Wound healing of the pelvic floor concerning pelvic organ prolapse – What do we know?

Cicatrização do assoalho pélvico e relação com prolapso de órgão pélvico – O que sabemos?

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ABSTRACT: Pelvic organ prolapse (POP) is a result of the pelvic's floor supportive tissues weakening, including levator ani muscles, endopelvic fascia, ligaments, and the vaginal wall. The objective of this review is to describe the wound healing physiology in tissues that might be injured in the pelvic floor and to discuss the factors that affect wound healing. Since the most important risk factors for POP, such as pregnancy, vaginal delivery, and increased intra-abdominal pressure, trigger tissue damage, *i.e.* a wound in the pelvic floor tissues, we hypothesize that a frustrated wound healing process could affect the tissue homeostasis and promote POP. MEDLINE database was searched to review the literature up to 2017. As with skin, the wound healing in the pelvic floor tissues takes place in four phases (hemostasis, inflammation, proliferation and remodeling), however the duration of each phase is longer in the different structures of the pelvic floor compared to skin. Mechanical loading in the pelvic floor negatively affects healing and is associated with increased collagenase activity, whilst estrogen seems to improve the mechanical properties of the stretched tissue and could be beneficial for vaginal wound healing. Neither damaged muscle, nerves, ligaments nor vaginal wall will fully recover their pre-wounding characteristics. We postulate that a frustrated wound healing of the tissues of the pelvic floor generates tissues with altered composition and mechanical properties which could lead to the incidence or progression of POP.

Key words: Pelvic floor; Wound healing; Pelvic organ prolapse.

RESUMO: O prolapso de órgão pélvico (POP) é resultado do enfraquecimento dos tecidos de sustentação e de suspensão dessa região, incluindo os músculos levantadores do ânus, a fáscia endopélvica, os ligamentos e a parede vaginal. Sabe-se que os fatores de risco mais importantes para o POP, como gravidez, parto vaginal e aumento da pressão intra-abdominal, causam lesão nos tecidos do assoalho pélvico. Assim, é possível que um processo de cicatrização imperfeito desses tecidos possa afetar sua homeostase e contribuir para o desenvolvimento do POP. Dessa forma, o objetivo deste artigo é revisar a fisiologia da cicatrização de tecidos do assoalho pélvico e discutir os fatores que interferem nesse processo. Para isso, foi feita uma revisão da literatura na base de dados do MEDLINE até 2017. Percebeu-se que, assim como na pele, a cicatrização das lesões do assoalho pélvico ocorre em quatro fases (hemostasia, inflamação, proliferação e remodelação), porém a duração de cada fase é maior nas diferentes estruturas do assoalho pélvico. Além disso, a pressão constante no assoalho pélvico está associada ao aumento da atividade da colagenase, afetando negativamente a cicatrização, enquanto o estrogênio melhora as propriedades mecânicas do tecido estirado e parece ser benéfico para a cicatrização das lesões na parede vaginal. De acordo com a literatura, os músculos, nervos, ligamentos ou parede vaginal danificados não recuperam totalmente suas características prévias. Desse modo, conclui-se que uma cicatrização imperfeita no assoalho pélvico resulta em tecidos com composição e propriedades mecânicas alteradas, o que pode levar à incidência ou progressão do POP.

Descritores: Diafragma da pelve; Cicatrização; Prolapso de órgão pélvico.

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INTRODUCTION

Pelvic organ prolapse (POP) is a disorder characterized by the downward displacement of a pelvic organ, such as vagina, uterus, bladder and/or rectum, from its natural anatomic location[1]. This condition affects women's daily activities and decreases their quality of life[2], which makes POP a growing public health concern worldwide. For example, in the United States alone, over 300,000 prolapse surgeries are performed annually[3] and the lifetime risk of surgery for POP in the general female population is around 19%[4].

POP is a consequence of the weakening of the pelvic floor supportive tissues. The abdominal and pelvic organs are mainly supported through an interaction between the pelvic diaphragm and the endopelvic fascia[5]. The pelvic diaphragm consists of the levator ani muscles and their coverings, whereas the endopelvic fascia forms consists of connective tissue that covers the pelvic organs and forms ligaments responsible for its lifting[5,6].

