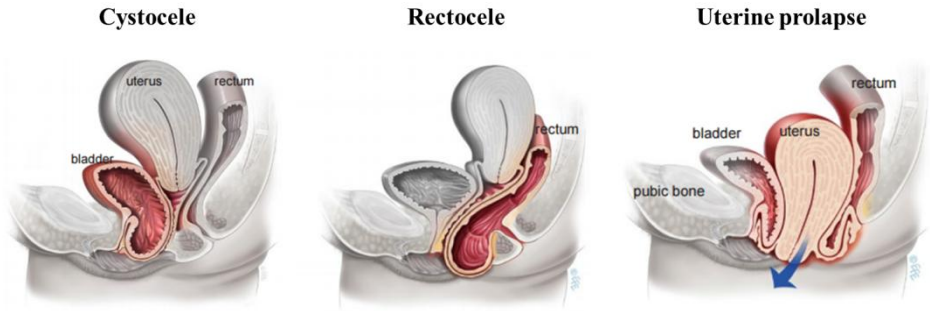
CHAPTER 1

GENERAL INTRODUCTION

Pelvic organ prolapse (POP)

Pelvic organ prolapse (POP) is a condition characterized by the weakening of the pelvic floor supportive tissues and subsequent protrusion of the pelvic organs outside the vaginal hiatus. Different kinds of prolapse can occur depending on which organ or organs protrude (Figure 1). The most common type is cystocele which occurs when the bladder prolapses outside the body as a consequence of the weakening of the anterior vaginal wall[1-3]. Weakening of the posterior compartment can lead to prolapse of the rectum (rectocele), sometimes also including the small intestines, *i.e.* enterocele. Failure of the apical compartment causes a prolapse of the uterus or vaginal vault.

Figure 1. Types of pelvic organ prolapse



Source: International urogynecology association (IUGA).

POP is associated with serious inconvenience and reduced quality of life in almost half of all women over 50 and remains a great therapeutic challenge as there is currently no optimal treatment. In an ageing population, prolapse is still one of the most common reasons for urogynaecological surgery that continuously causes a great burden to global health services. In The Netherlands alone, it has been projected that one in five women will undergo surgery for POP and/or urinary incontinence during their life time[4]. Women with POP suffer from dyspareunia, chronic pain, incontinence and social isolation[1,2].

In most cases, conservative therapies using ring pessaries or pelvic floor exercises are used. Nevertheless, almost 11% of women with POP will require reconstructive surgery[5,6], with a 10-year re-operation rate of 17%[7]. Since the 1970s, synthetic polypropylene meshes that provide good anatomic outcomes have been used to repair POP. However, the use of such meshes has been questioned due to reported irreversible problems, such as mesh exposure (18%) and erosion (11.4%)[8]. These issues lead to the safety communication warning of the US Food and Drug administration (FDA)[9]. This was a wake-up call to everyone involved in the field who now realise that the development of effective therapeutic options depends on more research on the pathophysiology of POP[3,10].

Epidemiological studies have identified the risk factors for prolapse but the aetiology is still unclear[11]. The cause of POP is likely to be multifactorial, and variable from patient to patient. The risk factors can be divided into genetic and acquired[12,13]. Some women with prolapse have been found to have a strong genetic predisposition, as seen in some cases where one in three females within the same family are affected by POP[14]. Connective tissue disorders such as cutix laxa, Marfan and Ehlers-Danlos syndromes have been also strongly linked to POP. Ethinicity is a major contributory factor in the differing predisposition to prolapse, with black women having the lowest incidence, whereas Caucasian, Latin and Asian women have higher prognoses of cystocele[2]. On the other hand, acquired environmental factors seem to play a major role in the weakening of the pelvic floor supportive tissues. The risk factors can be categorised into four groups: predisposing, inciting, promoting and decompensating (Table 1). The most common inciting and promoting risk factors (pregnancy, parturition, obesity, and pulmonary diseases) are related to excessive tissue strains caused by extreme increases in the intra-abdominal pressure. The intra-abdominal pressure is a physiological load that will be discussed later in the section "tissue biomechanics".

