REVIEWS

The mechanisms and potential of stem cell therapy for penile fibrosis

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Abstract | Fibrosis is often caused by chronic tissue injury leading to a persisting inflammatory response with excessive accumulation of extracellular connective tissue proteins. Peyronie's disease, urethral stricture and penile (corpora cavernosa) fibrosis are localized fibrotic disorders of the penile connective tissues that can substantially impair a patient's quality of life. Research over the past few decades has revealed the ability of stem cells to secrete a wide range of paracrine factors, a characteristic that could be exploited therapeutically to prevent and treat several inflammatory and fibrotic diseases. In preclinical studies, mesenchymal stem cells (MSCs) have proven to be the most effective and readily available type of stem cells for therapeutic use. An important advantage of MSCs is their ability to circumvent the immune system and function as immunomodulatory 'drug stores' to influence multiple cell types simultaneously. Many studies using stem cells have been applied exclusively to corpora cavernosa fibrosis owing to its well-established disease models. A plethora of preclinical data suggest the benefit of stem cells for use in penile fibrosis. However, their exact mechanism of action and optimal timing and mode of administration must be determined before clinical translation.

Tunica albuginea
The connective tissue sheath surrounding the erectile tissue (corpora cavernosa) of the penis.

Corpora cavernosa
Sponge-like regions of the erectile tissue that contain most of the blood during a penile erection. (Singular: corpus cavernosum).

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Fibrosis is a wound-healing disorder that often occurs in response to chronic tissue injury and that is defined by a persisting inflammatory reaction and the subsequent excessive accumulation of extracellular connective tissue proteins such as collagen, elastin and fibronectin (collectively termed the extracellular matrix (ECM))[1,2]. Typically, inflammation and ECM aggregation is an essential and reversible phase of the normal wound-healing process[3]. However, if the initial injury (for example, infection, mechanical stress or autoimmune reaction) is not resolved in a timely manner or the wound-healing process itself becomes deregulated, this phase can gradually evolve into a permanent fibrotic reaction and lead to fibrogenesis, a process termed 'fibrosis'[4]. Importantly, fibrosis is the conclusive pathological consequence of many chronic inflammatory disorders and can lead to a progressive loss of tissue and/or organ function[5].

Localized fibrotic disorders of the penile connective tissues such as Peyronie's disease[6–8] and urethral stricture disease[9] are thought to occur as a result of impaired wound healing. Peyronie's disease is considered to be caused by lesions in the tunica albuginea following intercourse-related repetitive microtrauma caused by buckling of the penis, whereas urethral strictures can be caused by trauma to the urethra as a result of instrumentation (iatrogenic: bladder catheters) and/or infectious and inflammatory disorders (for example, sexually

transmitted diseases)[8,9]. Penile fibrosis occurs as a diffuse fibrotic process in the corpora cavernosa of the penis and is the result of various conditions associated with erectile dysfunction (ED) such as diabetes mellitus, atherosclerosis, iatrogenic pelvic nerve damage (after radical prostatectomy for prostate cancer) and even ageing-related ED[8]. Additionally, severe corporal fibrosis can occur acutely, in which case it is frequently the result of episodes of ischaemic priapism[10]. These fibrotic disorders can severely decrease the quality of life of patients by causing lower urinary tract symptoms (LUTS), the onset or worsening of ED, painful and deformed erections, major depressive disorders and relationship issues[11,12].

At present, very few therapeutic strategies are available for the treatment of fibrotic conditions; however, the search for novel treatments has led to the discovery of the immunomodulatory capacities of stem cells. Stem cells are well known for their ability for self-renewal and differentiation into a diverse set of mature cell populations[12]. Moreover, the secretion of a wide range of paracrine factors, including growth factors, cytokines, chemokines and even functional small RNAs (via extracellular vesicles), makes stem cells appealing for therapeutic application. These secreted factors enable stem cells to influence and modify their host environment, particularly during and early after tissue injury[13–15]. In recognition of these unique properties, a growing body of (preclinical) evidence has demonstrated the potential

Key points

- Fibrosis is a state of excessive wound-healing leading to the replacement of the local parenchyma with stiff and afunctional extracellular matrix, resulting in loss of organ function in advanced stages.
- Owing to the complex network of cell types and interactions involved in fibrosis, very few effective medical treatment options are currently available for patients with fibrotic diseases, including penile fibrosis.
- Stem cells can interrupt several key processes in the fibrotic cascade simultaneously and, therefore, have great potential as novel antifibrotic therapies.
- Penile fibrosis can occur in the corpora cavernosa, tunica albuginea or urethra.
- A large number of preclinical studies have investigated the effect of stem cells for the treatment of penile fibrosis, and have reported encouraging results.
- Certain limitations (such as isolation, timing, administration and dosage) need to be addressed before preclinical findings can be translated into the clinical setting.