Throughout a woman's life, the vaginal wall is constantly being remodeled in response to several stimuli. Parturition is probably one of the most challenging stimuli to induce vaginal tissue remodeling, as it is strongly associated with the incidence of POP. Also, pregnancy itself, previous surgery and increased intra-abdominal pressure due to obesity, chronic constipation, or chronic coughing, are important risk factors for the development of POP[7]. Micro-injury to the pelvic floor tissues caused by increased intra-abdominal pressure might have the same impact as obstetric injury in the development of pelvic floor damage[8]. This might explain why also nulligravidas can develop POP[9].

What all risk factors have in common is that any injury caused by these stimuli triggers a wound healing process that aims to restore the tissue integrity[10]. In addition, the mechanical loading in the pelvic floor caused by the prolapse itself could overstretch the wound and affect the healing process. Therefore, an impaired wound healing process possibly affects the tissue homeostasis. This, in turn, might cause a permanent impairment on the pelvic floor strength that could contribute to the development and progression of POP. Thus, the purpose of this article is to review the current literature regarding wound healing of the pelvic floor supportive tissues and the factors that affect it. This review will help to clarify the physiology of the tissue repair process and the factors affecting wound healing, especially of the soft tissues of the pelvic floor. This knowledge could advance the understanding of extracellular matrix (ECM) abnormalities seen in prolapsed tissues, contribute to the development of treatments in an early stage after wound repair in the pelvic floor, and might lead us towards new preventive strategies.

METHODS

The primary investigator and a clinical librarian searched the computerized database MEDLINE 2017, using terms such as "pelvic organ prolapse", "uterine prolapse", "cystocele", "rectocele", "pelvic floor", "parturition", "wound healing", "wound repair", "wound closure", "tissue healing", "tissue remodeling", "supportive tissue" and "extracellular matrix". Not only free text terms but also Mesh terms were used. Only data published in full article form were included. Additionally, articles were obtained by reviewing reference lists of pertinent studies and reviews. No articles written in languages other than English provided additional information.

Wound healing physiology

Wound healing is a biological process that aims to restore tissue integrity after an injury. The wound healing process for skin is well described and has four overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling[10,11].

The hemostasis starts with the vascular damage that occurs immediately after an injury. It consists of clot formation and constriction of damaged arterial vessels to avoid blood loss. The clot formation is achieved through three mechanisms: the intrinsic pathway of the clotting cascade (contact activation pathway), the extrinsic pathway of the clotting cascade (tissue factor pathway), and the platelet activation[10]. Besides being responsible for clot formation, platelets produce growth factors and cytokines that regulate the healing cascade[10]. Simultaneously, the constriction of damaged vessels reduces the blood flow and leads to tissue hypoxia and acidosis. This results in the production of vasoactive metabolites, such as nitric oxide and adenosine, which, in addition to histamine released from mast cells, aims to increase dilatation and vascular permeability to facilitate the entry of inflammatory cells into the extracellular space near the wound[10].

The inflammatory reaction aims to prevent infection and to clear the debris from the wound through the action of neutrophils, macrophages, and lymphocytes[10]. Neutrophils infiltrate the wound within the first 48 hours in response to chemical signals such as the complement cascade, interleukin activation, and transforming growth factor-β (TGF-β)[10]. Neutrophils also destroy debris and bacteria through phagocytosis, and by secreting toxic substances and reactive oxygen radicals[10]. Macrophages infiltrate the wound between 48 and 72 hours after injury attracted by chemical stimuli released from platelets and injured cells[10]. Macrophages are a source of growth factors, such as TGF-β and epidermal growth factor (EGF), which contribute to stimulate angiogenesis and enhance the formation of granulation tissue. Lymphocytes produce ECM and remodel collagen from 72 to 120 hours after the injury occurs[10].

Various signaling molecules are produced to

initiate the proliferation phase, which is characterized by fibroblast migration, deposition of ECM, the genesis of granulation tissue, and epithelialization[11]. Fibroblasts, attracted by factors; such as platelet-derived growth factor (PDGF) and TGF-β[11], appear in the wound around 2 to 4 days after an injury. They proliferate and differentiate into myofibroblasts[12]. Myofibroblasts are "transient" cells characterized 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 rapid deposition of repair matrix to fill the gaps within the tissues[13]. The myofibroblasts produce matrix proteins that contribute to the construction of the new ECM such as fibronectin, hyaluronan, "repair" collagens (III and V), and proteoglycans. Besides providing turgor to the tissue, the ECM supplies substratum for cell adhesion and regulates growth, movement, and differentiation of cells[11]. Moreover, the granulation tissue is well established by 3 to 5 days after injury and it is characterized by angiogenesis, which is induced by several factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), PDGF, and TGF-β[11]. The epithelialization consists of migration and proliferation of epidermal cells from the wound edges and it requires a moist environment, adequate nutrition, and bacteriological control[11]. This process is modulated by a number of growth factors, including keratinocyte growth factor (KGF), EGF, and bFGF[11].

