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Ex vivo aorta patch model for analysis of cellular adhesion

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ABSTRACT

The vascular endothelium as well as subendothelium are objects of many researches as it is directly involved in a multiplicity of physiological and pathological settings. Detailed study of endothelial function became feasible with the development of techniques to culture endothelial cells (EC) *in vitro*. Limitations of this approach have become apparent with the realization that cell culture dedifferentiate with time and do not exhibit properties of intact tissue. Here we describe the development of a novel *ex vivo* tissue model to study cell–vascular wall interactions by using isolated mouse aorta patches. Validation of this model was performed by demonstrating cell attachment and changes in cell shape typical for cell spreading during adhesion. A major advantage of this model is that cell–endothelium interaction and its molecular backgrounds can now be studied more feasibly on an intact and native tissue.

1. Introduction

The functions of the endothelium (EC) and subendothelium (SEC), which built up the inner layer of all blood vessels, are multiple and do not just form a passive barrier. EC and SEC are actively involved in fluid and solute exchange, regulation of perfusion, inflammatory responses, blood coagulation and not at last in malignant cell arrest and extravasation processes (reviewed in Cines et al., 1998; Pinkney et al., 1997). Aberrant EC function can therefore lead to pathological conditions involving atherosclerosis, hypertension, vasculitis, thromboembolic diseases, tumor onset and metastasis (reviewed in Bouïs et al., 2001; Cines et al., 1998; Gallagher and Sumpio, 1997; Kramer and Nicolson, 1979; Ruoslahti and Rajotte, 2000). The growing interest for the endothelium in physiological and pathological conditions has led to an increased demand for representative model systems. Unfortunately, the direct assessment of vascular tissue *in vivo* was shown to be difficult. But without such tissue, the endothelial cell's contribution to disease development can only be deduced. In the last decades, *in vitro* EC cultures have emerged to be important tools for studying vascular physiology and disease pathology (Jaffe et al., 1973; Lewis et al., 1973; Gimbrone et al., 1974; Bachetti and Morbidelli, 2000; reviewed in Cines et al., 1998). Due to numerous transgenic mouse lines, the isolation and culture of mouse ECs is of particular interest. And as many *in vivo* data were derived from mice, the results obtained with animal cells may be more easily compared with. However, the isolation and maintenance of primary mouse endothelial cells (MEC) continues to be challenging and time-consuming 1 (Bachetti and Morbidelli, 2000). Moreover, primary EC feature a series of disadvantages, such as cell senescence, heterogeneity of EC donors, the loss of their primary characteristics and responsiveness to various stimuli (Erusalimsky, 2009; Bouïs et al., 2001). Therefore, development of stable, immortalized MEC lines that retain the characteristics of endothelial cells was expected to greatly facilitate endothelial biology and pathology research (reviewed in Bouïs et al., 2001). Since in general immortalization is achieved by transfection with an oncogene, immortalized cells show tumor cell characteristics. Thus, an immortalized cell line is a compromise between the presentation of the desired primary characteristics and tumor cell traits (Hohenwarter et al., 1994).

Abbreviations: EC, endothelial cells; SEC, subendothelial cells; MEC, primary mouse endothelial cells.

