

ONLINE APPENDIX

Online Supplementary Figure 1. Schematic diagram showing all the steps and techniques performed in the study.

Isolation of T-MSCs

- Digestion of newborn pig thymus
- Expansion of the obtained cell line
- Characterization of the T-MSCs by:
 - Flow Cytometry
 - Differentiation into three mesodermal lineages
 - Cytokine secretion
 - Interaction with cardiomyocytes



Engineering of the SIS-ECM

- Seeding onto the scaffold
- 2 weeks culture under static and dynamic conditions
- Characterization of the graft
 - Cell attachment (SEM and Histology)
 - Cell viability (Live/Dead Assay)
 - Mechanical properties (Tensile Testing)



In vivo implantation

Seeded or unseeded grafts were used to reconstruct the RVOT of a growing swine



Recovery of the animal

- 24-48 hours intensive care and 4.5 months of follow-up
 - Animal weight
 - Echocardiography
 - Cardiac magnetic imaging



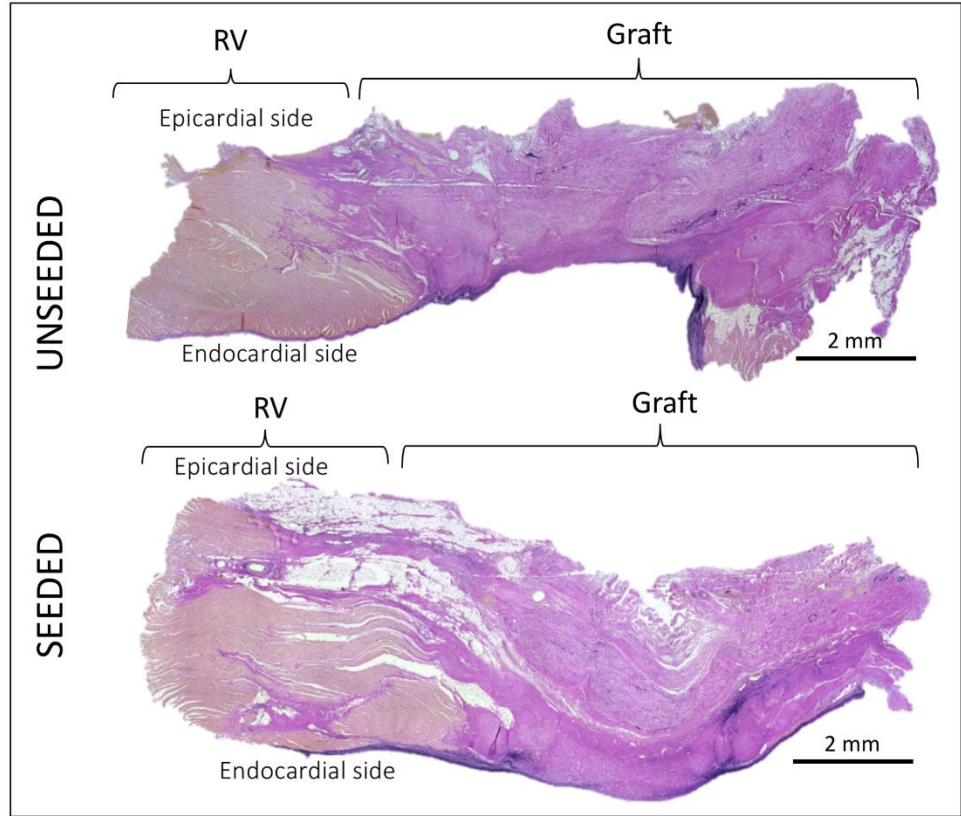
Termination

- Harvesting of the implants for further analysis
 - Endothelialisation (SEM/Histology)
 - Cardiac regeneration (Histology, IF)
 - Vascularisation (IF)
 - Fibrosis (Histology, IF)

Online Supplementary Figure 2. Histological stains of the unseeded and seeded grafts. (a) Elastic van Gieson stain shows a collagen-rich tissue that constitute the graft side of the explants. The endocardial side also developed an endothelium containing a layer of elastin. (b) Von Kossa stain demonstrates lack of calcification in both the unseeded and seeded samples.

a

Elastin van Gieson



b

Von Kossa

