Video Article

An *In Vitro* Model of a Parallel-Plate Perfusion System to Study Bacterial Adherence to Graft Tissues

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Abstract

Various valved conduits and stent-mounted valves are used for right ventricular outflow tract (RVOT) valve replacement in patients with congenital heart disease. When using prosthetic materials however, these grafts are susceptible to bacterial infections and various host responses.

Identification of bacterial and host factors that play a vital role in endovascular adherence of microorganisms is of importance to better understand the pathophysiology of the onset of infections such as infective endocarditis (IE) and to develop preventive strategies. Therefore, the development of competent models to investigate bacterial adhesion under physiological shear conditions is necessary. Here, we describe the use of a newly designed *in vitro* perfusion chamber based on parallel plates that allows the study of bacterial adherence to different components of graft tissues such as exposed extracellular matrix, endothelial cells and inert areas. This method combined with colony-forming unit (CFU) counting is adequate to evaluate the propensity of graft materials towards bacterial adhesion under flow. Further on, the flow chamber system might be used to investigate the role of blood components in bacterial adhesion under shear conditions. We demonstrated that the source of tissue, their surface morphology and bacterial species specificity are not the major determining factors in bacterial adherence to graft tissues by using our in-house designed *in vitro* perfusion model.

Video Link

The video component of this article can be found at https://www.jove.com/video/58476/

Introduction

Staphylococcus aureus (*S. aureus*) employs a variety of virulence strategies to circumvent the host immune defense system colonizing biological or non-biological surfaces implanted in the human circulation, which leads to severe intravascular infections such as sepsis and IE[1,2,3,4,5]. IE remains an important treatment associated complication in patients after implantation of prosthetic heart valves while individual factors contributing to the onset of IEare not yet fully understood[6,7]. Under flow conditions, bacteria encounter shear forces, which they need to overcome in order to adhere to the vessel wall[8]. Models, which allow studying the interplay between bacteria and prosthetic valve tissue or endothelium under flow, are of interest as they reflect the *in vivo* situation more.

Several specific mechanisms facilitate bacterial adherence to endothelial cells (ECs) and to the exposed subendothelial matrix (ECM) leading to tissue colonization and maturation of vegetations, being essential early steps in IE[9]. Various staphylococcal surface proteins or MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) have been described as mediators of adhesion to host cells and to ECM proteins by interacting with molecules such as fibronectin, fibrinogen, collagen and von Willebrand factor (VWF)[8,10,11]. However, in view of intra-molecular folding of some virulence factors, mostly studied in static conditions, many of these interactions may have different relevance in endovascular infections in circulating blood.

Therefore, we present an in-house designed *in vitro* parallel-plate flow chamber model, which allows the assessment of bacterial adherence to different components of ECM and ECs in the context of tissue grafts implanted in the RVOT position. The overall purpose of the method described in this work is to study mechanisms of interaction between bacteria and underlying endovascular tissues in flow conditions, which are

closely related to the *in vivo* environment of bloodstream pathogens such as *S. aureus*. This novel approach focuses on the susceptibility of graft tissue surfaces to bacterial adherence to identify potential risk factors for the development of IE.

Protocol

1. Preparing Graft Tissues for *In Vitro* Studies

Note: Three types of tissues were used: Bovine Pericardium patch (BP), Cryopreserved Homograft (CH) and Bovine Jugular Vein grafts (BJV). In case of BJV conduit and CH (tissue processed by the European Homograft Bank (EHB) and stored in liquid nitrogen prior to use), both the wall and valvular leaflets were used. BP patch and BJV conduit were purchased from the manufacturers. Prior to use, thaw the CH following the EHB instructions[12].

1. Rinse all tissues with 0.9% NaCl prior to use.
2. Prepare tissue biopsies using a disposable skin biopsy punch to cut circular tissue pieces (10 mm in diameter).
3. Cut all tissue patches to the same height using disposable sterile scalpels.
4. For tissues fixed with glutaraldehyde (*for example* BJV conduit), incubate graft pieces overnight at 4 °C with 200 g/L of human albumin to neutralize the fixative.
5. Wash out residues of glutaraldehyde with 0.9% NaCl in a microtiter plate. Repeat 3 times for 1 minute.

2. Preparing Bacteria for Perfusion Experiments

Note: Three bacterial isolates were used: *S. aureus* Cowan (ATCC 12598), *S. epidermidis* ATCC 149900 and *S. sanguinis* NCTC 7864. *S. aureus* and *S. epidermidis* were grown at 37 °C in tryptic soy broth (TSB) and *S. sanguinis* was grown at 37 °C with 5% CO_2 in brain heart infusion broth (BHI).

