Horserace Betting Levy Board
5th Floor
21 Bloomsbury Street
London WC1B 3HF

Tel: 020 7333 0043
Fax: 020 7333 0041
Web: www.hblb.org.uk
Email: equine.grants@hblb.org.uk

Equine herpesvirus 1 induced disease in the horse

Developing a model to study equine herpesvirus 1 infected cells with blood vessels

Prj:763

Horserace Betting Levy Board
5th Floor
21 Bloomsbury Street
London WC1B 3HF

Tel: 020 7333 0043
Fax: 020 7333 0041
Web: www.hblb.org.uk
Email: equine.grants@hblb.org.uk

Modelling EHV-1 induced abortion – factors affecting virus spread from leukocytes to endothelial cells

Dr. Lara Compston-Garnett, Dr. Julia Kydd, Dr. Lutz Goehring, Prof. John Sinclair, Dr. Debra Elton, Dr. Neil Bryant

Animal Health Trust

Prj:763

Reason for study

- Equine herpesvirus type 1 (EHV-1) is a virus that can cause respiratory disease in equines worldwide.
- Infection of pregnant mares can lead to abortion.
- More rarely, EHV-1 infection can result in neurological disease, which can be fatal.
- Commercially available vaccines in the UK do not totally protect against abortion or neurological disease.
- There is a need for a more effective vaccine to protect horses from these serious clinical signs.
- We need to further our understanding of the disease process in order to design a better vaccine.

What we know about EHV-1 infection

- EHV-1 initially infects the upper respiratory tract where it replicates.
- Here it is able to infect white blood cells which then move to the lymph nodes, where the virus infects further white blood cells.
- These white blood cells leave the lymph nodes and move throughout the blood stream, where they can come into contact with other tissues, including cells lining the blood vessels known as endothelial cells.
- This interaction of EHV-1-infected white blood cells with endothelial cells is thought to be a critical step in developing the serious clinical signs of disease.

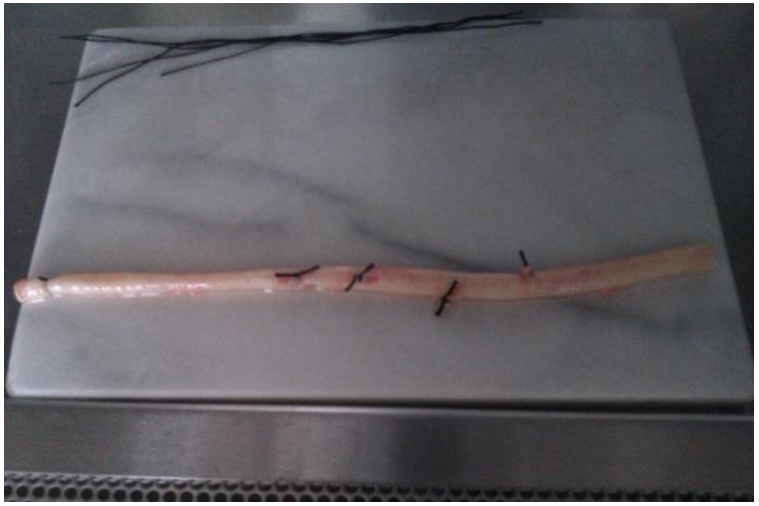
Objectives of the study

- The overall aim was to develop a model flow system to study the interaction of EHV-1 infected white blood cells with endothelial cells without conducting animal experiments.

- Smaller objectives within this overall aim included:
  - Optimisation of endothelial cell isolation from post-mortem tissue.
  - Characterisation of the endothelial cells from different anatomical sites.
  - Determine the effect of EHV-1-infection on the white blood cell-endothelial cell interaction.

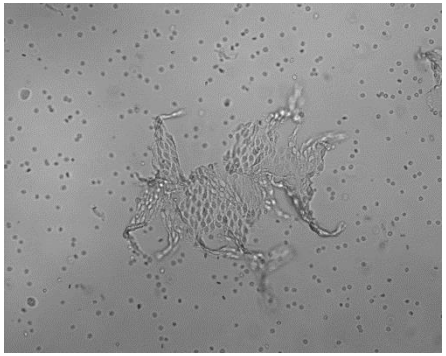
Our findings

- Endothelial cells could be isolated from a variety of fresh tissues including carotid arteries (below) using enzymatic digestion and EndoGRO-LS media (Millipore).