Priapism
A condition whereby the penis remains in an erectile state without any stimulation, or after the stimulation has ended, for more than 4 hours.

Mesenchymal stem cells
(MSCs). Multipotent stromal cells with the ability to differentiate into several cell types within their germ layer (osteoblasts, chondrocytes, myocytes and adipocytes).

Corpus spongiosum
Spongy tissue surrounding the male urethra within the penis.

Totipotent stem cells
These stem cells have the capacity to divide and develop into cells from all three germ cell layers and into extra-embryonic tissues (for example, placenta). The zygote is an example of such a cell.

Pluripotent stem cells
These stem cells have the capacity to divide and develop into cells from all three germ cell layers, but not into extra-embryonic tissues (for example, placenta). Embryonic stem cells are examples of such cells.

Multipotent stem cells
These stem cells have the capacity to divide and develop into cells from a specific tissue or organ. Most adult stem cells are examples of such cells.

therapeutic role of stem cells in alleviating fibrosis[16–20]. Mesenchymal stem cells (MSCs) have been commonly used in this therapeutic context and have been shown to have a role in reducing fibrosis in animal models of lung[21,22], liver[23–25], kidney[26–28], heart[29,30], corpus spongiosum and urethra[31], corpus cavernosum[32,33] and tunica albuginea[34] fibrosis. As of September 2018, ~60 clinical trials (active or recruiting) are evaluating the efficacy of MSCs for the treatment of various fibrotic disorders (for example, hepatic, Crohn's disease-related intestinal, cardiac and pulmonary fibrosis).

The precise mechanisms governing the antifibrotic properties of MSC therapy are yet to be elucidated. However, the leading theory is that MSCs function as 'drug-stores', influencing several cell types (for example, cells from the innate and adaptive immune system, resident fibroblasts and smooth muscle cells) and the production of several profibrotic and antifibrotic factors simultaneously[12]. Most preclinical studies suggest that MSCs exert their antifibrotic functions through immuno-modulation, thereby limiting the host's response to injury and preventing the onset of fibrosis[12,15]. Another putative mechanism is the attenuation of profibrotic phenotypic changes of resident fibroblasts into the contractile and ECM-producing myofibroblasts[35]. Furthermore, stem cells can directly modulate ECM composition on the basis of their ability to secrete high levels of matrix metalloproteinases (MMPs) and other matrix-modulating enzymes (for example, through inhibition of tissue inhibitors of metalloproteinases (TIMPs))[15]. Nonetheless, these hypotheses have not yet been proven and additional studies focusing on the mechanisms of MSC function are ongoing[12,15].

In the past decade, stem cells have also been evaluated for the prevention and treatment of fibrosis of the male genitourinary tract (Peyronie's disease, urethral stricture and corpora cavernosa fibrosis). In this Review, we provide an overview of current research on stem cells for the treatment of penile fibrosis, with an emphasis on the specific mechanisms of antifibrotic activity.

Pathophysiology of fibrotic disorders

Fibrotic diseases embody a spectrum of disorders (pulmonary, renal, hepatic, cardiac and dermal fibrosis) that are characterized by an excessive accumulation of ECM[3]. Fibrosis is considered to be the result of an abnormal wound-healing process, and is often the end stage of disorders caused by chronic tissue injury and inflammation[36,37]. Wound-healing is an intricate and finely tuned process that involves the coordinated production of growth factors, cytokines, ECM proteins and crosstalk between many cell types. This process is divided into four steps that are tightly regulated — haemostasis, inflammation, proliferation and remodelling[2] (FIG. 1). Fibrosis is characterized by the disproportionate accumulation of ECM components, such as interstitial collagens (types I and III), cellular fibronectin, elastin and basal membrane proteins (such as laminin and collagen IV)[5]. The fibrotic process is driven by a complex and ubiquitous molecular pathway that is shared by all fibrotic diseases, regardless of the aetiology, trigger or organ affected[1,2,17,37–39] (FIG. 1). However, the precise mechanism remains poorly understood despite extensive study. The intricate interplay between the innate and acquired immune system and resident cell types within a tissue (for example, endothelial cells, pericytes and fibroblasts) makes the fibrotic process difficult to study owing to a lack of in vitro models mimicking these interactions and the fact that in vivo models do not sufficiently represent the human disease[17,39,40]. Furthermore, tissue resection and surgical intervention is rarely required in the clinical context (in contrast to cancer), leading to inadequate characterization of the fibrotic process. These limitations have had important repercussions in the search for a common and effective treatment for fibrotic disorders. To date, only a few drugs targeting fibrosis are clinically available (for example, nintedanib and pirfenidone for idiopathic lung fibrosis). However, their use is usually limited owing to their modest clinical effects and/or unfavourable toxicity profiles, leading to a considerable proportion of patients who do not receive therapy[3,41,42].