Table 1. Risk factors for pelvic organ prolapse[12]

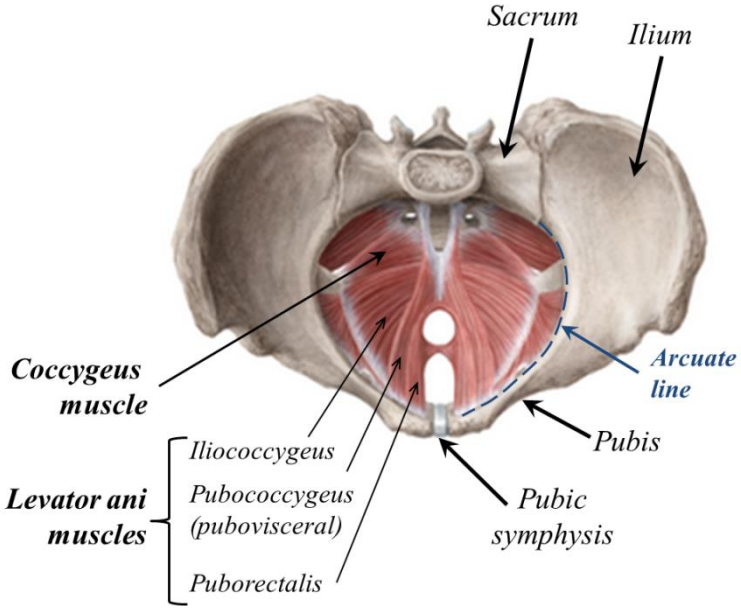
Predisposing	Inciting	Promoting	Decompensating
Genetics	Pregnancy	Obesity	Ageing
Ethnic origin	Parturition	Smoking	Menopause
Gender	Myopathy	Constipation	Neuropathy
	Neuropathy	Pulmonary diseases	Myopathy
	Pelvic surgery	Chronic strain	General health

The pelvic floor: anatomy and support

The pelvic floor is a combination of muscles, fascias and ligaments that form a hammock at the bottom of the abdomino-pelvic cavity and are attached to the pelvic bones (Figure 2). They have two basic functions: (1) to provide support to the pelvic organs, *i.e.* the bladder, the uterus and the rectum; and (2) to facilitate intercourse, vaginal delivery, and voluntary defecation and urination.

The pelvic floor is located within the cavity of the pelvis and between the *arcuate line* of hip bones, the *sacrum* and the *coccyx*. The muscles of the pelvic floor are collectively known as the pelvic diaphragm and consist of the *levator ani* muscles and the *coccygeus* muscles together with their fascias. The *levator ani* is the most important muscle of the pelvic floor and is divided into *iliococcygeus*, *puborectalis* and *pubococcygeus* (*puboviscera*l) muscles. These muscles maintain a constant state of contraction that keeps the urogenital hiatus closed and supports the weight of the pelvic organs against the opening action of the intra-abdominal pressure.

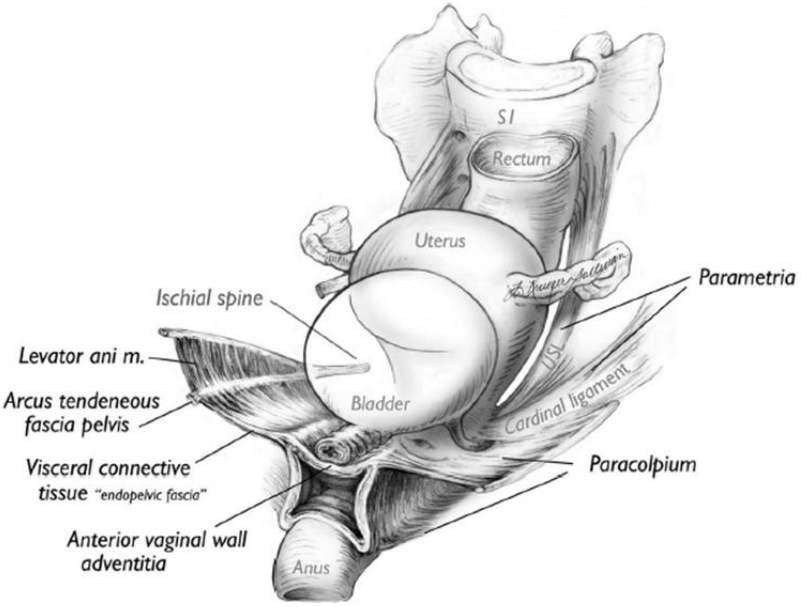
Figure 2. Bony pelvis and pelvic diaphragm