1. Prepare overnight culture of bacteria on a solid blood agar plate.
    1. Use a sterile loop to scrape the frozen bacteria off and inoculate onto a Mueller-Hinton blood agar plate for overnight culture at 37 °C.
2. Use a sterile inoculation loop to pick up a single colony from the overnight blood agar culture and inoculate into 10 mL of TSB or BHI in a 14 mL tube and culture overnight at 37 °C.
3. Centrifuge overnight cultures (3000 x g, 4 °C, 10 min) and resuspend the pellets in 10 mL of phosphate buffered saline (PBS). Place 14 mL tubes on ice.
4. Prepare an aliquot of 3.7 mg/mL solution of 5(6)-carboxy-fluorescein N-hydroxy-succinimidyl ester (CF) in ethanol and store at -20 °C. Further dilute the stock of CF to 150 µg/mL using 'ultrapure' water.
   Note: Protect tubes from light using aluminum foil and store at -20 °C.
5. Centrifuge the bacteria (3000 x g, 4 °C, 10 min) and resuspend the pellets in 800 µL of PBS and add 200 µL of the 150 µg/mL CF solution (final concentration of 30 µg/mL used for perfusion experiments). Protect tubes from light with aluminum foil and incubate for 30 min using an orbital shaker.
6. After labeling, block with 2% of bovine serum albumin (BSA) solution in PBS and spin (3000 x g, 4 °C, 10 min). Follow with a wash step using 10 mL of PBS and pellet bacteria by centrifugation (3000 x g, 4 °C, 10 min).
7. Dilute bacteria with PBS to obtain 10^7 colony-forming units (CFU)/mL (verified by CFU counting on Mueller-Hinton blood agar plates), which corresponds to an OD_{600} (optical density) of 0.65. Keep the tubes in the dark on ice prior to perfusion experiments.
   Note. Keep in mind that OD_{600} measurements reflect the approximate number of bacteria. To count the effective inoculation dose, the serial dilution method is an additional necessary step to verify the OD based numbers as described in section 3.8.

3. *In vitro* Perfusion Experiments using a Parallel-Plate Flow Chamber

1. Mount tissue biopsies of 10 mm in diameter and the same thickness (prepared in the steps 1.1 - 1.5) into a flow chamber system with the inner surface facing up to get in contact with the bacterial suspension.
   Note: The same tissue thickness across various grafts ensures that the same tissue height is reached in the channel allowing laminar flow in all conditions. All elements of the flow chamber are presented and described in **Figure 1**.
    1. To begin the protocol, place the round tissue piece between a microscope slide with an 8 mm circular perforation and a rubber gasket.
       Note: The microscope slide possesses the ultra-thin bottom film to allow the generation of the 8 mm hole. Together with the rubber sheet, it fixes the tissue to enable the direct contact between the specimen and the flowing medium and also prevents the dislocation of the biopsy during the experiment. The surface of the investigated tissue, which is exposed to the flow (smaller diameter) cannot be manipulated by the forceps.
    2. Insert the holder with the tissue into the gasket sheet that is embedded in the bottom metal frame of the chamber.
    3. Attach the upper metal frame with the corresponding gasket sheet onto the bottom part of the chamber with the previously inserted tissue holder. Subsequently mount the entire chamber with eight screws and screw nuts. Make sure that the chamber height is always the same across grafts.
       Note: The chamber height should be determined always upon tightening the screws. Use a caliper or ruler.
2. Connect the flow chamber with a peristaltic pump and the fluid reservoir with the tubes.
3. Perfuse the tissues with suspensions of 10^7 CFU/mL (verified by CFU counting and related to OD_{600} measurements) fluorescently-labeled bacteria in PBS with a shear stress of 3 dyne/cm^2 (dyne per square centimeter pressure unit) by means of the peristaltic pump (flow rate 4 mL/min) for 1 h using a 400 mL bacterial reservoir (**In-house design, Figure 1**) conditioned at 37 °C using a plate thermostat (**Table of Materials**).
4. Recirculate continuously the 100 mL bacterial suspension using the same collection reservoir.

