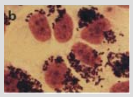
Heart valve tissue-engineered matrices attenuate monocyte binding and procoagulant responses in human endothelial cell cultures exposed to *S. aureus, S. Sanguis and S. epidermidis.*

Heying, R. (1,4), Eyskens B. (4), Cools B. (4), Wolf C. (2), Beekhuizen H. (3), Jockenhoevel S. (5), Hoylaerts M.F. (6), Schmidt K.G. (1), Schroten H. (2,7)

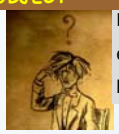
Department of Pediatric Cardiology (1), Pediatric Infectious Diseases, Department of General Pediatrics (2), University Children's Hospital, Duesseldorf, Germany, Department of Infectious Diseases, Leiden University Medical Center, The Netherlands (3), Department of Pediatric Cardiology, UZ Leuven, Belgium (4), Department of Applied Medical Engineering, RWTH Aachen, Germany (5), Center for Molecular and Vascular Biology, KU Leuven, Belgium (6), Pediatric Infectious Diseases, Department of Pediatrics, University Hospital Mannheim, Heidelberg University, Germany (7)

BACKGROUND



- Infective endocarditis (IE) remains a clinical challenge. Bacterial metastasis involves the preferential interaction of disseminating bacteria with endothelial cells and monocytes leading to subsequent endothelial inflammatory and procoagulant activation.

SUBJECT



Does the fibrin or collagen gel matrix on which endothelial cells are cultured influence monocyte binding and endothelial procoagulant activation?

METHODS / TISSUE ENGINEERED HEART VALVES

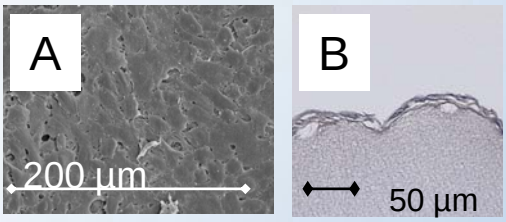
Goal of tissue – engineering:

- autologous valves, improved compliance
- decreased risk of complications
- fibrin and collagen gel are used as a basic matrix

Experiments:

- Human venous endothelial cells (ECs) were isolated with 0,2 % collagenase.
- ECs were cultivated on the two gels and polystyrene tissue culture plates as a control matrix with a cell density of 2 x 10^5 cells/cm^2.
- Probability of infection (infection assay), adhesion molecules (FACS analysis), monocyte adhesion and tissue factor dependent coagulation (FXa assay) were measured.

RESULTS



Endothelial cell outgrowth on fibrin gel
Scanning electron microscopy image (A) and HE staining (B) demonstrating a confluent endothelial cell layer without any cell migration into the gel.

RESULTS

- *S. aureus* exhibited a similar pattern of EC infection when seeded on collagen and fibrin gel with 3.7-4.5 % of the inoculum remaining bound, i.e. considerably higher than for control ECs: 1.5 % of the inoculum bound ($p < 0.01$).

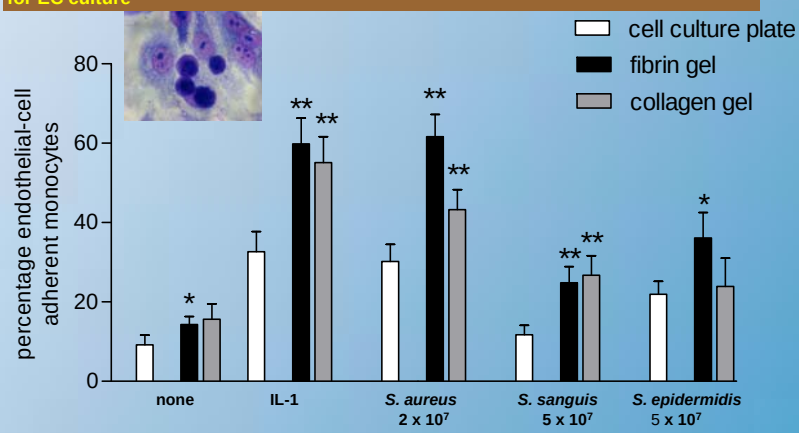
- *S. sanguis* and *S. epidermidis* were less potent infectants of ECs (0.6 – 1.3 % of the inoculum bound) grown on the gel matrices.

- In association we found higher monocyte adhesion on ECs infected with *S. aureus* (61% on fibrin and 43% on collagen) than in the control cultures (30%, $p < 0.01$), even when the EC surface expression of ICAM-1 and VCAM-1 remained comparable. Moderate monocyte adhesion was seen upon infection with *S. sanguis* and *S. epidermidis* for both gel matrices. Data are shown in Figure 1.

- Despite increased monocyte adhesion, monocytes do not enhance pro-coagulant activity when ECs cultured on the gel matrices. In contrast, a marked increase in tissue factor mediated coagulation activity was seen on tissue culture plates. Data are shown in Figure 2.

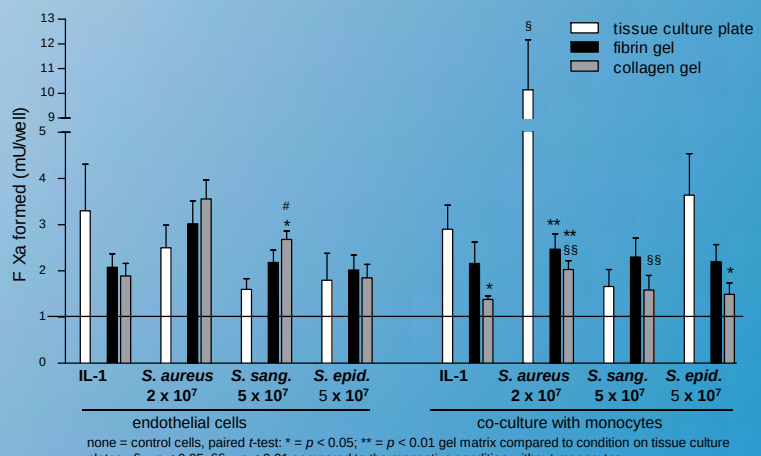
- The collagen matrix attenuated the *S. aureus* induced MCP-1 expression 2.0 fold, compared to control ECs. This reduction prominently coincided with a 4.2-5.0 fold reduction in the procoagulant activity.

Figure 1: Monocyte adhesion after bacterial contact influenced by the matrix used for EC culture



none = control cells, paired *t*-test: * = $p < 0.05$; ** = $p < 0.01$ gel matrix compared to condition on tissue culture plates;

Figure 2: Endothelial tissue factor activity after bacterial contact influenced by the matrix used for EC culture



none = control cells, paired *t*-test: * = $p < 0.05$; ** = $p < 0.01$ gel matrix compared to condition on tissue culture plates; § = $p < 0.05$; §§ = $p < 0.01$ compared to the respective condition without monocytes

CONCLUSION

Fibrin and collagen gel matrices serving as a basic structure for tissue-engineered heart valves equally increase bacterial adhesion and subsequent monocyte binding to infected ECs. In contrast, these matrices modulate EC responses to these stimuli, resulting in attenuated cytokine production and attenuated adherent monocyte-dependent tissue factor production by ECs. Further investigations will need to confirm that also in vivo, EC-matrix interactions can attenuate EC responses to bacteria and inflammatory cells to reduce IE at infected endovascular sites.