Gel casting as an approach for tissue engineering of multilayered tubular structures: Application for urethral reconstruction

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Background
Our group is interested in tissue engineering for urethral reconstruction performed in patients with urethral strictures or congenital disorders. As the corpus spongiosum (CS) is an integral part of the urethra and important in supporting the function of the urethra, tissue engineering of the urethra should be combined with reconstruction of the CS [1]. We showed that the CS is composed of a three-layered, highly vascularised structure with distinct distribution of extracellular matrix (ECM) components (Fig. 1A). Currently there is a lack of tissue-engineered solutions for replacement/regeneration of urological tissues, like ureters and the urethra/CS. Such tissues present a complex tubular organisation with different cell layers. Tissue engineering of complex, clinical relevant tubular constructs is not possible yet. Here we propose an innovative gel casting approach to engineer multilayered tubular constructs based on fibre-reinforced hydrogels to generate a CS tissue construct which mimics the structure/organisation of native tissue.

Methods
A mold with three chambers, representing the three layers of the CS (Fig. 1B), was designed, and fabricated using polydimethylsiloxane (PDMS) molding [2]. The chambers were loaded with gelatin-transglutaminase hydrogels (mTG gel) containing a coculture of endothelial cells and pericytes (layer 1 and 3) and smooth muscle cells (SMCs, layer 2). A melt-electrospun poly(caprolactone) (PCL) fibre mesh was incorporated at the base of the construct to serve as a porous support for the gels and to roll the construct into a multilayered tubular construct (Fig. 1C). The hydrogels were mechanically tested and compared to native tissue (equine urethra).

Results
The mTG gel can be successfully casted and rolled (Fig. 3A-B). The encapsulated cells were cultured up to 2 weeks and showed good cell viability (Fig 2A-C) and functionality. Within 2 weeks little capillary-like structures were formed in layer 1 and 3 (Fig 2D-F) and the SMCs express elastin (Fig. 3D). The compressive modulus of native tissue was similar to the mTG gel (Fig. 1B).

Conclusions
Our novel gel casting approach enables to construct tubular structures with distinct composition per layer. Cell survival up to 2 weeks has been achieved as well as functionality. Within 2 weeks, little capillary-like structures have been formed and SMCs express elastin. Furthermore, the compressive modulus of mTG gel is within the same range as native tissue. This approach towards tissue engineering of multilayered tubular structures may be applicable to the urological field (to help engineer ureters or urinary diversions), as well as in other fields of soft tissue engineering.

References:
2. WO 2013/085404 A1