

# Shear stress modulates tumour cell adhesion to the endothelium

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**Abstract.** The adhesion of breast adenocarcinoma cells (MDA-MB-231) to human umbilical vein endothelial cells (HUVEC) was studied in whole blood and under varying flow conditions. This study was done on HUVEC either kept under static conditions or pre-conditioned in flow for 2 hours at a shear stress of 5 or 13 dyn/cm<sup>2</sup>. Coverslips coated by HUVEC were placed in a parallel plate perfusion chamber and perfused at a shear rate of 300 or 1500 sec<sup>-1</sup> with heparin-anticoagulated blood containing <sup>111</sup>In labelled MDA-MB-231 cells. We report here the optimal conditions for studying the adhesion of MDA-MB-231 to endothelial cells under shear constraints corresponding to those observed into small and medium sized arteries.

## 1. Introduction

Blood and lymphatic vessels are major pathways for the dissemination of metastatic tumour cells from the primary tumour to distal sites. To form metastasis, cancer cells leave the primary tumour mostly by using the new vessels formed during tumour angiogenesis [5]. Tumour cells then come out of the vascular system during the extravasation, a multistep phenomenon including adhesion of tumour cells to endothelial cells in the microcirculation, dissociation and retraction of endothelial cells, adhesion of tumour cells to the subendothelium, vessel wall remodelling and finally tumour cell invasion of the tissue. Tumour cell adhesion to endothelial cells depends either on specific interactions between the two-cell types [11] and also on the contribution of products released by activated blood cells, particularly blood platelets [3,6]. Indeed, in blood, tumour cells interact with platelets and induce their aggregation. However, platelet activation depends on tumour cell lines [3]. Platelet aggregates trap tumour cells in the microcirculation, thus promoting the adhesion of tumour cells to the vascular endothelium [1].

Moreover, endothelial cells in the circulation are submitted to two types of mechanical forces: circumferential forces acting tangentially on the vessel wall, depending on blood pressure and vessel size (diameter and depth), and shear stress acting longitudinally at the interface between blood and endothelium, determined by the local flow profile [15]. Endothelial cells submitted to shear stress present two types of molecular responses (i) the regulation or the reorganisation of pre-existing proteins; these effects are rapid and linked to a critical alteration of flow; (ii) a regulation of gene expression consecutive to a chronic modification of flow [16]. Blood flow modifications can thus modulate functional properties of endothelial cells, particularly their interactions with circulating cells. To study tumour cell adhesion to the endothelium *in vitro* under conditions as close as possible from the physiology we have developed an adhesion assay using whole blood under well-defined flow conditions. To investigate the effect

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of flow variations on the reactivity of the endothelium with tumour cells, adhesion assays were done with endothelial cells grown under static conditions or pre-exposed for 2 hours to a shear stress of 5 or 13 dyn/cm<sup>2</sup>.

## 2. Methods

### 2.1. Cell culture

HUVEC were isolated and grown in M199 supplemented with 15 mM HEPES, 15 mM sodium bicarbonate, 2 mM glutamine, 100 UI/ml penicillin, 100 µg/ml streptomycin, and 20% foetal calf serum (FCS) according to Jaffe et al. [8]. Cells were harvested by a solution containing 0.05% trypsin and 0.02% EDTA and seeded onto Thermanox® (Nalgen Nunc International, USA) coverslips coated by 1% gelatin. Cells were grown until the confluence.

Breast adenocarcinoma cell line MDA-MB-231 was maintained in DMEM containing 10% FCS, 100 UI/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml fungizone and 15 mM HEPES. In order to quantify tumour cell adhesion, MDA-MB-231 were radiolabelled with <sup>111</sup>In as reported by Incardona et al. [7].

### 2.2. Perfusate

Blood obtained from consenting healthy human donors was anticoagulated with standard heparin (13 UI/ml). Radiolabelled MDA-MB-231 were added to blood at a final concentration of 10<sup>7</sup> cells/ml of blood.

