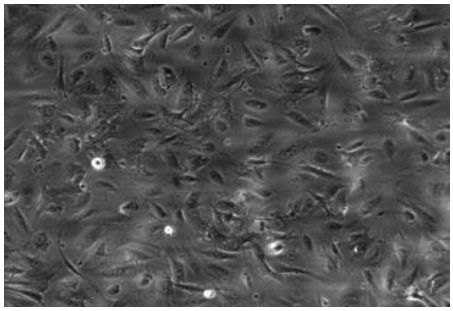
- Stimulation of primary HUVEC
  Primary HUVEC (90% confluent) were stimulated with 10 ng/mL rhTNF-α overnight (16–18 hrs) in T75 cm^2 flask.
- Coating Vena8 Endothelial+ biochips
  Vena8 Endothelial+ biochip was kept under UV for 20–30 mins and all the channels were coated with 12 µl of 100 µg/mL Laminin (Sigma, cat. no L-6274). The biochip was then kept in an opened humidified chamber in CO_2 incubator for 1–1.5 hrs.
- Preparation of primary cells
  Primary HUVEC were maintained in the recommended growth medium. Cell suspension of 1.5 x 10^6 per 100 µL was prepared for seeding in the channels.
- Cell seeding in Vena8 Endothelial+ biochip
  After the incubation period, 5 µl of 1.5 x 10^6 per 100 µL 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 40 µL of media. The biochip was kept for a further 1.5–2 hrs in the CO_2 incubator to obtain a primary cell monolayer inside the channels of the Vena8 Endothelial+ biochip.

Cellix Ltd.
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

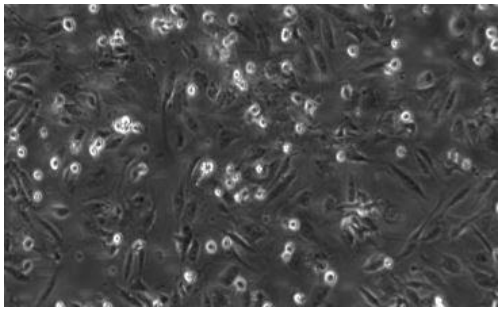
- Preparation of cell line
  THP1 cells were maintained in the recommended growth media. Cell suspension of 5 x 10^6/mL cells was prepared for the experiment.
- Adhesion assay
  VenaFlux assay software was used to perform experiments. After the incubation period, THP-1 cells were infused over the HUVEC monolayer inside the channels at a shear stress of 0.5 dynes/cm^2 using the Mirus Evo nanopump. The THP-1 cells were perfused for 3 mins per channel. Images were acquired at positions 3, 4 and 5 after 2 mins 40 secs of perfusion in every channel. Ten 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°C using Oko-lab's microscope cage incubator.
- Image analysis
  The adhered THP-1 cells on primary HUVEC were counted using the Image Pro Premier application software and 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%.



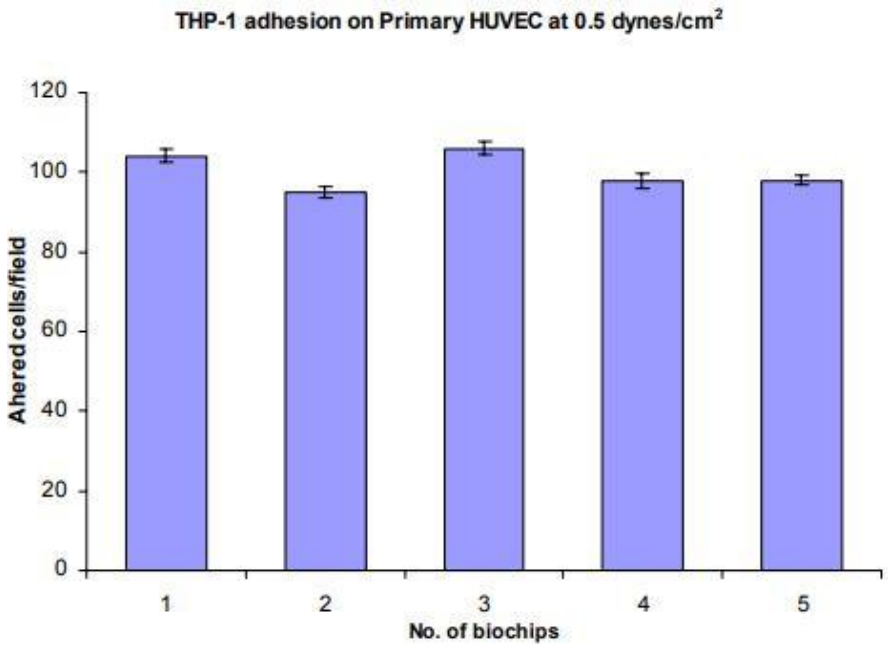
Primary HUVEC cultured inside Vena8 Endothelial+ biochip channels



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

Cellix Ltd.
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

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	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				



THP-1 cell adhesion to 10 ng/mL rhTNF-α stimulated primary HUVEC at a constant shear stress of 0.5 dynes/cm² on Vena8 Endothelial+ biochip

Cellix Ltd.
Unit 1, Longmile Business Park
Longmile Road, D12EK79
Dublin, Ireland

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