5. After perfusion, dismantle the chamber to release the graft and wash the tissue piece two times with 4 mL of PBS in a 12-well plate using the laboratory orbital shaker for 3 min each. Subsequently cut the inner part of the graft using a skin biopsy punch of a smaller diameter.
6. Place each tissue biopsy into a separate 14 mL tube containing 1 mL of sterile 0.9% NaCl. Label the tube as #1.
7. Detach the bacteria from the tissue using a sonication bath for 10 min (amplitude = 100% and frequency = 45 kHz).
   Note: Full detachment of bacteria from the tissue grafts should be evaluated upon incubation of patches overnight at 37 °C in TSB liquid medium followed by OD_{600} measurements compared to control patches treated with a bacteria free solution.
8. Use a serial dilution method on Mueller-Hinton blood agar plates to count CFUs.
   1. Prepare a single 14 mL tube with 10 mL of sterile saline to make serial dilutions of the bacterial suspension obtained after sonication. Label this tube as #2.
      Note: For each tissue experiment one tube with 10 mL of 0.9% NaCl is necessary.
   2. Prepare three 14 mL tubes with 10 mL of sterile 0.9% NaCl for serial dilutions of initial bacterial suspension from step 2.7. Label the tubes as follows #3, #4, #5.
      Note: This step is necessary to know the real CFU number in bacterial suspension used for the perfusion experiment.
   3. Vortex mix each tube for 15 s. Vortex the tubes with the tissue biopsy as well as the initial bacterial suspension to make serial dilutions.
   4. Prepare three agar plates, two for the tissue experiment (perfusion of bacteria and control perfusion of PBS) and the third one for the initial bacterial suspension used for perfusions.
   5. Label three sectors per plate for the tissue experiment in the following manner 10^{-1}, 10^{-3} and 10^{-4}. To count the number of CFUs in the bacterial perfusates, label the plate as follows: 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7}.
      Note: All indications on agar plates such as 10^{-1}, 10^{-3} and so on refer to the final number of CFU/mL calculated on the next day. Control plate does not require any sectors. Before use, blood agar plates should be placed under the laminar hood and opened to remove excess moisture.
   6. To continue preparing the serial dilutions, transfer 100 µL of tube #1 to tube #2 and mix vigorously with vortex.
   7. Spread 100 µL of the contents of tube #1 and #2 onto the corresponding sectors 10^{-1} and 10^{-3} of the agar plate. Likewise, spread 10 µL of tube #2 on the sector 10^{-4}, repeat this step 4 times to obtain 4 separate growths from each volume of 10 µL.
      Note: Due to the small volume used for plating onto the sector 10^{-4}, it is advised to have multiple number of droplets to make an average number of grown CFUs.
   8. To prepare the serial dilutions of the initial culture, transfer 100 µL of bacterial suspension from step 2.7 to tube #3 and mix vigorously with vortex. Add 100 µL of tube #3 to tube #4 and mix well, repeat the procedure for subsequent tube #5.
   9. Spread 100 µL of the contents of tubes #3, #4, #5 and the adjusted bacterial suspension (step 2.7), respectively, onto sectors 10^{-3}, 10^{-5}, 10^{-7} and 10^{-1} of the blood agar plate.
   10. Leave the blood agar plates in the laminar hood to air dry the bacterial spreads, typically for 10 minutes. Afterwards, place the plates at 37 °C for overnight incubation.
   11. After overnight incubation, count the bacterial colonies to obtain the number of CFUs resulting from the adhesion to the tissue biopsies as well as CFUs/mL in the starting bacterial suspension used for the perfusion. Express results as CFU/cm^2.

4. Fluorescence Microscopy of Adhered Bacteria to Graft Tissues upon Perfusion

1. After perfusion, wash tissue pieces with PBS (see step 3.5) and cut the inner part of a graft using a punch of a smaller diameter.
2. Prepare a 6-well plate and place droplets of mounting medium (**Table of Materials**).
3. Place each piece of tissue with its perfused surface downward on a single drop of mounting medium.
4. Read a plate using a fluorescence scanner (**Table of Materials**). Set parameters of excitation and emission wavelengths according to a fluorophore used for bacterial labeling.

Representative Results

To better understand the mechanisms behind IE development, this model enables the evaluation of bacterial and tissue associated factors present in the *in vivo* situation of infection onset.