### 2.3. Adhesion under flow conditions

#### 2.3.1. Flow chambers

We have used the parallel flow chamber described by Sakariassen et al. [14]. Coverslips coated with HUVEC were placed into the chamber and blood was perfused on the cells using a peristaltic pump (Masterflex, USA). Two rectangular flow chambers presenting a slit width of 0.8 mm and slit heights of 0.45 or 0.28 mm were used in order to obtain wall shear rates of respectively 300 and 1500 sec<sup>-1</sup> at a constant flow rate (10 ml/minute) according to the relation established for flow through tubes with square geometry [9]. In the case of the pre-treatment of endothelial cells by flow (see below), shear stress ( $\tau$ ) expressed in dyn/cm<sup>2</sup> is the product of the shear rate and the viscosity of the medium (0.0085 g/cm-s). Under these conditions, the shear stresses obtained in the chambers were of 5 ( $\tau_5$ ) and 13 ( $\tau_{13}$ ) dyn/cm<sup>2</sup>. The lower shear rate (300 sec<sup>-1</sup>) and stress ( $\tau_5$ ) are observed into medium arteries whether the higher shear rate (1500 sec<sup>-1</sup>) and stress ( $\tau_{13}$ ) are observed into small arteries.

#### 2.3.2. Preconditioning of endothelial cells by flow

Coverslips coated with HUVEC were either kept under static conditions or perfused with the culture medium containing 20% FCS at  $\tau_5$  or  $\tau_{13}$  shear stresses during 2 hours at 37°C in the above perfusion chambers.

#### 2.3.3. Interactions of tumour cells with HUVEC

Pre-treated HUVEC were then perfused for 5 minutes at 37°C with blood containing <sup>111</sup>In-labelled MDA-MB-231 at a shear rate of 300 or 1500 sec<sup>-1</sup>. Coverslips were then washed in PBS and cells were fixed during 10 minutes in 4% paraformaldehyde.

Tumour cell adhesion was determined by counting the <sup>111</sup>In in a  $\gamma$  counter (Wallach, Finland). Cells on the coverslips were stained with Diff'Quick (Dade, Switzerland) and observed by optical microscopy.

### 3. Results and discussion

Few studies have been reported on the adhesion of tumour cells to endothelial cells under flow conditions. Moreover, flow studies were generally performed with tumour cells suspended in buffered medium without other blood cells [12]. These conditions are far from the physiology and particularly disregard the role of other circulating cells that could react with the activated endothelial cells like leukocytes [13], or with the subendothelium like platelets and neutrophils [17]. Indeed, Bastida et al. [2] have shown that blood platelets promote tumour cell adhesion to the subendothelium under flow. In this study, we have used the parallel plate flow chamber largely used for studying platelet interactions with the subendothelium [14].

#### 3.1. HUVEC monolayer integrity under flow conditions

Endothelial cells grown under static conditions and exposed to shear stress are activated and this activation depends on the duration of shear stress exposure [16]. In order to study the effect of a long-term exposure to the flow, we have pre-exposed endothelial cells to shear stress in buffer for various periods of time between 2 and 24 hours. After 2 hours, the HUVEC monolayer appeared intact, whereas, under our conditions, after longer periods of time, the monolayer was damaged when observed by optical microscopy. As we wanted to measure tumour cell adhesion to an intact HUVEC monolayer, these cells were coated on 1% gelatin and pre-activated by an exposure of 2 hours to  $\tau_5$  or  $\tau_{13}$  dyn/cm<sup>2</sup> in presence of 20% SVF, before actual adhesion experiments with flowing blood.

#### 3.2. Effects of flow on tumour cell adhesion to HUVEC

When MDA-MB-231 tumour cells in blood were incubated for 5 minutes at 37°C under static conditions on HUVEC (Fig. 1),  $170 \pm 30$  tumour cells/cm<sup>2</sup> adhered to HUVEC. This indicated that tumour cells were able to interact with endothelial cells independently of any contribution of the flow. With blood perfusion, about 300 tumour cells/cm<sup>2</sup> adhered to HUVEC, independently of the blood shear rate ( $319 \pm 79$  at 300 sec<sup>-1</sup> and  $276 \pm 42$  at 1500 sec<sup>-1</sup>). This suggested that the short time (5 minutes) activation of endothelial cells during the blood perfusion induced an activation of HUVEC that favoured their interaction with tumour cells.

