



## Application Note O100

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Oncology: Melanoma; Metastasis

## Objectives

Melanoma is the most aggressive form of skin cancer, which invades deeper layers of the skin and has a propensity to metastasise early. In an effort to provide insight into the mechanism by which melanoma cells metastasise, we examined differential cell adhesion within an isogenic model of melanoma progression under physiological shear flow conditions using the Cellix VenaFlux platform.

## Introduction

The poor prognosis of cancer is associated with the ability of tumour cells to metastasize. During the process of metastasis, tumour cells circulating in the blood or lymph vessels can adhere to, and potentially transmigrate through, the endothelium and invade the connective tissue. Most cancer-related deaths are caused by metastasis formation; a process that starts with the dissociation of tumour cells from the primary tumour and is followed by tissue invasion, entrance into blood or lymph vessels (intravasation), and transport to remote sites. It is widely assumed that tumour cells can then escape from the microvasculature (extravasation), invade the target tissue and form a secondary tumour in distant organs. A potentially rate-limiting step in metastasis formation, therefore, would be the extravasation process that involves adhesion of tumour cells to endothelial cells, and the transmigration through the endothelial cell monolayer and basement membrane.

Previously, non-physiological conditions using transwell plates were the preferred option for in vitro studies of metastasis. In recent studies of extravasation, researchers are recognizing the importance of shear stress to mimic physiological conditions. The Cellix VenaFlux platform generates a physiological relevant environment, enabling the researcher to model survival in circulation and adhesion to endothelial cell-derived proteins. In this study, we determine if the steps in extravasation differed in an isogenic melanoma cell line model of progression.

## Methods

### 1. Cell Harvesting

The melanoma cell lines (1205-Lu, WM793, WM793-P1 and WM793-P2) and the green fluorescent protein-tagged melanoma cell line, GFP-1205Lu, was maintained in Dulbecco's Modified Eagle's Medium (DMEM) with GlutaMAX (Invitrogen), supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin and 4 µg/ml insulin (Sigma–Aldrich). The melanoma cell lines were kindly supplied by Prof. William Gallagher, University College Dublin. The Human Umbilical Vein Endothelial Cell line (HUVEC) was maintained in DMEM with 1 g/L glucose (Invitrogen), supplemented with 10% fetal bovine serum, 5 ml/L gentamicin and 10 ml/L amphotericin B solution.

## 2. Vena8 Fluoro+ Biochip Coating

Vena8 Fluoro+ biochip (400  $\mu\text{m}$  wide, 100  $\mu\text{m}$  deep) was coated overnight in humid conditions at 4°C with rhICAM-1 (10  $\mu\text{g}/\text{ml}$ ), rhVCAM-1 (10  $\mu\text{g}/\text{ml}$ ) or fibronectin (20  $\mu\text{g}/\text{ml}$ ) before being coated with BSA (10  $\mu\text{g}/\text{ml}$ ) to block non-specific binding. Two additional channels were coated with BSA for two hours at room temperature. Prior to shear experiments, all channels were washed thrice with medium.

## 3. VenaEC Biochip Culturing Procedure

The VenaEC biochips were placed in a 35 mm dish (area 9.61  $\text{cm}^2$ ) and UV sterilized for 20 mins prior to cell seeding. HUVEC cells were seeded on the biochips at a density of 75,000 cells/ $\text{cm}^2$  and allowed reach confluency for 48 hrs. The assembled biochips (microchannel 600  $\mu\text{m}$  wide, 120  $\mu\text{m}$  deep) were preconditioned under a shear stress of 10  $\text{dyne}/\text{cm}^2$  for 10 mins followed by a shear stress of 0.5  $\text{dyne}/\text{cm}^2$  for 10 mins.

## 4. Adhesion Profiles and Image Capture

The melanoma cell lines 1205Lu, WM793, WM793-P1 and WM793-P2 (concentration  $5 \times 10^6$  cells/ml), were infused into the coated channels under (A) a defined shear stress of 0.5  $\text{dyne}/\text{cm}^2$  for a time period of 5 min (accumulation assay) or (B) a declining gradient shear stress of 5, 2 and 0.5  $\text{dyne}/\text{cm}^2$  for a time period of 2 min/shear stress. Images were

captured using Cellix's VenaFlux assay software and analysed using Image Pro Premier software. Data was obtained from four experiments and exported into Excel to allow further analysis.

The adhesion profile of GFP-1205Lu cells was examined using the VenaEC Biochips. The GFP-tagged cells were subjected to a shear stress of 0.5  $\text{dyne}/\text{cm}^2$  over the confluent HUVEC monolayer. The monolayer was imaged using phase contrast on a Zeiss Axiovert 25 CFL microscope equipped with a LD A-Plan 20x/0.30 objective lens, 0.5x camera adapter and a DeltaPix DP200 camera. The fluorescently labelled melanoma cells were then imaged using Zeiss filter set #9 (excitation BP 450-490 nm, emission LP 515).