The last phase of the wound repair in soft tissues is the tissue remodeling of the repair matrix. This process involves wound contraction and continuous synthesis and degradation of collagen[11]. The degradation is achieved by enzymes, mainly matrix metalloproteinases (MMPs), that are produced by fibroblasts, neutrophils, and macrophages. The synthesis and secretion of MMPs are tightly regulated by growth factors, cytokines, and phagocytic stimuli to avoid degradation of essential ECM proteins, which could lead to impaired healing[11]. The main cells and growth factors involved in wound healing are listed in Tables 1 and 2.

Table 1. Cells involved in wound healing[10,11]

Cell type	Time of action	Function
Platelets	Seconds	Thrombus formation; release of inflammatory mediators.
Neutrophils	1-48h	Phagocytosis of bacteria; release of proteolytic enzymes; wound debridement; increase vascular permeability.
Keratinocytes	8h	Release of inflammatory mediators; neovascularization.
Macrophages	48-72h	Phagocytosis of bacteria; wound debridement; release of inflammatory mediators; stimulation of fibroblasts division, collagen synthesis, and angiogenesis.
Lymphocytes	72-120h	Not clearly defined; may produce cytokines.
Fibroblasts / Myofibroblasts	120h	Synthesis of granulation tissue; production of ECM components (collagen, fibronectin, hyaluronic acid, proteoglycans); release of inflammatory mediators.

Table 2. Growth factors involved in wound healing[10,11]

Growth factor	Major source	Action
VEGF	Platelets, neutrophils.	Stimulates angiogenesis.
FGFs	Fibroblasts, endothelial cells, smooth muscle cells, macrophages.	Genesis of granulation tissue, fibroblasts and epithelial cells proliferation, matrix deposition, wound contraction, angiogenesis.
KGFs	Fibroblasts.	Proliferation and migration of keratinocytes.
EGF	Platelets, macrophages, keratinocytes.	Formation of granulation tissue, proliferation, and migration of keratinocytes.
PDGF	Platelets, fibroblasts, macrophages, endothelial cells.	Chemotaxis, the proliferation of fibroblasts, smooth muscle cells, and endothelial cells, collagen deposition.
G-CSF	Monocytes, fibroblasts, lymphocytes.	Proliferation of keratinocytes, enhances function of neutrophils, and monocytes.
GM-CSF	Keratinocytes, macrophages, lymphocytes, fibroblasts.	Proliferation of epidermal cells.
TGF-α	Macrophages, platelets.	Proliferation of epithelial cells and fibroblasts, genesis of granulation tissue.
TGF-β	Platelets, macrophages, fibroblasts, neutrophils.	Chemotaxis, differentiation of fibroblasts to myofibroblasts, stimulate angiogenesis, collagen metabolism, wound contraction, release of other growth factors, stimulates MMP.
IL-1	Macrophages, lymphocytes, platelets.	Chemotaxis, proliferation of fibroblasts.
TNF-α	Platelets.	Chemotaxis, nitric oxide release, activation of other growth factors.
IGF-1	Fibroblasts, plasma, liver.	Proliferation of fibroblasts, synthesis of collagen, and proteoglycans.
HGF	Fibroblasts, keratinocytes, endothelial cells.	Re-epithelialization, neovascularization, formation of granulation tissue.

VEGF: vascular endothelial growth factor; FGFs: fibroblast growth factors; KGFs: keratinocyte growth factors; EGF: epidermal growth factor; PDGF: platelet-derived growth factor; G-CSF: granulocyte-colony stimulating factor; GM-CSF: granulocyte macrophage-colony stimulating factor; TGF-α: transforming growth factor-α; TGF-β: transforming growth factor- β; IL-1: interleukin-1; TNF-α: tumor necrosis factor-α; IGF-1: insulin-like growth factor; HGF: hepatocyte growth factor.

In the pelvic floor, pelvic organ support could be impaired due to micro- or macro- injuries of the levator ani muscle, the nerves, the endopelvic fascia, the suspending ligaments, and/or vaginal wall[14–16]. Injury to these different tissue types triggers a specific wound healing process[17].