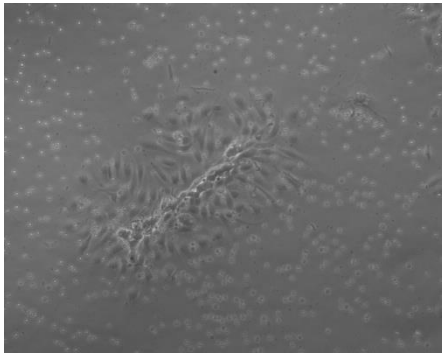


Our findings

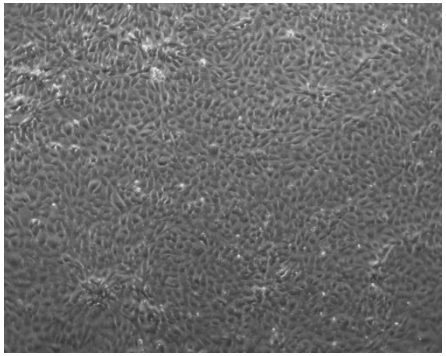
- Endothelial cells could be grown in a flask



1) Enzyme treatment of blood vessels to release cells.



2) Cells attach and begin to grow out.

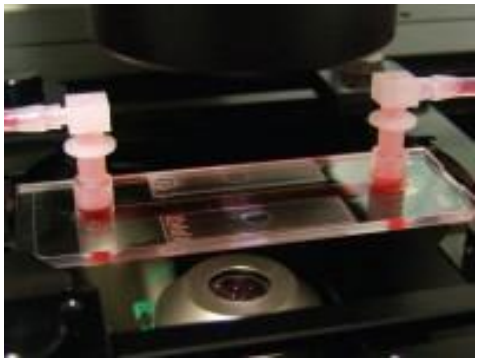


3) After 5 days endothelial cells cover the whole of the dish.

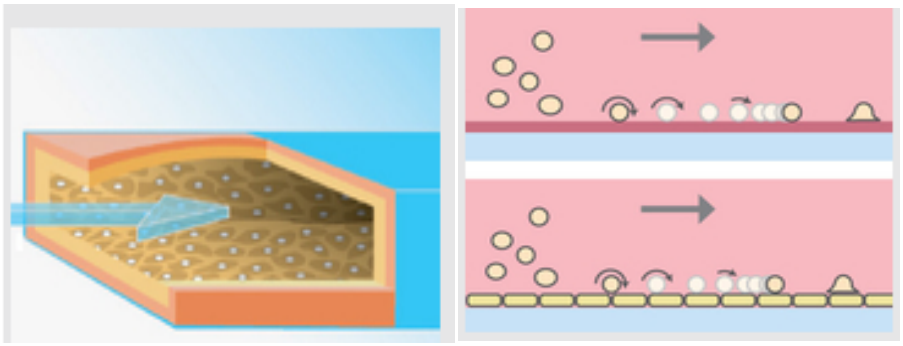
Our findings

- Endothelial cells could be transferred to a flow chamber (a) where they attached to the bottom and EHV-1-infected white blood cells could be pumped over the surface to study any interactions (b).

a



b

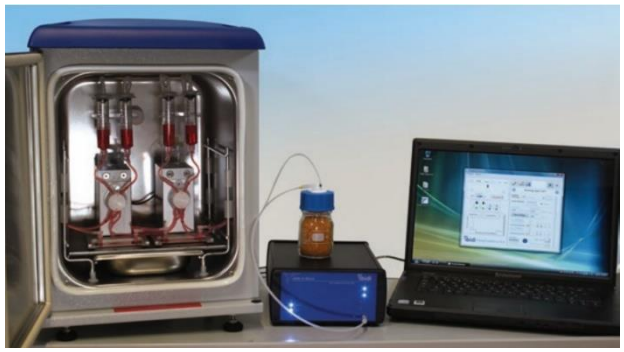


Picture credits: ibidi.com (with permission)

