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Staphylococcus aureus adhesion to tissues used for RVOT reconstruction under static and shear stress conditions

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Introduction: Patients after repair of congenital heart disease (e.g. Tetralogy of Fallot) frequently develop dysfunction of the right ventricular outflow tract (RVOT). Implantation of cryopreserved pulmonary homografts, bovine jugular valved conduits or stent-mounted valves are often used for reconstruction. This improves patients' quality of life but inherits an increased risk of infective endocarditis (IE) often caused by *Staphylococcus aureus*. In this work, we focus on the susceptibility of different graft tissues to *S. aureus* adherence.

Methods: Standardized tissue pieces prepared as for clinical use (cryopreserved pulmonary homograft, bovine jugular venous conduit and pericardium patch) were mounted in a 6-well plate and incubated for 1h at 37^{o}C with 10^{7} CFU/mL of *S. aureus* Cowan (labelled with carboxy-fluorescein) for static adhesion and adherence under laminar shear stress of 10 dyne/cm^{2} in a flow chamber. Bacterial adhesion was confirmed using the fluorescence microscope IN Cell Analyzer 2000 (GE Helathcare) and quantified by CFU count on blood agar plates (expressed as Mean Log CFU/mL ± SD). Tissue pieces were sonicated in 1 mL of 0.9% NaCl for bacterial detachment and serial dilution were spotted on blood agar plates. Immunohistochemistry was also performed in fixed tissue pieces.

Results: Using the IN Cell Analyzer 2000 we visualize equal bacterial attachment to all tested tissue surfaces. The graft tissues showed similar susceptibility for bacterial adhesion in static condition with an average of 3.52 ± 0.31 Log CFU/mL (*P>0.05*, one-way ANOVA). Moreover, the shear stress increased significantly bacterial adhesion for all graft tissues (4.81 ± 0.01 Log CFU/mL; *P<0.05*, one-way ANOVA) although there was no difference among them (*P>0.05*, one-way ANOVA). The immunohistochemistry revealed a "single-cell like" bacterial attachment in static conditions and a "cluster like" pattern under shear stress.

Conclusions: Our results indicate that all graft tissues tested have similar susceptibility to be colonized by *S. aureus*. The important influence of shear stress in bacterial adhesion has been verified. It seems that the tissue surface itself has no significant influence on bacterial adhesion, however it might modulate endothelial cell adhesion and inflammatory responses that are crucial for IE development. Our following studies will focus on these aspects.