Wound healing of the different structures of the pelvic floor

Muscle

The healing of a muscle injury has similar overlapping phases to the skin but with longer durations: destruction (1-3 days), repair (3-4 weeks), and remodeling (3-6 months)[18,19]. The destruction phase consists of rupture and necrosis of the myofibers, formation of hematoma, and inflammation[18]. The repair phase is characterized by phagocytosis of the necrotic tissue, regeneration of the myofibers, capillary ingrowth in the wound, and production of a connective tissue scar[18]. The remodeling phase includes maturation of the myofibers, contraction and reorganization of the scar tissue, and recovery of muscle functional capacity[18].

Muscle healing is a slow process that may result in incomplete functional recovery[20]. The main cause of suboptimal muscle repair is fibrosis[19], which is an abnormal and unresolvable chronic increase of ECM that affects tissue function[21]. The presence of fibrotic tissue restricts the regeneration of myofibers and reduces the elasticity of the native muscle and thus, reinjury may occur[20].

In a rat model, Kääriäinen et al.[22] observed that the muscle did not reach complete functional recovery by 56 days after laceration injury. Furthermore, in a study with 68 women, Miller et al.[23] found that levator injury from childbirth did not solve 8 months postpartum. Similarly, Shek et al.[24] assessed women's pelvic floor 3 to 6 months and 2 to 3 years postpartum and found no evidence of regression of pregnancy- and childbirth-related changes to levator distensibility. These data suggest that levator ani may not recover completely after trauma. Additionally, the muscle functional recovery depends on its reinnervation[25], since non-innervated muscles may undergo degenerative morphological and biochemical changes[26] associated with gradual atrophy[27].

Nerve

Nerve repair depends on the extent of the injury[28,29]. During labor, nerve injury occurs because of stretching and compression[14]. Based on Seddon[30] classification (Table 3), nerve compression is likely to fit in the neuropraxia group of nerve injuries[28]. Neuropraxia is the mildest type of nerve injury, which consists of local myelin damage with no loss of axon continuity[30]. In this case, the nerve does not undergo distal degeneration and there might be a transient functional loss[28,31]. Complete recovery is likely to occur within weeks to months[28].

Table 3. Seddon[30] nerve injury classification

Neuropraxia	Local myelin damage, no axon disruption.
Axonotmesis	Axon disruption, variable preservation of the surroundings connective tissue.
Neurotmesis	Complete disruption of the entire nerve trunk.

Research on pudendal nerve injury and regeneration after childbirth has been related to its association with stress urinary incontinence (SUI). Most of the available data comes from external urethral sphincter (EUS) electromyography (EMG) and urethral leak-point pressure (LPP) assessment. According to recent studies conducted in rats, functional recovery after pudendal nerve crush (PNC) could be observed from 2 to 3 weeks after injury[32,33], although the neuroregenerative process seemed to be near completion only after 6 weeks[32].

However, if PNC and vaginal distension (VD) were performed together (PNC+VD), which better simulates the injuries from vaginal delivery[33], the functional recovery seems to occur slower than PNC or VD alone, without complete recovery 6 weeks after injury[33]. The delayed neuroregeneration in this case may be explained by an insufficient upregulation of neurotrophins due to the muscle damage associated with VD[33]. In a study with 96 women, Allen et al.[34] found that vaginal delivery causes partial denervation of the pelvic floor in most women, and the pudendal nerve conduction tests showed no significant differences between the puerperium and 2 months postnatally. Furthermore, a study with rats[35] showed incomplete EUS neuromuscular innervation 9 weeks after PNC+VD despite functional recovery, which suggests a persistent neurogenic deficiency.

Endopelvic fascia and ligaments

After significant denervation or overstretching of the tissues of the pelvic floor, women might develop POP especially when there is a pre-existing weakness of ligaments and fascia[36]. The ligaments and fascia of the pelvic floor differ from those from other sites in the body because they are visceral connective tissue elements, more related to organ function than to locomotion[36]. Unfortunately,

much of the current knowledge about connective tissue is derived from orthopedic and sports medicine research[36], and the healing characteristics might be different between different ligaments[37]. Hence, we should bear in mind that only some of the current knowledge might apply to pelvic floor connective tissues.

