Simulation of blood flow-mediated shear stress in pneumococcal cell culture infection

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Introduction

Streptococcus pneumoniae (pneumococcus) belongs to the group of bacterial pathobionts sharing the ability to enter the bloodstream thereby causing life-threatening diseases such as septicemiae[1]. Bacterial circulation in the vasculature induces an endothelial response and triggers the immediate exocytosis of specialized storage vesicles - the Weibel Palade bodies (WPB, Fig.1). The WPB exocytosis leads to the release of procoagulatory von Willebrand factor (VWF) into the vessel lumen[2].

VWF is a mechanosensitive molecule, circulating as globular protein in the blood system with a physiological concentration between 6 and 14 µg/mL[2]. In response to vascular injury, VWF anchors thrombocytes to the subendothelium and delivers coagulation factor VIII, which is required for the incorporation of fibrin into the thrombus. VWF is also multimerizing to large protein strings on activated endothelial surfaces. Pneumococci bind to both, the globular VWF and the multimerized VWF strings via the surface exposed enolase[3]. The detailed characterization of the pathophysiological function of a mechano-responsive protein such as VWF in the infection process requires a model system, which simulates the shear stress environment present in the vascular system. Here, we present a microfluidic cell culture system which enables the investigation and microscopic visualization of the role of VWF in bacterial blood stream infections[4].

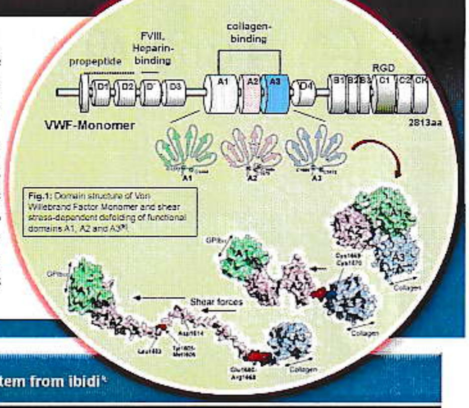
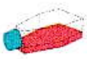


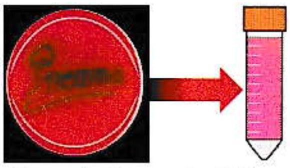
Fig.1: Domain structure of Von Willebrand Factor Monomer and shear stress-dependent defolding of functional domains A1, A2 and A3[5].

Step1 — Precultivation of primary endothelial cells

- Primary human vein endothelial cells (HUVEC) were cultivated in complex endothelium cell culture medium without antibiotics to sub confluence
- The cells were proliferated up to passage 5 at maximum

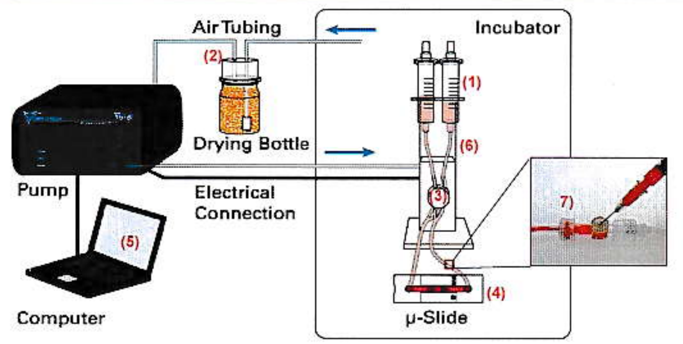


Step2 — Precultivation of *Streptococcus pneumoniae*



- RFP-expressing *S. pneumoniae* 35A[6] was cultivated o/n on blood agar followed by liquid culture at 37°C and 5% CO_2 in Todd Hewitt Broth with 5% Yeast-extract to mid exponential phase.

Step3 — Endothelial cell cultivation of HUVEC under microfluidic conditions using a pump system from ibidi*



(1) Microfluidic pump system composed of fluidic unit with two medium reservoirs, constantly moved by air pressure
(2) Silica-beads inside drying bottle, used to remove any moisture. Medium reservoirs are kept sterile by two filter caps
(3) Specific valve system ensures defined, constant, and unidirectional pumping of liquids over cell surface
(4) HUVEC are seeded within the channel of µ-slide, enabling real time microscopic visualization
(5) Pump system is controlled by flow software, offering different fluidic settings including continuous, pulsed or inverse flow
(6) Perfusion tubes available in different lengths and diameters for application of shear stress at specific dyn/cm²
(7) Additional injection ports can be incorporated into tubing for needle-injection of supplements and bacteria suspensions (inset)