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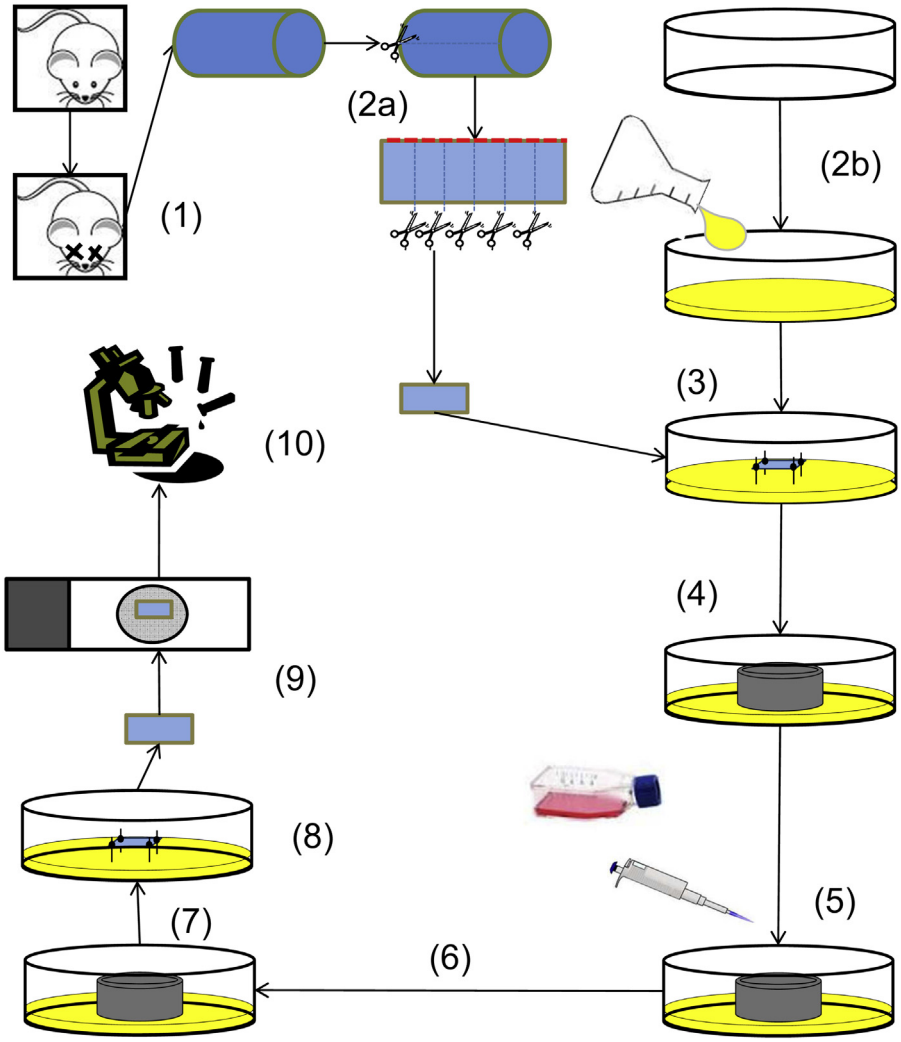

Fig. 1. Schematic illustration of the *ex vivo* aorta tissue model for analysis of tumor cell adhesion. (1) Isolation of Carotid artery from mice; (2a) cutting of blood vessel in roughly rectangular shapes; (2b) pouring of silicone elastomer; (3) using metal pins to secure blood vessel patches onto the wells; (4) patches were kept in DMEM (10% FCS, 1% P/S) at 37 °C and 5% CO_2; a metal ring was used to isolate the patches from the well; (5) cancer cells (HCT-116) were added to the patches; (6) incubation over several hours or overnight; (7) removal of metal ring; (8) careful staining of actin; (9) mounting of the patches on slides; (10) microscopy of slides.

The objective of this study was to develop a new model for investigation of mouse aortic endothelium by overcoming the disadvantages of hitherto primary and immortalized endothelial cell lines. For that purpose, we established an *ex vivo* aorta pin tissue model of mouse aortic patches to perform analysis of cell-to-endothelium interactions. We conclude that this model is not only applicable for analysis of cell adhesion processes on the vascular endothelium, but would also enable further studies with prospect of more intense investigations of the vascular endothelium.

2. Materials and methods

2.1. Cell line

HCT-116 cells were obtained from the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (Braunschweig, Germany). The cell line HCT-116 was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% (v/v) fetal calf serum (FCS), 100 μg/ml streptomycin and 100 units/ml penicillin. Cells were cultured at 37 °C in a humidified incubator with 5% CO_2.

2.2. Isolation and plating of mouse aortal patches

Mice (FVB/NCrl (Charles River, USA); male; 11 weeks) were obtained from the animal facility (UKE, Hamburg, Germany). All experimental procedures comply with the German Animal Welfare Act and the European Guideline EU 2010/63. The procedures have been approved by the Animal Welfare Officer of the Institution and the Authorities the íBehörde für Gesundheit und Verbraucherschutzí of the City of Hamburg. Mice were killed by CO_2 intoxication, the sternum was opened and blood was removed by injection of Ringer solution into the left ventricle after opening of the Vena cava inferior. The aorta thoracalis was detached from the spinal cord by cutting the branches and attaching tissue was removed with care. The aorta was collected in Ringer solution on ice, cut into three equal pieces and then the ring was opened carefully by a longitudinal incision. The aorta tissue from mice was transferred to a plate containing Hanks Balanced Salt Solution (HBSS). Extraneous tissue surrounding the aorta was removed, and the ends of the aorta were cut and discarded. Additionally the aorta was cut into three nearly identical pieces. The aorta pieces were then relocated into 6-well plates coated with a silicone elastomer (Sylgard^{TM} 184 Silicone Elastomer; Dow Corning; Belgium).