In detail, the novel *in vitro* approach allows to quantify bacterial adhesion in flow conditions to different graft tissues by perfusing fluorescently labeled bacteria over the tissues exerting the shear stresses in the physiological range of 3 - 10 dyne/cm^2 for the RVOT. In this work, we used a flow rate of 4 mL/min that corresponded to 3 dyne/cm^2. Taking into consideration the channel height of 0.3 mm across all tissue patches, the distance between the mounted graft and the medium inlet of about 39 mm, the perfusion chamber (shown in **Figure 1**) guarantees fully developed laminar flow (Re = 3.89 is significantly lower than 2000; the entrance length = 0.05 mm is significantly smaller than the distance `inlet-graft`, parameters necessary for assuming appropriate flow pattern).

Under shear stress conditions, a similar bacterial attachment across the various graft tissues was observed for both *S. aureus* and *S. epidermidis* infection (**Figure 2** and **Figure 3**). Although not significant, a trend towards higher adhesion of *S. aureus* to the CH leaflets was noticeable.

For *S. sanguinis* a significant reduction of adherence to the BJV wall was found when compared to the BP patch (**Figure 4**; $P < 0.05$). When comparing the 3 species of bacteria, *S. sanguinis* presents significantly lower adhesion to the BJV wall in relation to *S. aureus* and *S. epidermidis* ($P < 0.01$ and $P < 0.05$ respectively, see the video). In general, we observed a similar bacterial adhesion to all tissues investigated under shear stress.

Our data from CFU counting (**Figure 2**, **Figure 3**, **Figure 4**) are supported by fluorescence microscopy using a high throughput scanner (**Figure 5**, **Figure 6**, **Figure 7**). Images are presenting pronounced foci of labeled bacteria adhering to graft tissues. Due to this approach, we were able to directly visualize tissues upon perfusion without any post experimental processing for illustration purposes.

Results demonstrate that the source of a graft tissue, surface morphological differences as well as bacterial adhesins are not major determinants of bacterial adherence*to these biological materials.*

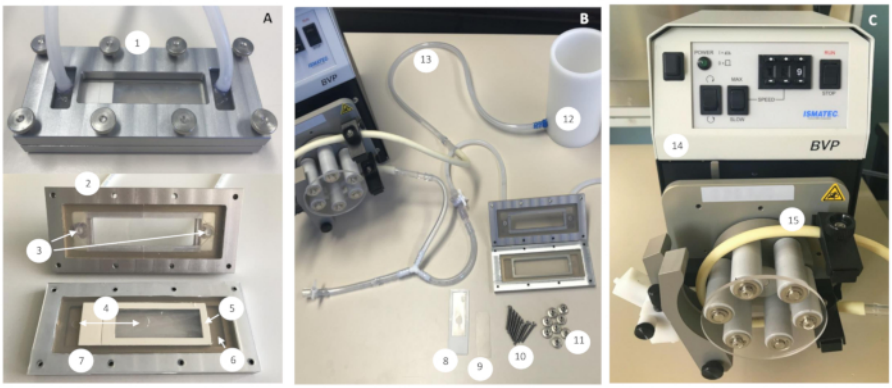

Figure 1: Image of a newly developed flow chamber system (in-house design by the Department of Biohybrid & Medical Textiles, AME - Helmholtz Institute for Biomedical Engineering, Aachen, Germany). A. The flow chamber (1) mounted flow set of dimensions LxWxH: 125 mm x 55 mm x 18 mm (screws in combination with screw-nuts hold the chamber`s parts together and put pressure *via* the metal frame on the gaskets to prevent leakage); **(2)** the upper part of the column; **(3)** the upper gasket sheet with two holes to fix tubing connectors, which connect the flow chamber with the pump and the fluid reservoir by means of the tubing system; **(4)** distance between the medium inlet and the tissue (the entrance length); **(5)** thin foil slide (with an 8 mm circular perforation to allow the exposure of the tissue to the bacterial suspension) (in a recess of the slide there is the space for a rubber gasket **B9**, to immobilize the tissue piece during the perfusion); **(6)** the bottom gasket sheet with a dedicated recess to place the tissue graft mounted between the microscope slide and the rubber gasket; **(7)** the bottom part of the flow chamber; **B. The full set-up (8)** the thin foil slide; **(9)** the rubber gasket; **(10)** eight screws with corresponding **(11)** eight screw-nuts; **(12)** the fluid reservoir (400 mL); **(13)** tubing system; **C. Perfusion unit (14)** the peristaltic pump; **(15)** dedicated tubing that withstands the rigors of peristaltic pumping action. Please click here to view a larger version of this figure.