With ligaments, wound healing is similar to the general wound healing process but with longer duration. Hemostasis and inflammation might take days to weeks; whilst matrix production may last weeks to months; and remodeling, months to years[37]. These timings could vary depending on the ligaments' size and on the mechanical forces applied to it during healing[36].

Several studies suggest that ligament healing results in weaker scar tissue, due to different biomechanical composition and structural organization[37,38]. In the early repair of the ligament tear, there are more collagen types III, V, and VI, besides less type I collagen than in the original tissue[38]. As the scar matures, collagen type I production increases and gradually replaces the weaker ones[38]. Moreover, a better alignment of the collagen fibers occurs and an improvement in collagen fibers orientation is likely to increase the tissue tensile strength[39]. A long-term study assessing rabbits' medial collateral ligament showed that the scar tissue quality seems to improve 2 years after injury[40]. Afterwards, no further improvement seems to be noted and the healed ligament appears to remain weaker and less stiff than normal[37,40].

Studies of the ligaments from fresh female cadavers showed that the uterosacral ligaments were stiffer than the round ligaments and that the rigidity increased with age[41]. Others have also reported that parous women had higher stiffness and maximum stress of the uterosacral ligaments than nulliparous women[42] and that the cardinal and uterosacral ligaments seem to be longer in women with POP[43].

Vaginal wall

The literature on vaginal wall healing is limited. By comparing abdominal skin vs. vaginal wall surgical wound healing, a study conducted on rabbits showed that apart from a fibrinous crust, which was evident in the abdominal skin but not in the vagina between 4 to 7 days after injury, most of the histological parameters did not differ significantly between both tissues[44]. However, the vaginal wound seemed to close and contract faster than the abdominal skin wound[4]. This might be due to the inherent and environmental differences between these tissues: (1) higher vascularization in the vagina; (2) keratinized epithelium in skin vs. non-keratinized epithelium in the vagina; (3) possible different concentrations of growth factors; (4) wound unexposed to air and in a moist environment in the vagina, vs. wound exposed to air and in a dryer environment in the skin; (5) different bacterial flora between tissues[45]. Abramov et al.[45] hypothesized that the faster wound contraction rate in the vagina could be due to the different mechanical forces applied to these tissues in the resting state, or due to distinct levels of myofibroblasts activity, possibly related to a distinct expression of/or response to TGF-β1.

Studies conducted on rabbits showed that TGF-β1[46] and PDGF-B mRNA[47] expression play a role in cutaneous and vaginal wound healing. However, it was also shown that oophorectomised rabbits have protracted vaginal wound healing associated with increased TGF-β1 transcription, suggesting that overexpression of this factor may lead to delayed vaginal wound healing[46]. TGF-β1 promotes fibroblast to myofibroblast differentiation and plays an important role in abnormal fibrotic conditions[13]. Studies with fibroblasts from prolapsed vaginal wall tissues have shown that these cells have lower contractile capacities[48], altered mechanoresponses[48,49], lower responses to TGF-β1[49], and altered matrix production[50]. The relation between TGF-β1 and POP, if any, still needs to be elucidated.

An assessment of the biomechanical properties of vaginal vs. abdominal surgical wound healing in rabbits showed that neither vaginal nor abdominal tissues had completely recovered their tensile strength and elasticity to pre-wounding values[45]. Indeed several studies have shown that anterior vaginal tissues from women with POP have altered ECM composition[6,51–54] and mechanical properties with a higher stiffness than controls[55–57]. Additionally, it has been shown that the vaginal wall of women with POP is more extensible than those without POP[58], probably due to the variations in collagen and elastin metabolism. The protracted extensibility was associated with increased the severity of POP[58]. Another study concluded that vaginal parity resulted in decreased collagen alignment and progression to inferior mechanical properties, which were highly correlated with POP[59]. Kim et al.[60] provided evidence that POP tissues possess a non-stretchable and fragile collagen matrix with reduced load-bearing capability due to collagen fibrils that are stiffer, bulkier, and with decreased braiding.

A summary of wound healing parameters on the different structures of the pelvic floor is found in Table 4.