This support system is active and depends on the proper function of the muscles, but even the strongest muscle cannot exert any force without its connective tissue attachment to a fixed bony structure[15]. In the pelvic floor, the supportive connective tissues form a continuous, interdependent sheet which supports the vagina and the pelvic organs (Figure 3). According to DeLancey[16], there are three levels of support of the pelvic floor: Level I contains the uterosacral ligaments; Level II is the endopelvic fascia, which corresponds to the connective tissue from the middle part of the vagina which is attached to the *arcus tendineus fasciae pelvis* (ATFP), and the superior fascia of the *levator ani* muscles; and Level III corresponds to the lower third of the vagina wall that is directly attached to the surrounding structures, the perineal membrane and the perineal body. This suspensory system prevents the uterus and vagina from falling out while the hiatus is open[17].

The most prevalent manifestation of prolapse is cystocele, which is the weakening of the anterior vaginal wall with subsequent prolapse of the bladder. The vaginal wall has four different tissue layers: a superficial layer of epithelium, a mucosal layer made of dense connective tissue, a muscularis layer made of smooth muscle and an adventitia layer which is primarily connective tissue[17]. The vaginal mucosal and muscularis form a fibromuscular layer that intermingles with the endopelvic fascia, providing longitudinal and central support and keeping the bladder in place[18].

Figure 3. Connective tissues supporting the pelvic organs (Levels I and II). Note that the urethra and the vagina were removed just above the pelvic floor muscles[18].



Tissue biomechanics

As mentioned above, the pelvic floor is a group of muscles and connective tissues that work together, providing support and keeping the pelvic organs in place. Because of its anatomical location, and the daily activities it performs in the upright position, the pelvic floor is constantly loaded by the intra-abdominal pressure (IAP). IAP is a physiological load that is transmitted from the lungs to the diaphragm into the abdominal cavity to the vaginal wall. This load can be passive or active compression of the abdominal wall for breathing, load bearing, coughing, laughing, etc. Higher loads would mean higher force increments in the IAP and therefore changes in the mechanical loadings to the pelvic floor.

Changes in the IAP have been recorded in the bladder and are assumed to be a good representation of the pressures received by the pelvic floor. The maximum intra-bladder pressure for a non-pregnant woman has been reported to be as high as 30 kPa (or 225 mmHg) during coughing and vomiting[19]. Straining a stool typically leads to peaks of pressures of 9.8 kPa over several seconds[20]. Obesity can further increase the base-line of the IAP by 1.9 kPa[21]. These conditions are related to clearly identified risk factors of pelvic floor disorders such as chronic coughing, constipation, and obesity. Pelvic organ prolapse has also been strongly linked with injury incurred during parturition[22]. Unsurprisingly, the maximum pressures exerted on the pelvic floor muscles are during the second stage of labour, and have been reported to raise pressures by up to a further 19 kPa[13]. Such pressures

are higher than the increased intra-vaginal pressures for coughing and straining, and may last for as long as an hour. In fact, by the end of the second stage of vaginal birth the pelvic floor *levator* muscles should be able to stretch to 3.5 times their original length without rupturing. This presents a high risk factor, but not all parous women develop prolapse and indeed there are other risk factors involved (Table 1).