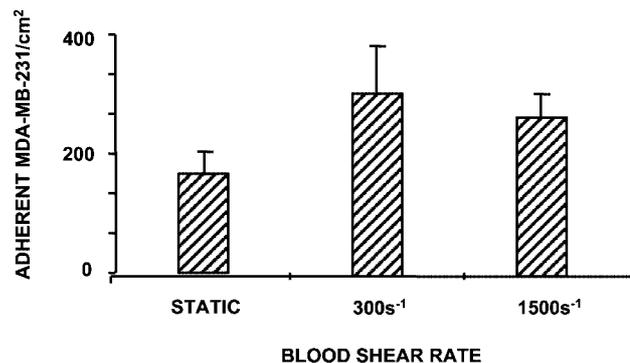


Fig. 1. <sup>111</sup>In-labelled MDA-MB-231 were added to heparinized blood (10<sup>7</sup> cells/ml) and incubated (static) or perfused at blood shear rates of 300 and 1500 sec<sup>-1</sup> for 5 minutes at 37°C on HUVEC. Adherent tumour cells were counted. Results are the mean ± SEM of 5 experiments.

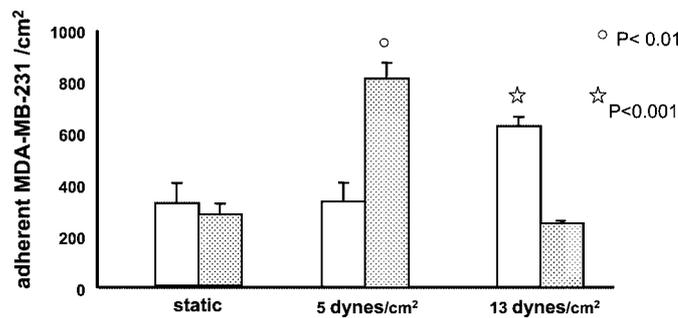


Fig. 2. Adhesion of  $^{111}\text{In}$ -labelled MDA-MB-231 to HUVEC kept under static conditions or pre-activated at 5 or 13 dyn/cm $^2$ . Blood containing tumour cells was perfused at shear rates of 300  $\square$  or 1500 sec $^{-1}$   $\square$  for 5 minutes at 37°C. Adherent tumour cells were counted. Results are the mean  $\pm$  SEM of 5 experiments.

### 3.3. Effect of shear stress on HUVEC reactivity with tumour cells

In the circulation, endothelial cells can be activated by flow modifications inducing a sustained endothelial cell gene regulation [16]. To investigate the effect of this activation on their interaction with tumour cells, HUVEC were submitted to shear stress of 5 ( $\tau_5$  HUVEC) or 13 ( $\tau_{13}$  HUVEC) dyn/cm $^2$  for 2 hours at 37°C and tumour cell adhesion to these pre-conditioned cells was measured under blood flow conditions.

#### 3.3.1. Tumour cell adhesion to pre-conditioned HUVEC

At a blood flow rate of 300 sec $^{-1}$  (Fig. 2), tumour cell adhesion to  $\tau_5$  HUVEC was equivalent ( $334 \pm 73$  cells/cm $^2$ ) to that observed for HUVEC kept under static conditions ( $319 \pm 79$  cells/cm $^2$ ). However, the adhesion of MDA-MB-231 was significantly enhanced on  $\tau_{13}$  HUVEC ( $626 \pm 37$  cells/cm $^2$ ). When the blood was perfused at a shear rate of 1500 sec $^{-1}$ , a significantly higher number of MDA-MB-231 were counted on  $\tau_5$  HUVEC ( $811 \pm 62$  cells/cm $^2$ ), whereas a basal MDA-MB-231 number ( $250 \pm 10$  cells/cm $^2$ ) was observed on  $\tau_{13}$  HUVEC. This indicates that when HUVEC are exposed to changes into their environment:  $\tau_5$  HUVEC exposed to a blood shear rate of 1500 sec $^{-1}$  or  $\tau_{13}$  HUVEC to 300 sec $^{-1}$ , cells likely reply to these changes by the induction of cell surface adhesion molecules that would enhance tumour cell adhesion to the endothelium [4,10]. The nature of these adhesion molecules remains to be determined.

## 4. Conclusion

Our results indicate that the arrest of tumour cells in the microvasculature is not only due to an occlusion of small vessels by tumour cells, but to a specific interaction of tumour cells with endothelial cells. Furthermore we show that shear stress activation of endothelial cells increases their interaction with tumour cells under shear conditions that are representative of the physiological situation in medium sized vessels.

The experimental system described here to measure tumour cell adhesion to endothelial cells in whole blood and under varying flow conditions, allows the investigation of several parameters such as: the activation of endothelial cells and the role of blood cells in the metastasis dissemination, and could be useful for pharmacological applications.

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