Table 4. Wound healing parameters on the different structures of the pelvic floor

Parameter	Muscle[18,19]	Nerve[28,31]	Endopelvic fascia and ligaments[37,38,61]	Vaginal wall[44,45,47]
Timing	Destruction: 1 to 3 days; Repair: 3 to 4 weeks; Remodeling: 3 to 6 months.	Weeks to many months.	Hemostasis and inflammation: days to weeks; Proliferation: weeks to months; Remodeling: months to years.	Approximately one month.
Cells	Neutrophils, macrophages, fibroblasts, myofibroblasts, satellite cells, myoblasts.	Schwann cells, macrophages, mast cells, fibroblasts.	Neutrophils, macrophages, fibroblasts, myofibroblasts.	Neutrophils, macrophages, fibroblasts, myofibroblasts, smooth muscle cells.
GF	TNF-α, FGF, IGF, TGF-β, HGF, PDGF, EGF, VEGF.	NGF, BDNF, ciliary neurotrophic factor, FGF, IGF.	IGF-I, TGF-β, VEGF, bFGF, EGF, PDGF.	VEGF, TGF-β, PDGF,
ECM	Fibronectin, tenascin-C, followed by collagen III and collagen I.	Laminin with collagen IV, proteoglycan and entactin; fibronectin and fibrinogen.	Increased amount of minor collagens (types III, V, and VI), decreased collagen cross-links, increased amount of glycosaminoglycans.	Collagen deposition increases until postwounding day 35.

GF: growth factors; ECM: extracellular matrix; TNF: tumor necrosis factor; FGF: fibroblast growth factor; IGF: insulin-like growth factor; TGF: transforming growth factor; HGF: hepatocyte growth factor; PDFG: platelet derived growth factor; EGF: epidermal growth factor; VEGF: vascular endothelial growth factor; NGF: nerve growth factor; BDNF: brain-derived neurotrophic factor.

Factors affecting wound healing

Wound healing is a complex process that depends on a precise integration between its phases of hemostasis, inflammation, proliferation, and remodeling. Some factors can interfere in one or more phases of the process, leading to improper or impaired tissue repair[62]. These factors are summarized in Table 5.

Table 5. Factors affecting wound healing

Factor	Action
Oxygen	regulates some GF production (TGF-β, VEGF); induces angiogenesis, fibroblast proliferation, and collagen synthesis; prevents infection through oxidative killing[75].
Infection	upregulates pro-inflammatory cytokines (IL-1, TNF-α) and increase levels of MMPs, which may result in excessive degradation of ECM and impaired healing[62,76].
Age	leads to enhanced platelet aggregation, increased levels of inflammatory mediators, delayed infiltration of macrophages and lymphocytes, impaired macrophage function, reduced levels of GF, delayed re-epithelialization, delayed angiogenesis and collagen deposition, decreased collagen turnover, and remodeling, reduced wound strength[77], slower healing[78].
Obesity	increases risks of complications (infection, dehiscence, hematoma, seroma, venous ulcer, pressure ulcer)[79] due to hypoperfusion and decreased diffusion of antibiotics[62]; increases risks of impaired mononuclear cell function, decreases lymphocyte proliferation, and alters cytokine levels[62].
Nutrition	nutrient deficiencies prolong inflammation, inhibit fibroblast function, reduce collagen deposition, and angiogenesis[10].
Diabetes	microvascular damage affects tissue oxygen levels and nutrients supply[10]. Diabetic neuropathy impairs neuropeptides functions: promote cell chemotaxis, induce GF production, and stimulate cell proliferation[62].
Alcohol consumption	impairs early inflammatory response, inhibits wound closure and angiogenesis, inhibits collagen production, alters protease balance at wound site[62], and increases the incidence of infection[80].
Smoking	decreases wound tensile strength and increases risks of complications (infection, wound rupture, necrosis) due to tissue hypoxia[81,82]. Low doses of nicotine enhance angiogenesis, and may improve healing[83,84].
Estrogen	regulates ECM production, protease inhibition, epidermal function, and genes related to regeneration and inflammation[85].
Mechanical loading	inhibits apoptosis in the proliferative wound environment[64], and upregulates expression of genes related to inflammation and ECM production[63], resulting in an excessive and disorganized accumulation of cells, and ECM that compromises tissue strength[64].

GF: growth factor; TGF: transforming growth factor; VEGF: vascular endothelial growth factor; IL-1: interleukin-1; TNF-α: tumor necrosis factor; MMP: metalloproteinase; ECM: extracellular matrix.