Understanding the pelvic floor biomechanics will improve clinical practice. To that end it is necessary to have the biomechanical properties of the "normal" tissues well defined. Unfortunately no studies have been performed with large populations of women and therefore no mean values are available for the mechanical properties of the female pelvic floor. It has been reported that the stiffness and the maximum stress of the vaginal wall varies between the anterior and the posterior compartment, and increases with the presence of pelvic organ prolapse[23]. These changes in tissue mechanical properties seem to be a consequence, rather than a cause of prolapse but further information is required.

The extracellular matrix

The bladder is kept in place by the connective tissue layer of the anterior vaginal wall, which is a dense extracellular matrix (ECM) with relatively few cells. The ECM obtains its mechanical properties from the fibrillar proteins: collagen I, collagen III, and elastin[24]. Collagen fibres provide tensile strength to the tissues because they are rigid and usually organized into large fibres. The elastin fibres provide some of the flexibility and resilience to the tissues because they follow the direction of stretch in tissues and regain their original shape after loading has been released[15].

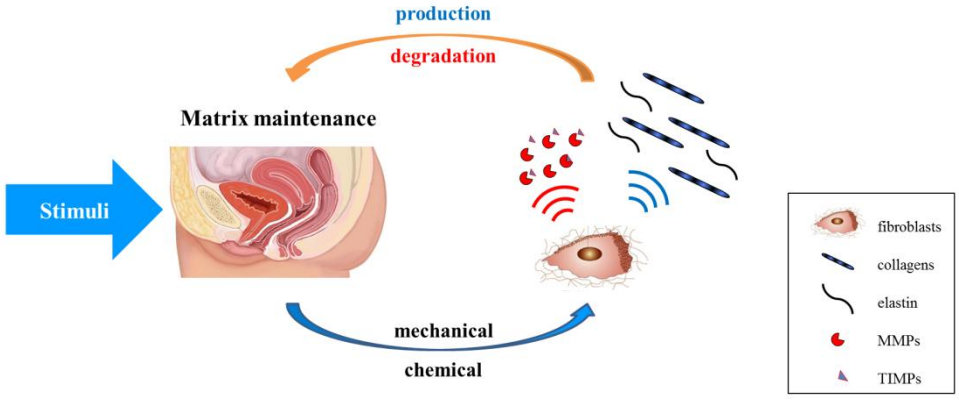
The integrity of the ECM depends on homeostasis between protein synthesis and degradation. In prolapsed tissues, however, such balance seems to be lost, as studies indicate that the metabolism of collagen and elastin is altered[25]. In patients with cystocele the prolapsed anterior vaginal wall tissues were shown to have altered morphology (*i.e.* disorganized collagen and elastin fibres[26]), tissue remodelling (*i.e.* increased enzymatic activity[27,28]), protein content (collagen[27,28,30,31], elastin[29] and elastin cross-linking[27]), and mechanical properties (*i.e.* increased stiffness[23]). Whether these alterations are a cause or an effect of pelvic organ prolapse still needs to be elucidated.

For years it was believed that the ECM was just a ground material with a filling purpose and only a passive role in soft tissues. Nowadays it is widely recognized that the ECM has an active role in keeping soft tissue homeostasis as it can dictate cell behaviour both chemically and mechanically. The ECM is a reservoir of growth factors which can be released into the cellular microenvironment under certain conditions thereby modulating cell behaviour. The fibrillar proteins of the ECM not only provide structure to the tissues, but can also transmit mechanical forces from the extracellular microenvironment to the intracellular space[32]. The effect of the altered prolapsed extracellular matrices on cell behaviour has not been studied.