Two presentations are common in patients with penile fibrosis. Patients might present with a fibrous plaque that develops in the tunica albuginea in Peyronie's disease or corpora cavernosa fibrosis, both of which can lead to ED, or with fibrosis of the corpus spongiosum, which is responsible for urethral strictures (spongiofibrosis) (FIG. 2). These processes differ in their aetiology, time course and treatment, but have many features in common with other fibrotic disorders that develop through similar molecular pathways (such as activation of the fibrin cascade leading to chemotaxis of macrophages and other cells of the innate immune system, together with induction of a phenotypic switch of resident fibroblasts into profibrotic myofibroblasts)[8].

Stem cells

Stem cells are capable of self-renewal and their potential for differentiation can give rise to mature cell populations. Depending on the environmental conditions, stem cells can follow different paths of differentiation; they can remain in a dormant (quiescent) state, undergo apoptosis or elicit cell division to yield either two daughter stem cells, one daughter stem cell and one differentiated cell, or two differentiated cells[43,44].

Depending on their potential for differentiation, stem cells are classified as totipotent stem cells, pluripotent stem cells or multipotent stem cells. The stem

cells of the morula have totipotent characteristics and can differentiate into any completely differentiated organ or extra-embryonic tissue. Embryonic stem cells (ESCs) are pluripotent and have the ability to produce all germinal layers[45,46], whereas multipotent stem cells (such as MSCs; also termed multipotent stromal cells) can be isolated from organs and can differentiate into any cell type within their germ tissue[47]. ESCs have two main advantages over MSCs, the first of which is their ability to proliferate for longer periods of time, and the second is their capacity to differentiate into a broader range of cell types. However, owing to the ethical conflict that surrounds ESCs, their use in research has been limited and, as such, MSCs are a more feasible option for research and therapeutic applications[12–15,46].

MSCs
MSCs were first described in 1968 as fibroblast-like cells derived from the bone marrow[48], and were subsequently isolated from a variety of other adult tissues, including adipose tissue, periosteum, synovial fluid, periodontal ligament, fallopian tube, brain and muscle tissue recovering from injury[49]. A growing body of evidence indicates that a substantial fraction of cells that exhibit the in vitro characteristics of isolated MSCs arise from vascular pericytes in vivo, suggesting that a common precursor cell type exists in a wide variety of adult and fetal tissues[17,39,50–53].

