

## New non-degradable polyurethane scaffolds for aortic valve tissue engineering

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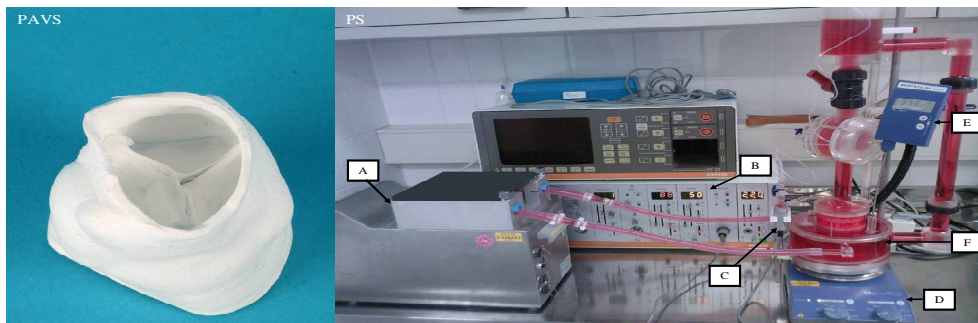
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### Introduction

Currently available heart valve prostheses comprise mechanical, biological and homograft components which are associated with a number of limitations, such as their thrombogenic potential, unfavourable hemodynamic properties, little durability and lack of availability [1, 2]. Tissue engineering of heart valves has been proposed to be the ultimate treatment solution by both clinical and experimental medicine [3-5]. The purpose of this study was to evaluate the cell colonization efficiency and the cell adhesion of human vascular cells seeded on newly designed polyurethane aortic valve scaffolds (PAVS) with similar dimensions as nature human aortic valve under pulsatile flow conditions.

### Material and methods

Human vascular fibroblasts (FBs) and endothelial cells (ECs) were isolated from saphenous vein segments and expanded in culture. PAVS (Fig. 1, n= 4) were primarily seeded with human FBs (mean  $56.3 \pm 6.8 \times 10^6$  cells) for  $3 \pm 0.5$  days and secondarily seeded with FBs (mean  $55.4 \pm 7.18 \times 10^6$  cells) for  $8 \pm 1$  days, followed by a colonization with ECs (mean  $80.2 \pm 22 \times 10^6$  cells) for  $9.25 \pm 1.75$  days. Seeded PAVSs were then exposed for 24 hours to pulsatile flow ( $V= 0.8$  L/min,  $P= 40$  mmHg,  $n= 60$  RPM) in a closed perfusion system (Fig. 1) filled with M-199 medium (Cell culture medium, Biochrom AG, Berlin, Germany). The temperature (mean  $37.1 \pm 0.4$  °C) was controlled and regulated simultaneously with a hot plate and a temperature sensor (IKA Works, Staufen, Germany).



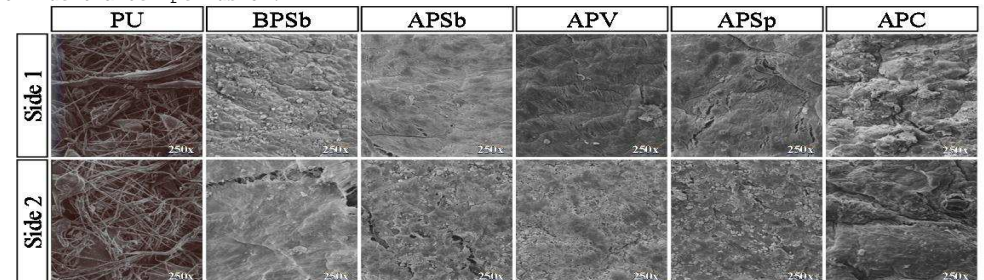
**Fig. 1** PAVS: Polyurethane Aortic Valve Scaffolds, PS: Perfusion system, A: pulsatile roller pump (Stoekert, Munich, Germany), B: pressure monitor (Siemens, Munich, Germany), C: pressure transducer (GOULD Statham, CA, USA), D: hot plate (IKA Works, Staufen, Germany), E: temperature sensor (IKA Works, Staufen, Germany), F: Bioreactor.

Samples were taken before (subvalvular part of the aorta) and after perfusion (cusp, subvalvular, valvular and supra- valvular part of the aorta) and evaluated by scanning electron microscopy (SEM) and immunohistochemical staining (IHC).

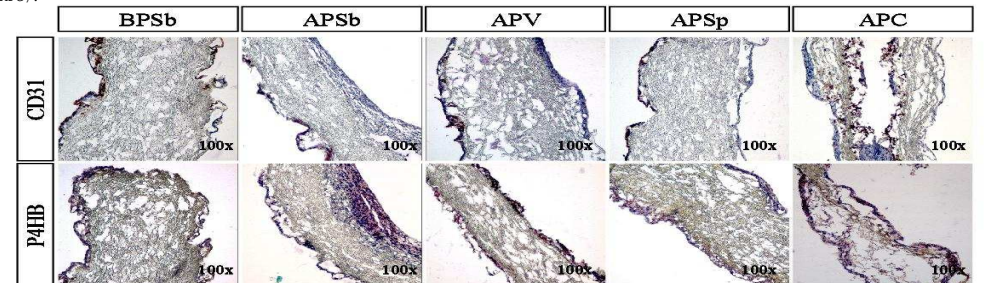
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### Results

SEM results showed a confluent cell layer on all PAVSs before perfusion (Fig. 2). IHC staining with CD31 (EC-Antibody, DAKO GmbH, Germany) and P4hb (FB-Antibody, Acris Antibodies GmbH, Germany) revealed a positive reaction of both cell layers before perfusion, whereas no or little CD31-positive reaction could be observed after perfusion (Fig. 3). ECs were not able to resist to 24h shear stress and detached from the PAVS. The FBs-layers were still confluent after perfusion.



**Fig. 2** SEM, magnification: 250x, native PU scaffolds (PU), scaffolds seeded with FBs and ECs before perfusion subvalvular part of the aorta (BPSb) and after perfusion: subvalvular (APSb), valvular (APV), supra- valvular (APSp) part of the aorta and cusp (APC).



**Fig. 3** IHC, peroxidase reaction, magnification: 100x, staining of PU scaffolds seeded with FBs and ECs with CD31 and P4hb before perfusion subvalvular part of the aorta (BPSb) and after perfusion: subvalvular (APSb), valvular (APV), supra- valvular (APSp) part of the aorta and cusp (APC).

### Conclusion

After 24h perfusion, ECs detach from tissue-engineered cardiovascular prostheses pre-seeded with human FBs. FBs remain their ability to react to shear stress and maintain their confluence. ECs adhesion can be increased by splitting the ECs-colonization into two steps and/or by pre-conditioning the seeded PAVSs with low flow conditions and gently raising the flow up to the required conditions.