Soft tissue remodelling cells: fibroblasts and myofibroblasts

The anterior vaginal wall connective tissues are made primarily of extracellular matrix ground material and fibroblasts, fat cells and mast cells[17]. Fibroblasts are the cells in charge of making and remodelling the extracellular matrix. They respond to external stimuli and keep the mechanical properties of the tissues by maintaining a homeostasis between tissue production and degradation (Figure 4). Fibroblasts can produce extracellular matrix proteins, such as collagen and elastin, they can activate catabolic enzymes such as matrix metalloproteinases (MMPs), and they can also secrete anabolic compounds such as tissue inhibitors of matrix metalloproteinases (TIMPs). Fibroblasts play a critical role in tissue repair as they can differentiate into myofbroblasts. Myofibroblasts are "transient" cells characterised by the presence of α-smooth muscle actin (α-SMA) in the cytoskeleton and play an important role in wound healing by closing wounds in two ways: (1) by contracting the ECM, and (2) by secreting large amounts of new matrix to fill the gaps within the tissues[33]. The connective tissue layer of the anterior vaginal wall is made and remodelled by fibroblastic cells, *i.e.* fibroblasts and myofibroblasts. Nevertheless, their role in the development and progression of prolapse is still unknown. The study of cell-matrix interactions in a disease-specific manner should shed light into the pathogenesis and pathophysiology of prolapse.

Figure 4. Extracellular matrix remodelling of soft tissues. During normal conditions, the extracellular matrix of the supportive soft tissues from the pelvic floor is remodelled by fibroblasts. This mechanosensitive cells respond to mechanical and chemical stimuli and keep homeostasis between tissue production and degradation by secreting and activating different proteins and factors. *MMPs: matrix metalloproteinases; TIMPs: tissue inhibitors of metalloproteinases.*



Hypothesis and thesis outline

The aim of this thesis is to gain an insight into the pathogenesis of POP by identifying the differences between extracellular matrix composition, cell behaviour and cell-matrix interactions in material derived from women with and without pelvic organ prolapse. It was hypothesized that most patients with prolapse acquire defects on fibroblast behaviour and extracellular matrix composition.

In this thesis the following specific goals were addressed:

- To compare healthy fibroblasts with fibroblasts isolated from women with prolapse, with regards to mechanical stimuli and their reaction to two matrix substrates *in vitro* (**chapter 2**).
- To determine whether defects in tissues from premenopausal women can be attributed to intrinsic or acquired properties of fibroblast behaviour (**chapter 3**) or extracellular matrix composition (**chapter 4**).
- To identify specific biological molecular pathways related to prolapse by using microarray technology (**chapter 5**).
- To identify the effects of prolapse and age on vaginal fibroblast matrix production and remodelling *in vitro* (**chapter 6**).
- To develop a culture system in which to investigate the effect of prolapse and non-prolapsed matrices on vaginal fibroblast to myofibroblast differentiation in prolapsed and non-prolapsed cells (**chapter 7**).

In **chapter 2**, a model is proposed to study fibroblast mechanoresponses *in vitro* on two different surface substrates and with a continuous loading mimicking respiration. To study the effect of POP on matrices and cells, whilst ruling out the effects of ageing, we designed a study including Caucasian premenopausal women with and without cystocele. To distinguish between acquired and intrinsic defects we also included biopsies from prolapsed and non-prolapsed tissues from the same women, in order that each patient be her own control. We evaluated the effect of prolapse on fibroblasts functional characteristics *in vitro* (**chapter 3**), and on matrix composition *in vivo* (**chapter 4**). In **chapter 5** we used a novel microarray analysis to identify specific biological pathways related to POP. In **chapter 6**, we investigated the effects of POP and menopausal status on the capacity of vaginal fibroblastic cells to produce and remodel matrix *in vitro*. In **chapter 7**, we proposed a novel cell culture system to study cell-matrix interactions in a disease-specific manner. We evaluated the effects of POP and non-POP tissues in the phenotype transition to myofibroblast of fibroblasts derived from POP and non-POP tissues. Finally, in **chapter 8** the information gathered in this thesis is integrated into a general discussion on the changes seen in cell behaviour, matrix composition and cell-matrix interactions in the progression of pelvic organ prolapse.

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