

Materials List for:

Pneumococcus Infection of Primary Human Endothelial Cells in Constant Flow

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Materials

Name	Company	Catalog Number	Comments
1 mL Luer-syringe	Fisher Scientific	10303002	with 1 mL volume for gelatin injection using the luer-connection of the slides
2 mL Luer-syringe	Sarstedt	9077136	For pipetting/injecting fluids into the luer connections of the channel chamber slides
Accutase	eBioscience now thermo fisher	00-4555-56	protease mix used for gentle detachment of endothelial cells
AlexaFluor350-conjugated Phalloidin	Abcam	ab176751	no concentration available from the manufacturer, stock solution is sufficient for 300 tests, company recommends to use 100 µL of a 1:1,000 dilution, blue fluorescence (DAPI-filter settings)
AlexaFluor488-conjugated goat-derived anti-mouse antibody	Thermo Fisher Scientific	A11001	stock concentration: 2 mg/mL for immunostaining of human VWF in microfluidic slide after pFA fixation, green fluorescence
AlexaFluor568-conjugated goat-derived anti-rabbit-antibody	Thermo Fisher Scientific	A-11011	stock concentration: 2 mg/mL for immunostaining of pneumococci in microfluidic slide after pFA fixation, red fluorescence
Bacto Todd-Hewitt-Broth	Becton Dickinson GmbH	BD 249210	complex bacterial culture medium
Bovine Serum Albumin (BSA)	Sigma Aldrich	A2153-25G	solubilized, for preparation of blocking buffer
Cell culture flasks with filter	TPP	90026	subcultivation of HUVEC in non-coated cell culture flasks of 25 cm ² surface
Centrifuge Allegra X-12R	Beckman Coulter Life Sciences	392304	spinning down of bacteria (volumes of >2mL)
Centrifuge Allegra X-30	Beckman Coulter Life Sciences	B06314	spinning down of eukaryotic cells
Centrifuge Z 216 MK	Hermle	305.00 V05 - Z 216 M	spinning down of bacteria (volumes of less than 2 mL)
Chloramphenicol	Carl Roth GmbH + Co. KG, Karlsruhe	3886.2	used in a concentration of 0.2 mg/mL for cultivation of pneumococci transformed with genetic construct carrying red fluorescent protein and chloramphenicol resistance cassette

Clamp for perfusion tubing	ibidi	10821	for holding the liquid in the tube befor connecting the slide to the pump system
CO ₂ -Incubator	Fisher Scientific	MIDI 40	incubator size is perfectly adapted to teh size of the fluidic unit with connected channel slide and was used for flow cultivation at 37 °C and 5% CO ₂
CO ₂ -Incubator	Sanyo	MCO-18 AIC	for incubation of bacteria and cells in a defined atmosphere at 5% CO ₂ and 37 °C
Colombia blood agar plates	Becton Dickinson GmbH	PA-254005.06	agar-based complex culture medium for <i>S. pneumoniae</i> supplemented with 7% sheep blood
Computer	Dell	Latitude 3440	Comuter with pressure pump software
Confocal Laser Scanning Microscope (CLSM)	Leica	DMi8	An inverse microscope with a stage covered by a heatable chamber and with a fluorescence unit equipped with fluorescence filter, Xenon-light source (SP8, DMi8) and DFC 365 FX Kamera (1392 x 1040, 1.4 Megapixel)
Di Potassium hydrogen phosphate (KH ₂ PO ₄)	Carl Roth GmbH + Co. KG, Karlsruhe	3904.1	used for PBS buffer
Drying material	Merck	101969	orange silica beads for drying used in a glass bottle with a tubing adaptor
ECGM supplement Mix	Promocell	C-39215	supplement mix for ECGM - medium, required for precultivation of endothelial cells: 0.02 mL/mL Fetal calf serum, 0.004 mL/mL endothelial cell growth supplement, 0.1 ng/mL epidermal growth factor, 1 ng/mL basic fibroblast growth factor, 90 µg/mL heparin, 1 µg/mL Hydrocortisone
ECGMS	Promocell	C-22010	ECGM supplemented with 5 % [w/w] FCS and 1 mM MgSO ₄ to increase cell adhesion
Endothelial Cell growth medium (ECGM, ready to use)	Promocell	C-22010	culture medium of HUVECs, already supplemented with all components of the supplement mix
Fetal Calf Serum (FCS)	biochrome now Merck	S 0415	supplement for cell culture, used for infection analyses
FITC-conjugated goat anti-human VWF antibody	Abcam	ab8822	stock concentration: 10 mg/mL, for immunodetection of globular and multimerized VWF in flow
Fluidic Unit	ibidi	10903	fluidic unit for flow cultivation
Gelatin (porcine)	Sigma Aldrich	G-1890-100g	for precoating of microslide channel surface
Histamine dihydrochloride	Sigma Aldrich	H-7250-10MG	for induction of VWF secretion from endothelial Weibel Palade Bodies
Human Umbilical Vein Endothelial Cell (HUVEC)	Promocell	C-12203 Lot-Nr. 396Z042	primary endothelial cells from pooled donor, stored crycoserved in liquid nitrogen
Human VWF-specific antibody derived from mouse (monoclonal)	Santa Cruz	sc73268	stock concentration: 200 µg/mL for immunostaining of VWF in microfluidic slide after PFA fixation