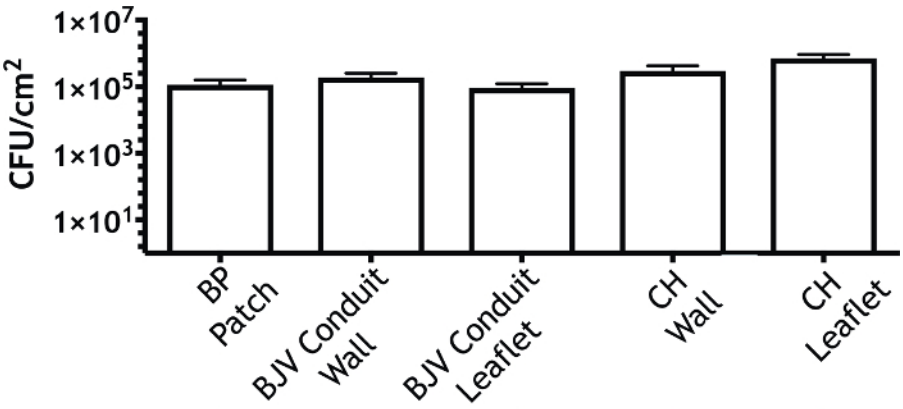

Figure 2: Adhesion of *S. aureus* Cowan to graft tissues under flow conditions. Fluorescently labeled bacteria were perfused over 5 graft tissues (conduit walls or valvular leaflets) in PBS. Bacteria were detached from infected tissue pieces by sonication. Bacterial adhesion was evaluated by serial dilutions using the CFU counting method and indicated as CFU/cm^2. All results are expressed as mean ± SEM (n > 3 for valvular leaflets due to limitation of material; n > 5 for conduit walls). *CFU:* colony-forming unit; *BP:* bovine pericardium; *BJV:* bovine jugular vein; *CH:* cryopreserved homograft. This figure has been modified from Veloso *et al.* (*Journal of Thoracic and Cardiovascular Surgery* **155** (1), 325-332 (2018)). Please click here to view a larger version of this figure.

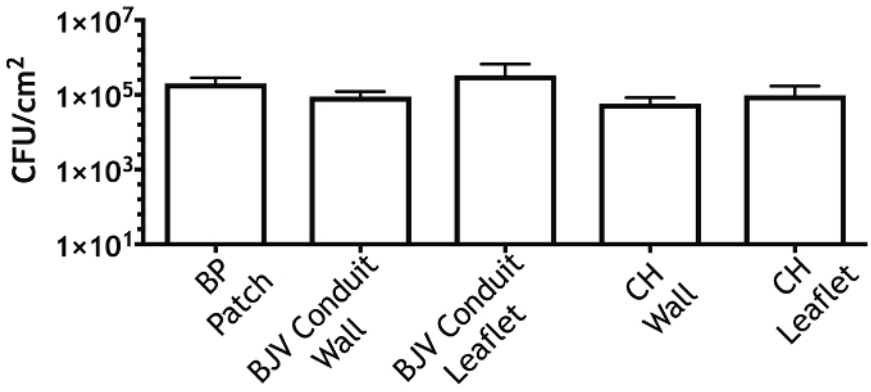

Figure 3: Adhesion of *S. epidermidis* to graft tissues under flow conditions. Fluorescently labeled bacteria were perfused over 5 graft tissues (conduit walls or valvular leaflets) in PBS. Bacteria were detached from infected tissue pieces by sonication. Bacterial adhesion was evaluated by serial dilutions using the CFU counting method and indicated as CFU/cm^2. All results are expressed as mean ± SEM (n > 3 for valvular leaflets due to limitation of material; n > 5 for conduit walls). *CFU:* colony-forming unit; *BP:* bovine pericardium; *BJV:* bovine jugular vein; *CH:* cryopreserved homograft. This figure has been modified from Veloso *et al.* (*Journal of Thoracic and Cardiovascular Surgery* **155** (1), 325-332 (2018)). Please click here to view a larger version of this figure.

