Studying Leukocyte Transendothelial Migration in Detail

After injury or infection with a pathogen, the human body reacts with a process known as inflammation. In this process, chemicals ('inflammatory cytokines') are released, resulting in local swelling, redness, heat and pain at the site of infection. Subsequently, leukocytes, the cells that are responsible for the defence against pathogens, will exit the bloodstream through the vessel wall and migrate into the underlying tissue towards the site of infection.

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The endothelial cells, the cells that line the inner side of the blood vessel wall, are activated by inflammatory cytokines and will express adhesion molecules such as selectins and Inter Cellular Adhesion Molecule-1 (ICAM-1). Circulating leukocytes detect these proteins, slow down and start to roll over the vessel wall. This causes activation of the leukocyte and subsequent firm adhesion. After initial contact between the leukocyte and an endothelial cell, ICAM-1 is recruited to the site of adhesion. This results in the formation of a cup-like structure, known as "docking structure" or "transmigratory cup". The clustering of ICAM-1 in these cups triggers a signalling cascade inside the endothelium. The result is a local loss of cell-cell contacts, permitting a leukocyte to migrate to the underlying tissue. In addition to migration between 2 endothelial cells (paracellular migration), leukocytes also can migrate directly through endothelial cells, this is called transcellular migration.

The Sanquin Research Dept. of Molecular Cell Biology is interested in the molecular basis of transendothelial migration, in particular in the events that result in the formation of docking structures and the signalling cascade that is activated by ICAM-1. In order to study this process, ICAM-1 was fused to Green Fluorescent Protein (GFP) so that we know where the protein localizes. To image these fluorescent fusion proteins, a Confocal Laser Scanning Microscope is used. This microscope uses lasers and filters to image a very thin slice of a tissue specimen or a cell at a time. By taking pictures at different heights, the software can reconstitute a 3D model of the tissue or cell imaged. To mimic leukocyte binding to the endothelium, polystyrene beads, coated with an antibody are used. Around these beads, endothelial cells form ring like structures, containing ICAM-1 GFP (Figure 1).

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Advanced as the confocal microscope may be, it still has a major drawback: a maximum magnification of 1000x and a limit in resolution of approximately 300nm. This makes it hard to study, for instance, membrane dynamics or submicron structures. The Phenom™ personal electron microscope enables us to study the endothelial docking structures in much more detail (Figure 2, 3). The Phenom is placed in our 'central facility', which also includes larger equipment (confocal microscopes, sequencing equipment, flowcytometers, a FACS sorter, etc.) used by different departments within Sanquin Research.

Possible research topics within Sanquin that can be studied with the Phenom are:

- Localization of adhesion molecules in microvilli
- Thrombocytes (platelets) and coagulation
- Erythrocyte-endothelium interactions
- Cell-cell contacts

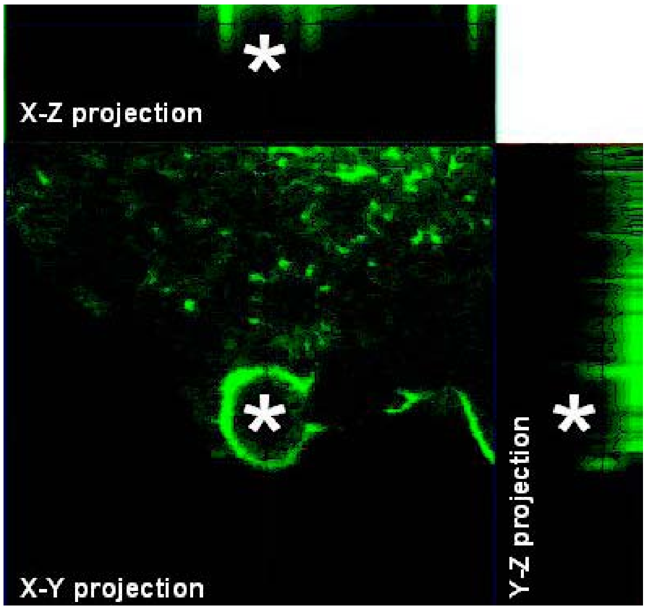
Method

Endothelial cells were cultured for 2 days on fibronectin-coated glass covers until confluency. After overnight stimulation with TNF-α to mimic inflammation, anti-ICAM-1 coated beads were added and incubated for 30 minutes. Specimens were fixed with a paraformaldehyde / glutaraldehyde fixation buffer to stop all cellular processes and crosslink proteins. Following washing with 70% ethanol, samples were incubated for 1hr each in increasing concentrations of ethanol (70%, 80%, 90%, 96% and absolute ethanol) to dehydrate the cells. After the absolute ethanol, samples were air-dried over night in a vacuum dessicator. Prior to analysis in the Phenom, samples were coated with a 10nm gold film using a sputter coater (Emitech).

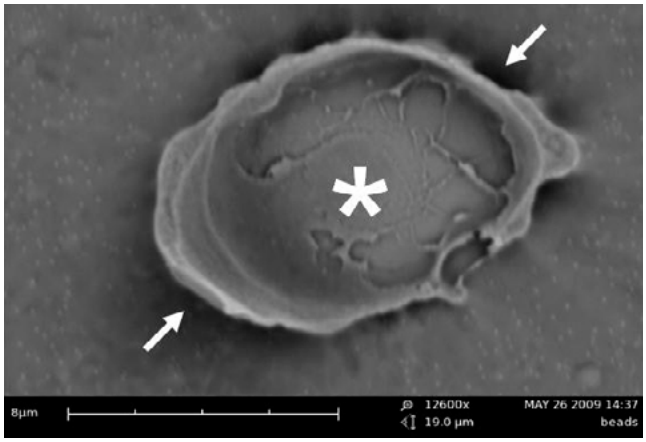
Conclusion

With the Phenom desktop scanning electron microscope, Sanquin scientists can quickly and easily make sub-micron images for detailed analysis of leukocyte transendothelial migration and similar processes. The speed and relative ease of producing these images enables researchers ultimately to advance the state of the art in their research.

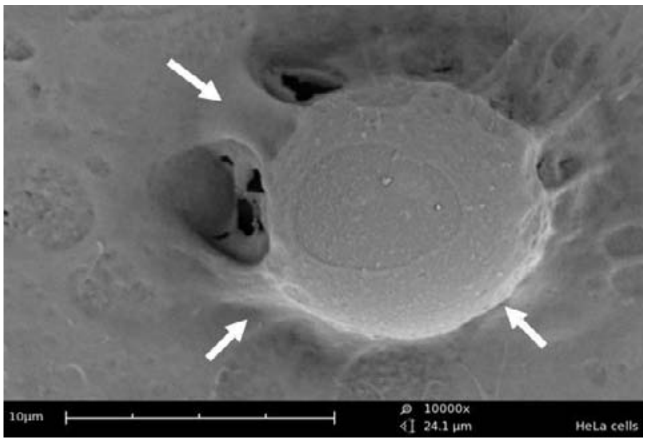
For more information about Sanquin research:
http://www.mcb.sanquin.nl/



Endothelial cells form ICAM-1-GFP positive ring structures around anti-ICAM-1 coated polystyrene beads (asterisk) (Figure 1).



Endothelial docking structure, membrane protrusions indicated by arrow. Bead (asterisk) has disappeared, probably during sample preparation and drying (Figure 2).



Anti Icam-1 coated bead adhered to an endothelial cell. Note the endothelial membrane protrusions covering the bead, indicated by arrows (Figure 3).

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