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Novel method to quantify bacterial adhesion to endovascular surfaces in vitro.

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Introduction: *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus sanguis* are frequent causes of native-valve infective endocarditis (IE). In addition, *S. aureus* is also implicated in about 50% of prosthetic-valve IE cases. In this work we present a novel *in vitro* method to quantify bacterial adhesion to heterologous bovine pericardium patch (PCP) used in congenital heart defects repair, in both static and shear stress conditions.

Methods: Tissue pieces (10 mm diameter) prepared as for clinical use were mounted in a 6-well plate, and incubated for 1h at 37^{o}C with 10^{7} CFU/mL of *S. aureus* Cowan, *S. epidermidis* ATCC 149900 and *S. sanguis* NCTC 7864 (labelled with carboxyfluorescein) for static adhesion. Similar tissue pieces, bacteria and bacterial inocula were used to study bacterial adherence under laminar shear stress of 10 dyne/cm^{2} in a newly developed flow chamber. After incubation, bacterial adhesion was confirmed using the fluorescence microscope IN Cell Analyzer 2000 (GE Helathcare) and the tissue pieces were sonicated in 1 mL of 0.9% NaCl for bacterial detachment and quantification by CFU count on blood agar plates (expressed as Mean Log CFU/mL ± SD).

Results: Using the fluorescence system IN Cell Analyzer 2000 it was possible to visualize bacterial attachment to PCP tissue surface. The different bacterial species showed similar adhesion capacity to the PCP tissue in static conditions, presenting in average 3.79 ± 0.10 Log CFU/mL (*P>0.05*, one-way ANOVA). Additionally, shear stress significantly increased bacterial adhesion (average of 5.02 ± 0.47 Log CFU/mL; *P<0.05*, one-way ANOVA) while there was no difference among bacterial species adherence (*P>0.05*, one-way ANOVA).

Conclusions: The IN Cell Analyzer 2000 system demonstrated to be the most efficient fluorescence method to visualize bacterial adhesion to PCP surface, overcoming the PCP auto-fluorescence issue due to glutaraldehyde crosslinking using other fluorescence microscopy techniques. In this work we were able to establish a novel and reliable method to quantify in vitro bacterial adhesion to endovascular surfaces. Although the bacterial species tested present different adhesion mechanisms and propensity to colonize these surfaces, in our results they presented similar capacity to attach to PCP tissue. In addition, shear forces increased bacterial adhesion.