Pelvic Organ Prolapse

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SUMMARY

Pelvic organ prolapse (POP) is a common multifactorial disease, affecting adult women of all ages. It decreases their quality of life considerably. In general, a prolapse is the result of mechanical failure in the pelvic floor connective tissues that support and suspense the abdominal and pelvic organs. For decades it has been speculated that a structural defect in the vagina or its supportive tissues predisposes women to POP. These changes can be in collagen content and collagen subtypes, changes in amount and quality of elastin, or in smooth muscle cells. Also the functionality of the vaginal fibroblasts may play a role in the pathophysiology of POP. These fibroblasts are the mechanosensitive cells responsible for maintaining extracellular matrix (ECM) homeostasis. To date is has been impossible to determine the cause-effect relationships in the development of POP. Problems could be induced by changes in the connective tissue, that are in turn induced by mechanical loading or stretch in the damaged vaginal tissue in patients with POP (acquired). Also, it could be that a structural defect of the fibroblasts contribute to changes in the functionality of the ECM (intrinsic).

POP is one of the most common reasons for gynecological surgery in women after the fertile period. The failure rate of native tissue repair is relatively high, though. In an attempt to improve surgical outcomes, synthetic meshes and biological grafts have been introduced in reconstructive pelvic surgery for repairing POP. Synthetic meshes significantly reduce POP recurrence compared with no mesh. However, in contrast to this clinical success rates, mesh exposure and dyspareunia are reported. Biological grafts appear to be insufficient for lack of support. Therefore, an alternative approach for reconstruction of the pelvic floor is urgently needed. This alternative could be sought in tissue engineering. It could provide attractive alternatives alone or as an adjunct to surgical reconstructive procedures in the pelvic floor and more specifically the anterior vaginal wall. Therefore we need additional information about the changes we see in the connective tissue and the functionality of the fibroblasts in women with POP: are they due to an intrinsic or an acquired defect?

In this thesis we seek to evaluate the composition of the anterior vaginal wall tissue and to evaluate the functionality of the fibroblast. This could help us to better understand the pathogenesis of the disease and to determine which kind of strategies can be used in the reconstruction of the pelvic floor, based on a tissue engineered construct.

After a general introduction in **chapter 1**, in **chapter 2** we provide an overview on the current literature on the connective tissue in women with POP. We evaluate literature in the light of an observation by Jackson in 1996 (Jackson, The Lancet 1996) that patients with uterine prolapse and cystocele have a reduced collagen content, with a relatively high content of immature collagen cross-links compared to non-POP patients. This newly formed collagen is degraded more easily than older, glycated tissue, by an increase in metalloproteinase activity, resulting in a decrease of collagen content. The old, glycated collagen that is left behind, results in brittle tissue with an impaired mechanical strength. This could be an important aetiologic factor in POP. Jackson found no change in the type I to type III collagen ratio. Most other studies, however, found an increase in type III or a decrease in type I, resulting in a decreased I/III ratio. This increase in collagen type III, together with an increase in enzyme MMP-9 is a characteristic of tissue that is remodeling after injury or accommodating to a progressively increasing mechanical load. Also, the functionality of the fibroblasts seems to be involved in the pathophysiology of POP, with less contractile capacity. This causes changes in interaction between the vaginal fibroblasts and the extracellular matrix. Despite numerous shortcomings in the available literature, the hypothesis of Jackson could still be valid. To date the aetiology of POP is not elucidated yet. More important, the question whether the changes seen in the tissue and the fibroblasts is due to an intrinsic or an acquired defect is not answered.

In order to shed light on the important question of cause and effect, we introduce a novel research approach in POP, in which not only the tissue of premenopausal age-matched women without POP, but also the unaffected tissue of a patient is the control for her prolapsed tissue. Biopsies were taken from the prolapsed anterior vaginal wall (POP site) and from the precervical non-prolapsed vaginal wall (non-POP site).

In **chapter 3** we describe the histological and biochemical features of the different components of the extracellular matrix. Our results show that the non-POP tissues in the vaginal wall of patients do not differ in any of the parameters evaluated from the same tissue in healthy, age- and parity matched controls. This indicates that for these parameters the non-POP precervical vaginal wall of the women with cystocele may be considered as a true control. We did observe marked differences between the prolapsed tissues of a POP-patient, compared to non-prolapsed tissues of the same patient. There was a significant increase in mature pyridinoline cross-links in collagen molecules, and in the number of smooth muscle cells in the muscularis layer of the anterior vaginal wall. These findings suggest that the

changes in connective tissue of the prolapsed vaginal wall are an acquired effect, rather than an intrinsic defect in the connective tissue.