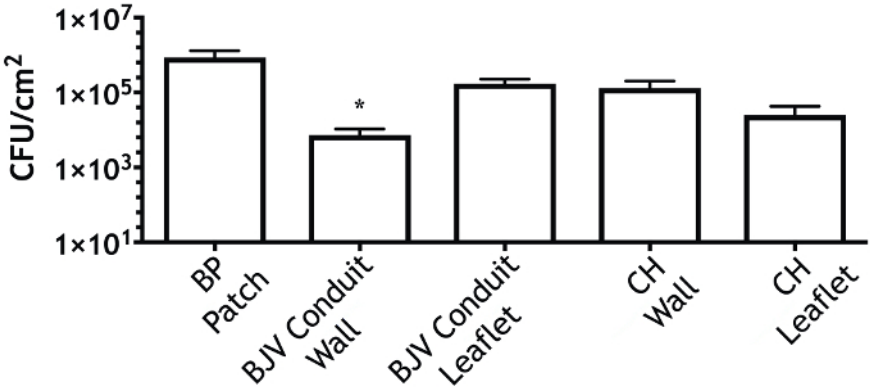

Figure 4: Adhesion of *S. sanguinis* to graft tissues under flow conditions. Fluorescently labeled bacteria were perfused over 5 graft tissues (conduit walls or valvular leaflets) in PBS. Bacteria were detached from infected tissue pieces by sonication. Bacterial adhesion was evaluated by serial dilutions using the CFU counting method and indicated as CFU/cm^2. All results are expressed as mean ± SEM (n = 3 for valvular leaflets due to limitation of material; n > 5 for conduit walls). *CFU:* colony-forming unit; *BP:* bovine pericardium; *BJV:* bovine jugular vein; *CH:* cryopreserved homograft. This figure has been modified from Veloso *et al.* (*Journal of Thoracic and Cardiovascular Surgery* **155** (1), 325-332 (2018)). *P < 0.05. Please click here to view a larger version of this figure.

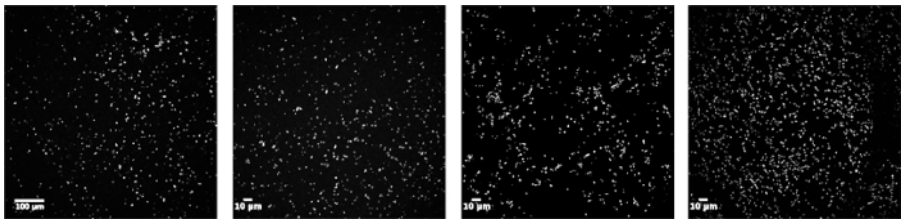

Figure 5: Visualization of *S. aureus* Cowan adherence to graft tissues by means of fluorescence microscopy. *Left to right:* BJV conduit wall, BJV leaflet, CH wall and CH leaflet. This figure has been modified from Veloso *et al.* (*Journal of Thoracic and Cardiovascular Surgery* **155** (1), 325-332 (2018)). Please click here to view a larger version of this figure.

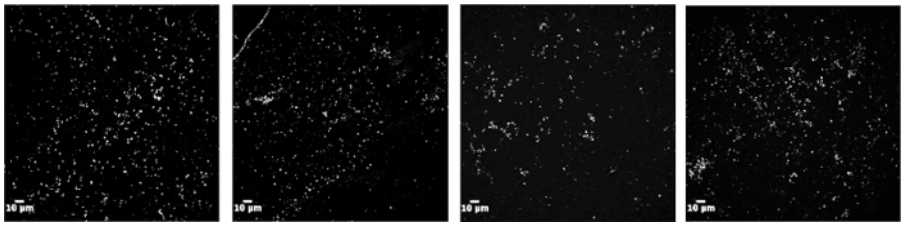

Figure 6: Visualization of *S. epidermidis* adherence to graft tissues using fluorescence microscopy. *Left to right:* BJV conduit wall, BJV leaflet, CH wall and CH leaflet. This figure has been modified from Veloso *et al.* (*Journal of Thoracic and Cardiovascular Surgery* **155** (1), 325-332 (2018)). Please click here to view a larger version of this figure.

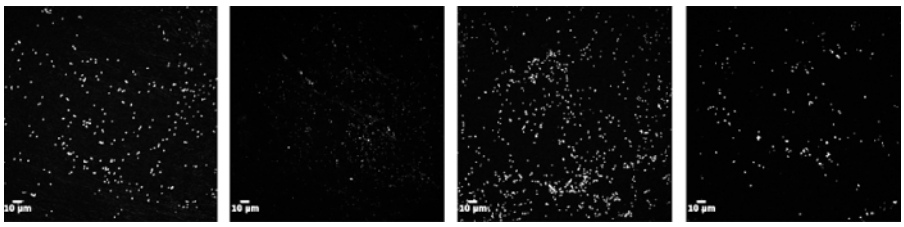

Figure 7: Visualization of *S. sanguinis* adherence to graft tissues using fluorescence microscopy. *Left to right:* BJV conduit wall, BJV leaflet, CH wall and CH leaflet. This figure has been modified from Veloso *et al.* (*Journal of Thoracic and Cardiovascular Surgery* **155** (1), 325-332 (2018)). Please click here to view a larger version of this figure.