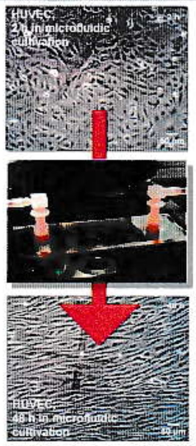


Fig 2: Microfluidic Cultivation of HUVEC. Top panel: the endothelial cells appear cobble-stone shaped and irregular distributed on the slide surface After microfluidic cultivation for 48 h at 10 dyn/cm² in an µ-slide (middle panel), the cells appear much longer and are tightly packed together to one layer (bottom panel). Pictures were used with permission from ibidi*

Step4 — Simulation of endothelial infection with RFP-expressing pneumococci in continuous flow

A)

B)

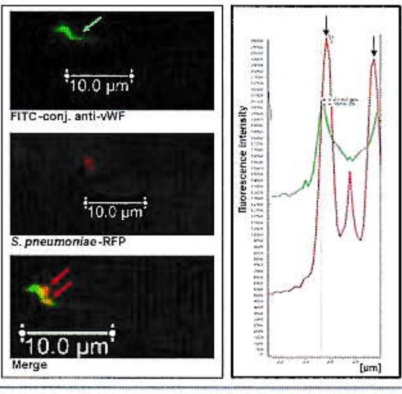


Fig.3 A, B:

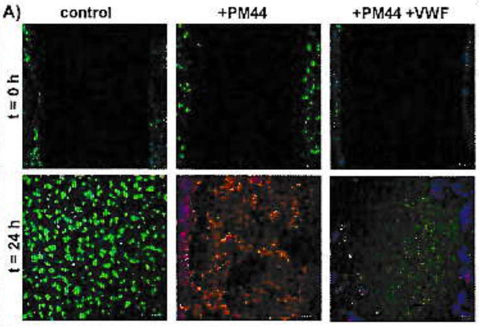
- Endogenous VWF secretion by HUVEC was stimulated by injection of 1 mM histamine
- VWF string formation was induced by applying shear stress of 10 dyn/cm²
- After injection of FITC-labelled VWF-specific antibody into fluidic circulation, VWF strings of up to 117 µm in length were visualized by fluorescence microscopy in real time (green arrow)
- After 30 min of VWF string formation, RFP-expressing pneumococci were injected into the microfluidic pump tubes and bacterial attachment was monitored by fluorescence microscopy (red arrow)
- Histogram of fluorescence overlay indicates colocalization of VWF strings and the two red fluorescent pneumococci (black arrows, B)

SUMMARY

- The microfluidic pump system enables the simulation of shear stress situation in human blood flow (Fig.2)
- Microfluidic cultivation supports a high differentiation status of primary endothelial cells (Fig.2)
- The pump system enables bacterial cell culture infection experiments in a closed, sterile environment under constant flow
- Microslides can be used for microscopic visualization in real time
- Antibodies, inhibitors, and other biochemical factors can be easily applied via the injection ports (Fig.2, 3)
- Microslide culture also enables post immunofluorescence staining after probe fixation via paraformaldehyde (Fig.3 A, B)
- Incubation with VWF reduces endothelial cell migration in static wound healing assays (Fig.4A,B)

Perspective: Use of the microfluidic system for analysis of pneumococcal effect on cell migration

A)



- cell migration assay with HUVECs, which were seeded in silicon-inlets on coverslips
- Effect of pneumococcus (PM44) and VWF-incubation on HUVEC migration was monitored at different time points
- differential immunofluorescence staining (DIF) revealed:
  blue = cytoskeleton (CS)
  green = VWF
  purple = CS + VWF + PM44
  orange/green = PM44 + VWF

Petri dish with coverslip and silicon inlet (ibidi*)

B)

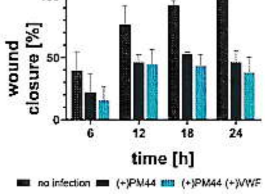


Fig.4: DIF for the cell migration assay after infection with PM44/PM44+VWF at MOI 25 showing a decreased wound closure (A) Process of wound healing after infection with *S. pneumoniae* (B)

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DFG Deutsche Forschungsgemeinschaft

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