Among those factors, estrogen and mechanical loading are worth mentioning in the context of pelvic floor wound healing. Although an appropriate amount of mechanical tension is necessary for myofibroblast differentiation and consequent wound closure[12], increased loading negatively affects the healing process (Table 5). Altered mechanical conditions are associated with fibroproliferative diseases and hypertrophy in multiple organ systems (*e.g.* pulmonary fibrosis, congestive heart failure, glomerulosclerosis)[63,64]. However, in the pelvic floor, mechanical stretch is associated with an increased collagenase activity by vaginal fibroblasts[65,66]. Vaginal cells from women with severe POP, in comparison to non-POP controls, react differently to in vitro mechanical stretch, showing downregulation of genes involved in ECM biogenesis and an upregulation of genes involved in ECM biodegradation[67].

Interestingly, the administration of estrogen could improve the mechanical properties of the stretched connective tissue by inhibiting the degradative behavior of the vaginal fibroblasts[65,68]. Vaginal estrogen application has been shown to promote the synthesis of mature collagen, decrease collagenase activity, and increase the thickness of the vaginal wall[69], thus appearing beneficial for wound healing[70]. Recent evidence suggests that local estrogen improves vaginal tissue quality in postmenopausal women after vaginal reconstructive surgery[71]. An animal study showed that bilateral oophorectomy and estrogen deficiency result in delayed vaginal wound healing, scar contraction and recovery of biomechanical properties[72], and was associated with enhanced inflammation[73]. Moreover, the systemic use of estrogen after a surgical injury to guinea pigs leads to greater amounts of collagen and elevated density of elastic fibers, resulting in higher distensibility of the vagina while maintaining its tissue strength[74].

Are impaired wound healing and POP interlinked?

POP is a multifactorial disease that occurs as a consequence of the weakening of the pelvic floor supportive tissues. According to literature, POP tissues differ from non-POP tissues in ECM composition[6,51-54], number and functionality of cells[48-50,86,87], and amount of growth factors[88,89]. Together, these differences affect the biomechanical properties of POP tissues, which seem to be more extensible, with stiffer matrices and reduced load-bearing capabilities[57,58,60].

Recent studies correlate POP with an injury of the pelvic floor's soft tissues, such as the levator ani muscle[15,90,91], the pudendal nerve[14,92], and the paravaginal fascias[16,36,93,94]. These injuries, in turn, seem to be related to parturition, constipation, and higher body mass indexes, which are among the main risk factors for POP[8,9,14,36,95,96].

Once an injury occurs, wound healing takes place, aiming to restore tissue integrity. Although the literature on wound healing of the different tissue types of the pelvic floor is scarce, several studies suggest that neither the damaged muscle, nerves, ligaments nor the vaginal wall are able to completely revert to their pre-wounding characteristics[22-24,33,35,37,40,45].

Hence, the changes in matrix and cells found in POP tissues could be the result of an impaired wound healing. The connective tissue with altered composition and biomechanical properties would thus be a cause of POP. However, it might also be that the remodeling in connective tissues is being triggered by the stretching due to the constant loading of the soft tissues of the prolapse itself, and the alterations are, in fact, a consequence of POP[97,98].

Regardless of being a cause or a consequence of POP, changes in POP tissue composition could affect a subsequent wound healing process. Fibroblasts, growth factors, and the ECM likely play an essential role in the proliferation and remodeling phases[11], and its altered functionality and composition would result in impaired healing. Thus, the improper healing could have an impact on the prognosis of POP, since it would negatively affect recovery after surgical treatment. Perioperative administration of estrogen in postmenopausal women increases synthesis of mature collagen and decreases degradative activity. Leading to an increased thickness of the vaginal wall, which might improve tissue quality postoperatively[69,71]. Identification of other factors which could be beneficial for wound healing on the pelvic floor should be an important topic for future research.

CONCLUSION

Compared to the skin, the duration of the wound healing process of the different tissues of the pelvic floor is longer. Mechanical loading on the pelvic floor negatively affects healing and is associated with increased collagenase activity, whilst estrogen seems to improve the mechanical properties of the stretched tissues and could be beneficial for vaginal wound healing. Neither damaged muscle, nerves, ligaments nor vaginal wall will fully recover their pre-wounding characteristics. Impaired wound healing of the tissues of the pelvic floor might generate tissues with altered composition and mechanical properties that could lead to the incidence or progression of POP. Further research is needed for better understanding of the wound healing of the different tissues of the pelvic floor, their healing times, and the factors involved to develop strategies to improve tissue quality. Overall, increasing the knowledge of POP's pathophysiology will contribute to improving its clinical management and development of new treatments.

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