Student project for collaboration between the Biofabrication facility and the Experimental Urology
(supervision Petra de Graaf, Urology (daily supervision) and Miguel Casthillo, Orthopeadics/Biofab)

Background and current status of the project:
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 organization with different cell layers. Our group is interested in tissue engineering for urethral reconstruction purposes, 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 have shown that the CS is a three-layered, highly vascularized structure with distinct distribution of extracellular matrix (ECM) components (manuscript in preparation). Biofabrication of clinical applicable tubular constructs is not possible yet. Therefore, we hypothesize that by an innovative casting approach to build multilayered tubular constructs, tissue engineered CS constructs can be generated that mimic the structure/organization of native tissue. The casting approach is based on micro-fiber reinforced hydrogels,
Based on an existing patent we have fabricated a mold with three chambers, representing the three layers of the CS using stereolithography and polydimethyl-siloxane (PDMS) molding [2]. The chambers were loaded with gelatin-based hydrogels containing endothelial cells and pericytes. A melt-electrospun poly(caprolactone) (PCL) fiber mesh was placed 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. This mold makes it able to engineer tubular structures with distinct composition in each distinct layer. 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.
Future perspectives:
We now like to vary the composition of the different chambers to generate a multilayered structure. In the first smallest chamber we need a microvascular network, for the second chamber elastic characteristics are required. Lastly, the largest chamber, corresponding to the spongy tissue in the corpus spongiosum, would need an open porous structure in the hydrogel. Lastly, we like to roll the construct and dynamically culture this construct, eventually with epithelial cells in the lumen of the construct.
Techniques used in the internship are molecular cell biology techniques like cell culture (3D in hydrogels, dynamic culturing), immunostaining, microscopy, western blot and biofabrication techniques, like biomechanical testing, electrospinning, PDMS molding, hydrogel casting, stereolithography

We are looking for either a Biofabrication master student with affinity for cell culture, or a Regenerative Medicine and Technology master student with affinity for biofabrication techniques. For more information, please contact Dr. Petra de Graaf (p.degraaf-4@umcutrecht.nl).

References:
[1] de Kemp, V., et al. (2015). Tissue engineering for human urethral reconstruction: systematic review of recent literature. PloS one *10*, e0118653
[2] WO 2013/085404 A1