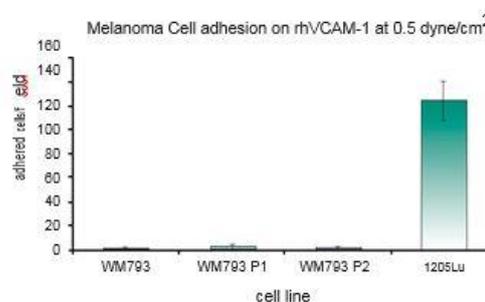


Figure 1

Figure 1: Melanoma cell adhesion to rhVCAM-1 at constant shear stress of 0.5  $\text{dyne}/\text{cm}^2$ . Melanoma cell lines WM793 and its derivatives WM793-P1, WM793-P2 and the metastatic derivative 1205Lu were subjected to a constant shear stress 0.5  $\text{dyne}/\text{cm}^2$  in Vena8 rhVCAM-1 coated channels.

## Results

In this study WM793, WM793-P1 and WM793-P2 cells did not adhere to the specified adhesion molecules at constant shear stress 0.5 dyne/cm<sup>2</sup>, whereas 1205-Lu cells adhered to V-CAM under similar shear (Figure 1). To determine the threshold shear stress that the 1205Lu cells adhere to V-CAM, a decreasing shear stress of 5, 2 and 0.5 dyne/cm<sup>2</sup> was applied which resulted in increased adhesion of 1205-Lu cells to V-CAM at shear stresses lower than 2 dyne/cm<sup>2</sup> (Figure 2). GFP-1205Lu cells were subjected to a shear stress of 0.5 dyne/cm<sup>2</sup> and adhesion to endothelial cells was recorded (Figure 3).

## Discussion

The isogenic model series was comprised of the poorly tumourigenic melanoma parental cell line WM793 and its derivatives WM793-P1, WM793-P2 (from tumours developed in mice at the site of WM793 injection) and 1205-Lu (from a spontaneous lung metastasis after subcutaneous injection of mice with WM793) which display increased growth, invasion and tumourigenicity in vitro, compared to the parental line [1, 2]. Our results showed that shear stress plays an important role in the extravasation process. The ability of 1205-Lu cells to attach to V-CAM under higher shear stress may contribute to its extravasation abilities, thus contributing to its high metastatic potential. Interestingly, all cell lines were highly adhesive under static conditions but displayed surprising inability to adhere under flow, with the exception of 1205Lu cells. It is envisaged that future work with the Cellix VenaFlux

platform will provide evidence of the timing and location of metabolic processes within the metastatic cascade enabling researchers to delineate novel markers associated with extravasation which may serve as specific targets for the treatment of cancer.

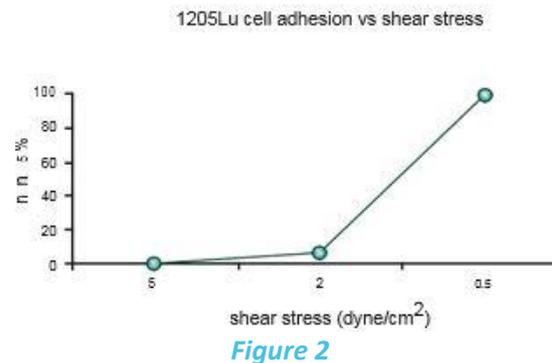


Figure 2

Figure 2: Threshold 1205Lu cell adhesion on rhVCAM-1. Melanoma 1205Lu cell line was subjected to a declining shear stress of 5, 2 and 0.5 dyne/cm<sup>2</sup> on rhVCAM-1 coated Vena8 Fluoro+ biochips. The number of 1205Lu cells adhered at each shear stress has been normalized to the number of cells adhered at 0.5 dyne/cm<sup>2</sup>.

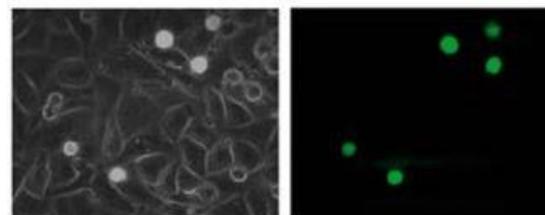


Figure 3

Figure 3: Representative microscope image of GFP-1205Lu adhesion on endothelial cells. The adhesion profile of GFP-1205Lu melanoma cells subjected to shear stress 0.5 dyne/cm<sup>2</sup> on VenaEC biochips on a confluent monolayer of HUVEC cells.

## Acknowledgements

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## References

1. Juhasz, I., et al., Growth and invasion of human melanomas in human skin grafted to immunodeficient mice. *Am J Pathol*, 1993. 143(2): p. 528-37.
2. Gallagher, W.M., et al., Multiple markers for melanoma progression regulated by DNA methylation: insights from transcriptomic studies. *Carcinogenesis*, 2005. 26(11): p. 1856-67.

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