Injection Port	ibidi	10820	for injection of histamin or bacteria into the reservoir tubing during the flow circulation
Light microscope	Zeiss	Axiovert 35M	inverse light microscope for control of eukaryotic cell detachment and cell counting using a 40x water objective allowing 400x magnification
Luer-slides I _{0.4} (ibiTreat472microslides)	ibidi	80176	physically modified slides for fluidic cultivation (μ -Slide I ^{0.4} Luer with a channel height of 0.4 mm, a channel volume of 100 μ l, a growth area of 2.5 cm and a coating area of 25.4 cm ²) suitable for all kinds of flow assay, the physical treatment generates a hydrophilic and adhesive surface.
Magnesium sulfate (MgSO ₄ , unhydrated)	Sigma Aldrich	M7506-500G	For preparation of ECGMS medium
Microfluidic Pump	ibidi	10905	air pressure pump
Neubauer cell counting chamber	Karl Hecht GmbH&Co KG	40442002	microscopic counting chamber for HUVECs
Paraformaldehyde 16% (PFA)	Electron Microscopy Sciences	15710-S	for cross linking of samples
Perfusion Set	ibidi	10964	Perfusion Set Yellow/Green has a tubing diameter of 1.6 mm, a tube length of 50 cm, a total working volume of 13.6 mL, a dead tube volume of 2.8 mL and a reservoir size of 10 mL. combined with the μ -slide L0.4Luer, at 37 °C and a viscosity of 0.0072 dyn x s/cm ² a flow rate range of 3.8mL/min up to 33.9 mL/min and shear stress between 3.5 dyn/cm ² and 31.2 dyn/cm ² can be reached. with 50 cm length for microfluidic
Phosphate-buffered saline (PBS)			the solution was prepared using the following chemicals: 0.2 g/L KCl, 1.44 g/L Na ₂ HPO ₄ , 0.24 g/L KH ₂ PO ₄ , pH 7.4
Plastic cuvettes	Sarstedt	67,741	(2 x optic) for OD measurement at 600 nm
Pneumococcus-specific antiserum	Pineda		raised in rabbit using heat-inactivated <i>Streptococcus pneumoniae</i> NCTC10319 and D39, IgG-purified using proteinA-sepharose column.
Polystyrene or Styrofoam plate			this is a precaution step to avoid cold stress on the cells seeded in the channel slides. Any type of styrofoam such as packaging box-material can be used. The plate might be 0.5 cm thick and should have a size of 20 cm ² .
Potassium chloride (KCl)	Carl Roth GmbH + Co. KG, Karlsruhe	6781.1	used for PBS buffer
Pump Control Software (PumpControl v1.5.4)	ibidi	v1.5.4	Computer software for controlling the pressure pump, setting the flow conditions and start/end the flow
Reaction tubes 1.5/2.0 mL	Sarstedt	72.706/ 72.695.500	required for antibody dilutions

Reaction tubes with 50 mL volume	Sarstedt	6,25,48,004	
RFP-expressing pneumococci	National Collection of Type Cultures, Public Health England	10,319	Streptococcus pneumoniae serotype 47 expressing RFP fused to a histone-like protein integrated into the genome
Serological pipets 5, 10 mL	Sarstedt	86.1253.025/ 86.1254.025	for pipeting larger volumes
Sodium Carbonate (Na ₂ CO ₃ , water free)	Sigma Aldrich	451614-25G	for preparation of 100 mM Sodium Carbonate buffer
Sodium dihydrogen phosphate (NaH ₂ PO ₄)	Carl Roth GmbH + Co. KG, Karlsruhe	P030.2	used for PBS buffer
Spectral Photometer Libra S22	Biochrom	80-2115-20	measurement of optical density (OD) of bacterial suspension at 600 nm
Sucrose	Sigma Aldrich	S0389-500G	for preparation of blocking buffer
Triton X-100	Sigma Aldrich	T9284-500ML	Used in 0.1% end concentration diluted in dH ₂ O for eukaryotic cell permeabilization after PFA fixation
Yeast extract	oxid	LP0021	bacteria are cultivated in THB supplement with 1% [w/w] yeast extract = complete bacterial cultivation medium THY