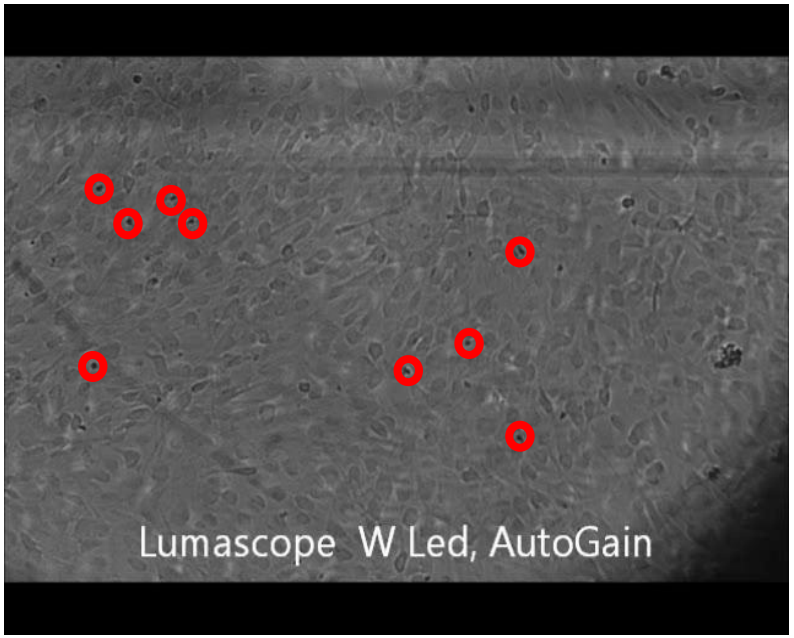
Our findings

- Using the Ibidi pump system (a) we could visualise white blood cells attaching to, and rolling on the endothelial cells by microscopy (b).

a



b

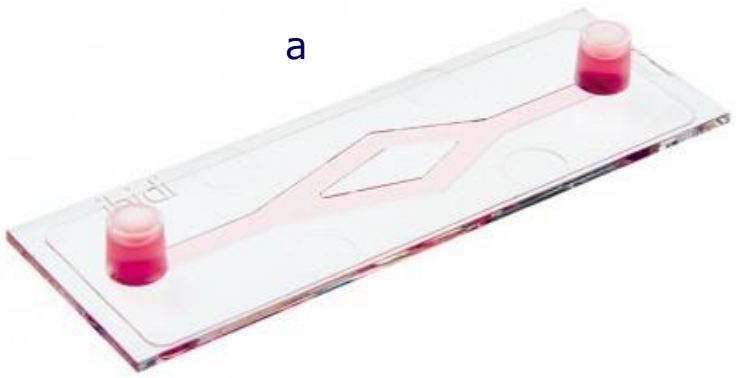


Picture credits: ibidi.com (with permission)

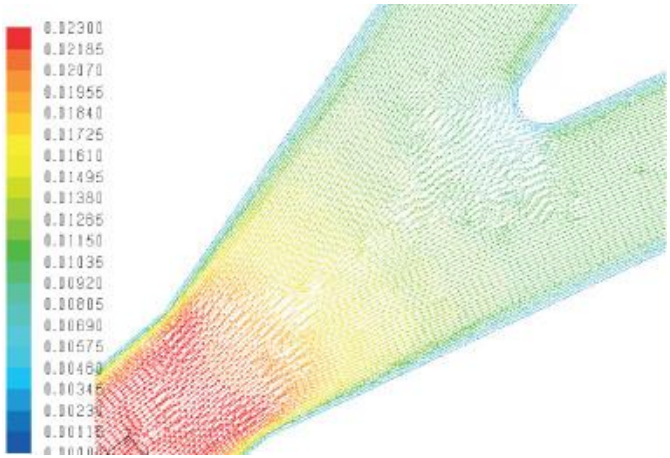
Our findings

- Using the Ibidi pump system with the endothelial cells growing in Y-shaped slides (a) allowed us to model non-uniform flow, known to occur at branch points in blood vessels (b).

a



b



Picture credits: ibidi.com (with permission)

Our findings

- We found that equine endothelial cells isolated from arteries and veins retained their cellular characteristics when grown under shear flow in the Ibidi pump system.

- Exposing the endothelial cells to shear forces in the flow chamber and infecting them with EHV-1 caused an increased production of specific proteins on the surface of the cells that are involved in cell-to-cell interactions.

- White blood cells infected with clinical strains of EHV-1 had a higher tendency to bind to and roll on the cell surface, and at a greater velocity, especially in turbulent flow regions.

Summary

- We have established an *in vitro* model to study the interaction of EHV-1-infected white blood cells with endothelial cells isolated from the blood vessels of horses.

- We have determined that EHV-1-infection of white blood cells alters the way they interact with endothelial cells, and potentially influencing the spread the virus throughout the horse.

- This model will be a valuable tool as we investigate the role of specific viral proteins in this interaction. It will help us design new improved and safe vaccines to protect horses against EHV-1 infection and prevent the serious clinical signs of abortion and neurological disease.