Discussion

Recent clinical observations give special awareness to IE as a complication in patients having undergone valve replacement of the RVOT[6,13]. Dysfunction of the implanted valve in IE is the result of bacterial interaction with the endovascular graft leading to extensive inflammatory and procoagulant reactions[1,14]. The presented novel *in vitro* model allowed us to investigate if differences in tissue structures and bacterial factors are likely to modulate the susceptibility to infections of *in vivo* used grafts[15]. BJV and CH graft tissue showed similar propensity towards bacterial recruitment in flow conditions. Therefore, data suggest that in general the source of the tissue and its surface structure as well as specific bacterial adhesive proteins *per se* are not the major determinant factors in initial bacterial adherence.

In general, pathways evoking inflammation, tissue damage, platelet and fibrin deposition at the infected endovascular site are activated by multiple players[1,16]. A major advantage of the developed *in vitro* model is the opportunity to analyze stepwise the contribution of involved players. Single bacterial factors can be investigated by using bacterial mutant strains or genetically modified bacteria expressing single adhesion proteins on their surface[14]. By choosing different perfusion media, plasma or blood, the involvement of plasma proteins and blood cells can be evaluated. Further studies will focus on tissue related factors for which tissues will be pre-incubated with *for example* plasma proteins before mounted in the flow chamber for subsequent perfusion. Since players contributing to the onset of prosthetic valve IE remain unclear, future studies might unravel the potential factors by building up to a more complex experimental setup. Furthermore, this experimental setup inherits the possibility that tissues can be seeded with an EC layer to analyze shear-dependent EC gene expression. The parallel-plate flow chamber also allows perfusion over EC-covered microscope slides due to a flexible inner height of the perfusion chamber. Different coatings of cover slips using various extracellular matrix proteins are also a possible option to assess important interactions with the subendothelial matrix. In addition, pharmacologic inhibitors or functional antibodies can be investigated for their effect in the respective condition in our flow chamber. In summary, various conditions can be studied by increasing complexity.

Inflammatory activation at the infected area of the graft is a crucial, shear-controlled step favoring deposition of activated platelets and monocytes. The impact of shear forces on bacterial adherence to tissue surfaces are of major concern. To address this issue, the novelty of the presented *in vitro* system focuses on the possibility to mount tissues in a flow chamber. This reinforces the significance of the method beyond existing alternatives, in which usually static interactions between bacteria and underlying tissues have been investigated. Even though shear stress was submitted by shaking or other external forces, it has not been standardized to the same level as we can gain from our uniform flow model.

In vivo, a non-physiologic flow pattern can favor bacterial adhesion as the onset of IE at the valve level of implanted conduits. Shear stress was found to up-regulate endothelial inflammatory parameters such as cytokine secretion and to increase tissue factor mediated coagulation[17]. The interaction of the underlying tissue used for valve prostheses with bacteria and their influence on EC gene expression under shear stress is important to construct a valve less capable of bacterial adhesion and chronic inflammation.

The basal technical issues of the fabricated chamber allow investigations under standardized conditions in the laminar flow[18]. To ensure the fully developed laminar flow at the site of the investigated tissue the chamber is constructed to mount the graft in a certain distance from the medium inlet (significantly longer than the computed entrance length, see Results and **Figure 1**). Using different pumps in the system would allow performing experiments under pulsatile or turbulent flow conditions in the future.

The flexible frame of the chamber prevents the chamber effectively from leaking and the internal height of the frame allows adapting for tissue thickness. The construction of the whole system enables a circulating flow, which is of importance to perform long lasting perfusions with using a respective amount of medium. Based on previous studies our adhesion protocol assumed a bacterial inoculation dose of 10^7 CFU/mL for a 1 h incubation[4,19]. By using these settings, adhesion levels were detectable, albeit low enough to be able to observe significant enhancement of bacterial adherence without saturation of the tissue graft surface. Moreover, in this period of time, it was feasible to notice potential differences in

binding across strains taken in this study. Shear parameters addressed here were in the physiologic range and optimized for the blood vessels, which were our target in respect to the RVOT.

