The use of equine decellularized extracellular matrix in producing hydrogels for corpus spongiosum tissue engineering

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Introduction
In males, a large part of the urethra is surrounded by the vascular corpus spongiosum, providing the urethra with nutrients and protection. In current reconstructive surgery for hypospadias or urethral stricture disease, the urethra is created from epithelial buccal mucosa or foreskin, without any support of the corpus spongiosum. There is no autologous tissue available mimicking the corpus spongiosum (CS). For tissue engineering purposes, hydrogels with different compositions are used to grow cells.
Our ultimate goal is to create a combination of a vascular corpus spongiosum with functional mucosa to restore the urethra.

Purpose:
The aim of this study is to generate hydrogels from native urethral and spongious tissue decellularized extracellular matrix (dECM) combined with silk fibroin in different ratios, for support. In this hydrogel, microvessel forming cells can be incorporated and epithelial cells can be seeded on top.

Study design, materials and methods
Tissue was obtained from deceased horses that had been donated to science by their owners in the veterinary hospital (waste material). From fresh corpses, the penis was dissected and transported to our institute, where it was frozen until further dissection. The urethra and CS were isolated and decellularized using sodium dodecyl sulfate, followed by pepsin digestion. Gels were tested on their sol fraction and swelling ratio and were taken into *in vitro* cell assays and *in vivo* Chick Chorioallantoic Membrane (CAM) assays.

Results
The isolated horse CS hydrogel showed low DNA content and abundance of ECM proteins as expected. In general, the hydrogels showed heterogenic crosslinking and high auto-fluorescence, which resulted in difficult analysis.
The hydrogels supported cell growth and cells were not able to grow on silk fibroin only (missing dECM, **Figure 1**).

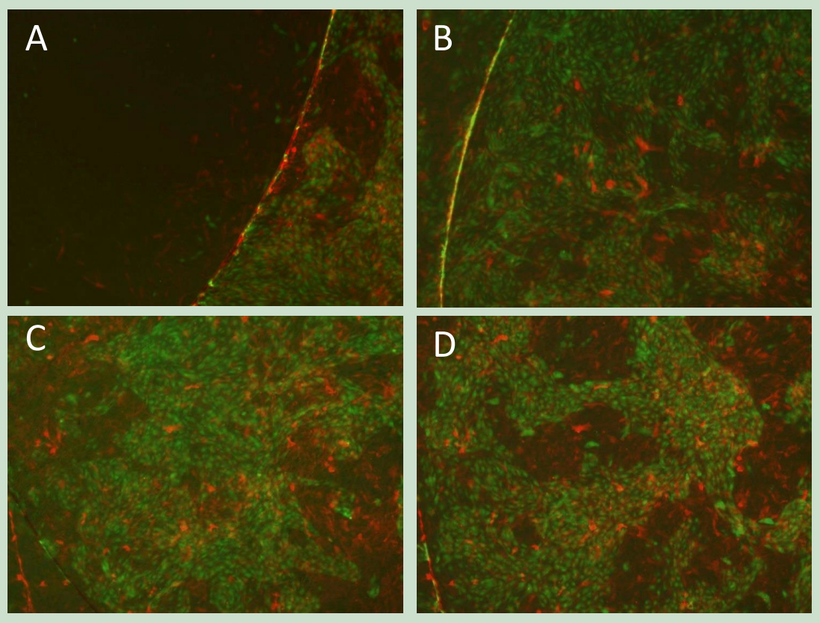

Figure 1: Cell compatibility with hydrogel components. Co-cultures of cells (HUVEC-GFP (green) and pericyte-DsRed (red)) on coverslips coated with the non-crosslinked gel solution, missing component: A) dECM, B) silk fibroin, C) riboflavin and D) SPS. No cell growth was observed in A (missing dECM)

Hydrogels grafted on the CAM showed survival of the chick embryos CAM vessels grow towards the hydrogel **(Figure 2)**. Vasculogenic index of the silk fibroin : dECM hydrogels showed higher counts compared to gelatin control gels, however, this was not significant.

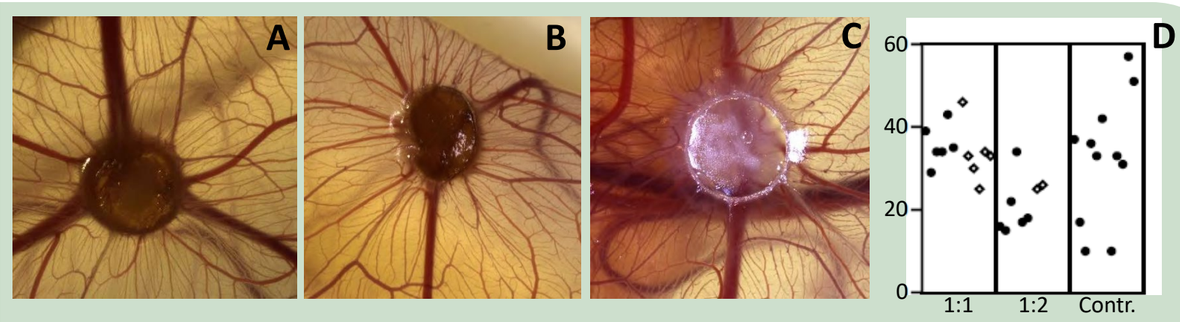

Figure 2: Hydrogels with surrounding vasculature on CAM. Ratio silk:dECM 1:1 (A) and 1:2 (B) and Gelatin (C), were photographed and analysed for vasculogenic index (D). No significant difference was found between the 1:1, 1:2 and control (gelatin) gels.

Discussion
Hydrogels could be formed from dECM from equine CS and support cell growth. Cells display less stress fibers when grown on dECM coating compared to gelatin, indicating the created niche is favored by the cells. In the CAM assay, the vessels of the CAM grow towards the hydrogel, showing vasculogenic properties of the hydrogel. Limitations in this study are that the biomechanical properties were hard to control. In addition, the gels were highly auto fluorescent, making them hard to analyse.

Conclusion:
Our Equine dECM : silk fibroin hydrogels showed good properties for gelation and endothelial and epithelial cell growth. However, the hydrogels were heterogenic and auto-fluorescent. This complicates cell biological analysis. We believe native dECM can potentially enrich hydrogels for better corpus spongiosum and urethral tissue engineering once the technical complications have been solved.

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