**Supplementary Material:**

***Staphylococcus aureus adheres avidly to decellularized cardiac homograft tissue in vitro in the fibrinogen dependent manner***

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**Material and Methods:**

***2.1 Parallel plate flow chamber***

We developed an *in vitro* model to investigate the propensity of different graft tissues
for bacteria and platelet adhesion and herewith their susceptibility for infection. The chamber has the following dimensions: LxWxH: 90 mm x 50 mm x 25 mm.

All investigated graft tissues were mounted into the micro-parallel flow chamber presented in Fig. 1 and perfused with bacterial suspensions in phosphate-buffered saline (PBS) at room temperature (RT) using a high accuracy multi-syringe pump (PHD 2000 Programmable; Harvard Apparatus, Cambridge, Mass) at a shear rate of 1000 s-1 that corresponds to a shear stress of 10 dyne/cm2.

All parameters of the chamber such as the channel height, the distance between the medium inlet and the mounted graft tissue guaranteed the full development of laminar flow 14 as this flow pattern was used throughout this study.

Compared to our previous system 14 we have now designed a set-up that allows to perfuse smaller volumes of liquids. Reduced volumes are important for perfusions with valuable fluids, such as blood, plasma or serum. Thereby, together with a substantial reduction of the perfusate consumption we can importantly perform short-term experiments more efficiently to assess different interactions of bacteria with cardiac graft tissues 9.

***2.2 Bacterial strains***

Graft tissues were perfused with bacterial suspensions of the *S. aureus* strain 8325-4. Bacteria were cultured in tryptic soy broth (TSB) liquid medium and on Mueller-Hinton blood agar plates at 37 °C. Bacteria were stored in liquid medium supplemented with 15 % (vol/vol) glycerol at -80 °C.

***2.3 Graft tissues***

Bacterial adhesion and fibrinogen binding were tested for bovine pericardium patch (BP; Supple Peri-Guard®; Synovis Surgical Innovations, St. Paul, Minnesota, USA), cryopreserved pulmonary homograft (CH; human origin, European Homograft Bank, Brussels, Belgium) and decellularized fresh pulmonary homograft (DPH; human origin, Corlife oHG, Hannover, Germany) under shear stress and static conditions, respectively. Tissue pieces, identically prepared as for clinical use, were cut with a 6-mm disposable skin biopsy punch (Acu-Punch, Acuderm Inc, USA). For DPH and CH, fragments of the valvular wall were used in all experiments. All tissue patches were adapted to have the same height using a disposable sterile scalpel if necessary and washed with 0.9 % NaCl (2 times 5 min each) prior to use. Tissue pieces were incubated overnight (O/N) at 4 °C with human plasma. Then, the graft discs were mounted in the holder of the micro-parallel plate flow chamber and submitted to perfusion.

***2.4 Bacterial adhesion to graft tissues***

Tissue pieces after O/N coating with human plasma were perfused in an in-house designed chamber at a shear rate of 1,000 s-1 for 10 min at RT with bacterial suspensions of 107 CFU/mL (verified by CFU counting) in PBS according to a modified protocol recently reported in detail 14. In brief, after perfusion, tissues were washed in PBS under flow and transferred to a tube containing 1 mL of 0.9 % NaCl. Samples were sonicated for 10 min to detach adherent bacteria using a sonication bath (VWR Ultrasonic Cleaner; VWR, Radnor, Pennsylvania). After serial dilutions 100-µL bacterial suspensions were spread onto Mueller-Hinton blood agar plates and incubated O/N at 37 o C to count colony forming units (CFUs).

***2.5 Fibrinogen binding to graft tissues***

Prepared graft tissues were incubated with 200 µL of human albumin for 2 h at 37 oC in a 96-well microtiter plate. After rinsing with PBS, tissue pieces were incubated with 100 µL of fluorescent human Fg (Alexa Fluor 488 conjugate, Invitrogen) diluted in human albumin (30 µg/mL) for another 2 h at 37 oC. Then, after 3 washes with PBS for 5 min., each circular tissue piece was placed up-side-down (treated surface downwards) on a single drop of antifade mounting medium (ProLong Diamond, Invitrogen) in a 6-well plate (Thermo Scientific Nunc). Images of samples were acquired using a high-throughput fluorescence scanner (InCell Analyzer 2000, GE Healthcare Life Sciences, Pittsburgh) using FITC excitation and emission parameters. Respective tissues incubated with human albumin without Fg served as an internal control of a background fluorescence. The absolute (auto)fluorescence units were quantified using ImageJ to express the degree of Fg binding as a relative signal to the internal control (fold change).