We hypothesize that due to increased mechanical load and stretch on the cells in the vaginal tissues different molecular mechanisms come into action. In **chapter 4** we aim to identify POP related dysregulated metabolic and signaling pathways by comparing gene expression profiles of prolapsed and non-prolapsed anterior vaginal wall tissues from women with cystocele, by means of whole-genome microarrays. In this so called 'fishing experiment', statistical analysis of micro-array and a cluster analysis is executed for the analysis of the 44.000 analysed genes. In the prolapsed anterior vaginal wall, we found dysregulation of general pathways related to signal transduction and transcription. These pathways are activated by mechanical load. The analysis suggests that beside a POP specific gene expression profile, at least two clusters of genes reflect variance in molecular processes between individuals. We therefore performed an additional cluster analysis of non-POP expression profiles. This analysis indeed divided the patient group into two subgroups with reciprocal clusters of genes. One subgroup is characterised by the ECM organization, integrin-1 and collagen formation pathways. The other cluster is characterised by genes involved in (smooth) muscle contraction. We therefore hypothesize that women with a predisposition for POP show two different compensatory mechanisms to adapt to physiological changes in mechanical load. This could also imply that different failure mechanisms ultimately lead to a 'common' POP disease gene expression pattern in more advanced stages of POP. To test this hypothesis, micro-array analysis in a validation cohort would be the next logical step to take.

The vaginal wall is one of the soft tissues that is constantly being remodelled in order to withstand the different forces that are applied to it during a woman's lifetime. The weakening of the pelvic floor seen in POP could be caused by an imbalance of this remodeling. Tissue remodeling is a well-balanced process involving several factors with different roles, and different cells as modulators. In the vaginal wall, fibroblasts (FB's) are the mechanosensitive cells responsible for maintaining homeostasis in the Extra Cellular Matrix (ECM). They produce molecules, and control anabolic and catabolic processes to remodel their surrounding matrix in response to mechanical and biochemical stimuli. Compounds particularly involved in ECM homeostasis include collagens (mainly type I and III), the collagen degrading matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs). It has been shown that the amounts of active MMP-2 or MMP-9 are increased in tissues from patients with POP in comparison to controls. We were interested to

see if these matrix metalloproteinases are also increased when cells are exposed to experimental cyclic mechanical loading in vitro. Furthermore we wondered whether this enzymatic activity is affected by the presence of artificial polymeric substrates. In **chapter 5** we describe an in vitro pilot experiment to test the hypothesis that fibroblasts from women with different degrees of prolapse, display different mechano-responses depending on the substrate encountered. This was done by subjecting fibroblasts from healthy, mild and severe POP women to cyclic mechanical loading on artificial polymeric membranes, uncoated as well as coated with collagen I. Changes in morphology and anabolic and catabolic compounds that may affect the remodeling of the extracellular matrix were evaluated at protein and gene expression levels. Our results show that on collagen I coated plates, alignment of fibroblastst from severe POP patients appears delayed in comparison to their mild counterparts. Released MMP-2 is lower in fibroblasts from POP patients compared to healthy control cells. These effects seem to disappear over time. On non-coated plates, POP fibroblasts show lower responses to extracellular matrix remodeling factors at both enzymatic and gene expression levels, compared to healthy fibroblasts. The data suggest that, although fibroblasts from POP patients seem to have lower mechano-responses, in the presence of collagen I coated plates, the system eventually reaches a balance. However, it appears that when cells are exposed to artificial polymeric substrates and stress is imposed, this balance is not reached. Fibroblasts from women with POP seem preconditioned by the abnormal prolapsed matrix.

The results of chapter 5 indicate that fibroblasts of woman with POP show altered characteristics, possibly due to the changes in the extracellular matrix. Our ultimate goal is to develop new surgical treatment for pelvic organ prolapse, using tissue engineering, combining biomaterials and growth factors with unaffected autologous cells, like fibroblasts or stem cells from vaginal tissue, which could stimulate vaginal tissue repair. These new approaches can only be implemented in patients without intrinsic defects. Therefore, in **chapter 6** we aim to investigate if alterations in fibroblast functions are acquired or intrinsic in premenopausal women with POP. To answer this question POP tissue is not only compared to biopsies from non-prolapsed anterior vaginal wall tissue but also to the same tissue in healthy controls. Additionally, we aim to confirm the data of the previous pilot experiment (chapter 5) by evaluation of vaginal wall fibroblasts mechanoresponses to different substrates under static or dynamic conditions. First we prove that the cell cultures studied were actually fibroblasts. Also no differences are seen in the proliferation rate of the cells studied, suggesting that the quality and not the quantity of the fibroblast are responsible for the results. The obtained data show

that fibroblasts mechanoresponses from the non-POP site of the anterior vaginal wall from patients with prolapse do not differ in any of the parameters evaluated with cells derived from the same tissue site in healthy controls. Moreover, we show clear differences between fibroblasts derived from prolapsed and non-prolapsed tissues within individuals. Fibroblasts from the POP site show delayed fibroblast-mediated collagen contraction and lower production of MMP-2 on collagen-coated plates. Mechanoresponses to cyclic mechanical loading on noncoated plates are also different: activation of MMP-2 is more pronounced in cells from non-prolapsed tissues, whereas up-regulation of MMP-2 and TIMP-2 gene expressions are only seen in POP-site fibroblasts. This study also indicates that collagen coating promotes cell attachment and alignment and increased gene expression of the extracellular matrix remodeling factors: collagen 3α1, TIMP-2 and MMP-9. This indicates that vaginal fibroblasts are mechanoresponsive and can sense and remodel their surrounding matrix. The current study also indicates that collagen coating improves cell-substrate interactions in vitro. The results support the results obtained in the previous pilot experiment. More important these results suggest that in the majority of the patients, the prolapse condition is an acquired rather than an intrinsic defect.

In **chapter 7** the combined results of the afore mentioned chapters are discussed in a broader perspective and future directions are given.