Further modifications of the method will focus on more efficient consumption of medium during the procedure as well as on simplification of mounting the setup. In addition, a new design including multiple slots for tissue assembly would ease an entire experiment in aspects such as efficiency.

At this stage our method is focused on the end-point results and was not tested for real time applications such as the time course of dynamic events occurring on the tissue surface. Thus, this broader application remains under consideration; however, issues such as tissue autofluorescence, optimization of an appropriate fluorescence microscope protocol as well as adaptations of the chamber need to be addressed. Further on, the method in its current state may be adapted to real-time monitoring of bacterial binding to EC layers on microscope slides by upright fluorescence microscope. Currently, we are able to visualize bacteria and other blood components/cells bound to tissues by confocal microscopy without a need for post experimental tissue processing, which is predisposing for the real-time visualization under flow by inverted fluorescence microscopes.

In this study, the quantification of bacterial adhesion was provided by CFU counting while fluorescence microscopy was a supportive, non-quantitative tool. Due to resolution issues resulting from the lack of an adequate microscope lens, fluorescence imaging turned out to be less reproducible in our hands than serial dilutions. Nevertheless, it is possible to use fluorescence scanning for quantification when suitable objective lens could illuminate the entire graft size of 8 mm in diameter for reliable foci quantification. Using an image processing program (such as ImageJ), absolute fluorescence units might be quantified for investigated tissue specimens and the bacterial adhesion might be expressed for example as a relative signal to the internal control (grafts perfused with non-labeled bacteria).

The major limitation of this experimental setting are the issues associated with *in vitro* studies in general. Results reached by using this *in vitro* flow chamber model could be transferred to an animal model for *in vivo* confirmation.

In conclusion, this *in vitro* model allows investigation of single bacterial, tissue and shear-based factors contributing to the onset of bacterial adhesion to tissues in a stepwise manner. The hereby enabled knowledge could contribute to the development of more effective prevention and treatment of IE.

Disclosures

None.

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Materials List for:

An *In Vitro* Model of a Parallel-Plate Perfusion System to Study Bacterial Adherence to Graft Tissues

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Materials

Name	Company	Catalog Number	Comments
Bovine Pericardium (BP) patch, Supple Peri-Guard Pericardium	Synovis Surgical Innovations, USA	PC-0404SN	
Bovine Jugular Vein conduits (BJV)	Contegra conduit; Medtronic Inc, USA	M333105D001	
CH cryopreserved homograft	European Homograft Bank (EHB)	-	
Acu-Punch	Acuderm Inc, USA	P850 (8 mm); P1050 (10 mm)	
human Albumin	Flexbumin; Baxter, Belgium	BE171464 LOT:16G12C	
Tryptic soy broth (TSB)	Fluka, Steinheim, Germany	22092-500G	
Heart infusion broth (BHI)	Fluka	53286-500G	
Phosphate buffered saline (PBS).	Gibco	14190-094	
5(6)-Carboxyfluorescein N-hydroxysuccinimide ester (CF)	Sigma-Aldrich, Germany	21878-100MG-F	
Peristaltic pump (MODEL ISM444B)	Ismatec BVP-Z Standard; Cole Parmer, Wertheim, Germany	631942-2	
Sonication bath	VWR Ultrasonic Cleaner; VWR, Radnor, Pa	142-6044	230V/50 -60Hz 60VA; HF45kHz, 30W
ProLong Gold Antifade Mountant	Invitrogen by ThermoFisher	P36930	
InCell Analyzer 2000 (fluorescence scanner)	GE Healthcare Life Sciences, Pittsburgh, Pa	29027886	
Arium Pro VF - ultrapure water - H_2O MilliQ	Millipore	87206462	
Microscopic slides - Tissue Culture Chambers (1-well)	Sarstedt	94.6140.102	
1-well on Lumox detachable	Sarstedt	94.6150.101	
Stainless Steel - surgical Blades	Swann-Morton	311	
Tygon Silicone Tubing, 1/8"ID x 1/4"OD	Cole-Parmer	EW-95702-06	Temperature range: –80 to 200°C Sterilize: With ethylene oxide, gamma irradiation, or autoclave for 30 min, 15 psi of pressure
PharMed BPT Tubing	Saint-Gobain	AY242012	Autoclavable 30 min at 121°C
Tygon LMT-55 Tubing	Saint Gobain Performance Plastics™	15312022	
Thermostat	BMG BIOMEDIZINTECHNIK	300-0042	230V, 90VA, 50Hz

