Pathophysiology of pelvic organ prolapse:

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GENERAL SUMMARY

Pelvic organ prolapse (POP) remains a great therapeutic challenge to urogynaecologists, gynaecologists and urologists worldwide as it is a common condition with no optimal treatment.

The most common kind of POP within Caucasian and Latin women is cystocele, *e.g.* the prolapse of the bladder. The connective tissue layer of the anterior vaginal wall keeps the bladder in place. This tissue is made of a dense extracellular matrix (ECM) that is constantly made and remodelled by fibroblasts. Tissue strength depends on a homeostasis between matrix production and degradation. This homeostasis seems to be lost in the weak tissues of patients with POP as they have been shown to have altered ECM composition and mechanical properties. Nevertheless little is known about the role of fibroblasts and their interactions with the ECM in the development and progression of POP. The aim of this thesis is to gain an insight into the pathophysiology of POP by identifying differences between cell behaviour, matrix composition, and cell-matrix interactions in human material derived from women with and without POP. It was hypothesized that most patients with prolapse acquire defects in cell-matrix interactions as a consequence of POP.

The hypothesis was tested using a multi-parameter study approach. In **chapter 2**, I presented an *in vitro* dynamic model using a continuous physiological stretching regimen mimicking respiration, which continuously loads the vaginal wall, to study vaginal fibroblasts mechanoresponses to synthetic collagen I coated and uncoated substrates. It was found that fibroblasts from two prolapsed tissues had lower mechanoresponses than cells from the control tissue, as shown by delayed cellular alignment on collagen I coated plates and lower secretion and activation of matrix metalloproteinase-2 (MMP-2) on uncoated plates. In **chapter 3**, vaginal fibroblasts functional characteristics were evaluated using the model from **chapter 2** and a contractility assay. Fibroblasts were derived from a very strict patient cohort including only Caucasian premenopausal women with and without cystocele to study the effect of POP while ruling out the effects of ageing. Moreover, to distinguish between acquired and intrinsic defects we also included biopsies from prolapsed and non-prolapsed tissues within the same women so that each patient was her own control. Results showed that fibroblast-mediated collagen contraction was lower in cells derived from prolapsed tissues compared to non-prolapsed tissues. Furthermore, fibroblasts from prolapsed tissues also secreted less MMP-2 on collagen I and uncoated plates than cells derived from non-prolapsed tissues. Mechanoresponses to mechanical loading on uncoated plates were also different as activation of MMP-2 was less pronounced in cells from prolapsed tissues. Together, the results from **chapters 2 and 3** showed that fibroblasts from women with POP have less contractile capacities and lower mechanoresponses than cells derived from non-prolapsed tissues and especially on uncoated plates. It was found that surface substrate affects cell behaviour, and that cell–matrix interactions seem to be impaired in fibroblasts from prolapsed tissues. Moreover, since no differences were found in any of the evaluated functional characteristics between cells derived from non-prolapsed tissues in women with and without prolapse (**chapter 3**), these defects seem to be acquired rather than intrinsic.

In **chapter 4** we also found acquired defects in the matrix composition of the prolapsed tissues compared to non-prolapsed tissues. Tissue biopsies from the same patient cohort as in **chapter 3** were compared histologically and biochemically, and as with cellular functionalities, no differences were found between non-prolapsed tissues in women with and without POP. Results showed increased pyridinoline collagen cross-links, higher quantities of smooth muscle cells and a tendency towards a higher amount of collagen III and elastin in the prolapsed tissues, compared to the non-prolapsed tissues within the same patient. Since most patients with prolapse that were included in the patient cohort from **chapters 3 and 4**, in **chapter 5** a microarray analysis was performed to identify specific biological molecular pathways related to POP. A cluster analysis revealed that most of the genes that showed a dysregulation were clustered in two molecular pathways: (i) "the ECM/integrin pathway cluster", or (ii) "the muscle cell/contraction pathway cluster". These results provide evidence for inter-individual differences in non-prolapsed and prolapsed tissues implying different possible failure mechanisms in different women leading to POP. In fact, the group of patients that showed an increase in smooth muscle (desmin-positive) cells by immunohistochemistry (**chapter 4**) were part of the "muscle contraction group". Therefore, women with prolapse showed evidence of two different compensatory mechanisms to adapt to physiological changes that could affect tissue adaptation in response to external stimuli such as mechanical loading.

The effect of prolapse in vaginal fibroblasts matrix production and remodelling is still unknown. Since most patients with prolapse are postmenopausal women, in **chapter 6** I investigated the effects of POP and menopausal status in the capacity of vaginal fibroblasts to produce and remodel matrix *in vitro*. It was found that POP decreases the quality of the matrix produced by vaginal cells. Affected fibroblasts from postmenopausal prolapsed tissues produced stiffer matrices with high collagen content and fibres with less anisotropic orientation than those produced by controls. Similarly to normal wound repair, there was a transient increase in myofibroblastic phenotype that was lost after the peak of tissue remodelling. These data suggest that the altered matrix production in prolapsed tissues does not appear to be a consequence of abnormal phenotypical changes towards the myofibroblastic lineage.

However, these results were obtained from cells cultured on artificial substrates. Therefore in **chapter 7**, I proposed a novel cell culture system using decellularized anterior vaginal wall tissues to study cell-matrix interactions in a disease-specific manner. The effects of POP and non-POP tissues in the phenotype transition to myofibroblast of fibroblasts derived from POP and non-POP tissues, were studied. In this pilot study we successfully used decellularized vaginal wall tissues as *in vitro* culture systems to study cell-matrix interactions in POP. Fibroblasts survived and migrated into the exogenous decellularized matrices with control cells attaching better than POP cells. The stiffer muscularis layer of the decellularized matrices induced myofibroblast differentiation of POP, but not of control fibroblasts, and was higher in the prolapsed matrices. Fibroblast to

myofibroblast differentiation seems to be altered in cells from prolapsed tissues and dependent on the origin of the extracellular matrix encountered.

Taken together, the results from this thesis provide evidence of acquired changes in cell behaviour, tissue composition and cell-matrix interactions in Caucasian women with pelvic organ prolapse of the bladder. We further identified two different molecular pathways that might have been part of the tissue adaptation process after POP. The acquired cellular defects seemed to be permanent as they were observed even after the cells were cultured for several passages *in vitro*.

This new knowledge has implications in clinical management for POP as well as for the development of new treatments. In particular, it gives hope for the use of autologous cell-based tissue engineering for pelvic floor repair since this treatment option would not be feasible in women with a genetic disease. Newly developed therapies should be tested in disease-specific models and should aim to restore not just the anatomical function but also optimize the local environment of the pelvic floor to re-establish proper functioning of the supportive tissues and the cells involved.