In the past few decades, many studies have shown that MSCs secrete cytokines that have trophic effects on cells, promoting cytoprotection, cell survival and immunomodulation[12]. Importantly, the therapeutic use of allogenic MSCs does not always require the use of immune suppression. MSC rejection and immunogenicity is poorly understood, but it seems that MSCs do not escape the immune system by default (that is, they are not 'immune privileged'); instead, they create an immunosuppressive environment and evade the immune system by impeding T cell activation and antigen-presenting cell (APC) maturation ('immune evasive')[54]. Thus, MSCs that do not manage to tip the balance towards an immunosuppressive state are still detected and destroyed by APCs (like any other allogenic cell), and debris from dead MSCs (either through apoptosis or phagocytosis) can further activate APCs in the context of a danger signal[54,55].
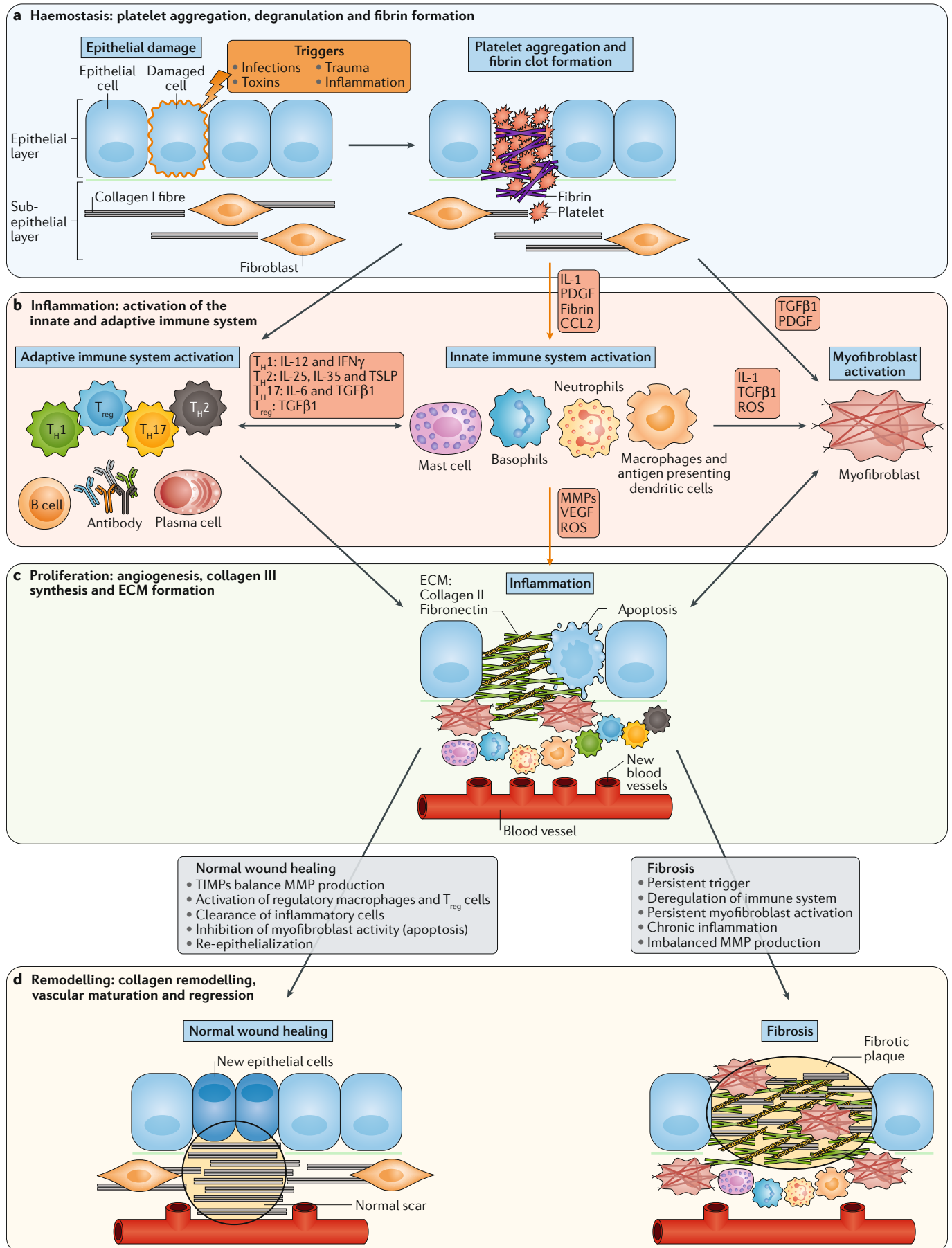
The considerable therapeutic potential of MSCs has generated increasing interest in a wide variety of biomedical disciplines[56]. However, investigators studying MSCs have used different methods of MSC isolation, expansion and characterization[57], which has generated a large number of ambiguities and inconsistencies in the field. To address this issue, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) have proposed minimal criteria to define human MSCs. Specifically, MSCs must be plastic-adherent in standard culture conditions (culture flasks, incubation at 37 °C, 5% CO_2); express CD105, CD73 and CD90 on 95% of the MSC population, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR (also known as CD74) surface molecules on <2–5% of the population (on flow cytometry analysis); and have the ability to differentiate into osteoblasts, adipocytes and chondroblasts under standard in vitro differentiating conditions[57].

Adult MSCs can differentiate into various types of terminally differentiated daughter cells within the mesodermal lineage[58]. Pluripotent and multipotent stem cells can give rise to hundreds of different cell types through distinct steps of differentiation[58]. Stem cell fate is determined by a complex network of epigenetic, transcriptional and post-transcriptional, and translational and post-translational regulatory mechanisms[59,60]. Importantly, a major role for epigenetic modifications through DNA methylation in the maintenance of dedifferentiated states has become clear. DNA (cytosine-5)-methyltransferase 1 (DNMT1) is an enzyme responsible for DNA methylation (and, therefore, transcriptional inhibition) of DNA regions that are important for cellular differentiation. DNMT1 loss leads to the failure of proliferative self-renewal and premature differentiation of stem cells[59]. Few studies have investigated the percentage of the stem cell genome that remains methylated once the cells are taken out of their in vivo niche to prepare them for therapeutic application. However, it seems that, when cultured, MSCs have a tendency to maintain their in vivo methylation patterns (90% of unmethylated genes remain unmethylated, and ~60% of methylated genes remain methylated)[59,60].

MSC differentiation and secretome. Until about a decade ago, the capacity of MSCs for multipotent division was thought to enable these cells to restore damaged tissues by replacing dysfunctional, damaged or dead cells[45,58]. Additionally, studies have increasingly shown that MSCs release a broad spectrum of regulatory and trophic factors (growth factors, cytokines and chemokines), suggesting a paracrine role for MSCs as 'site-regulated drug stores' in vivo, whereby MSCs can influence the local tissue environment even if they do not engraft or differentiate[12–14,49,61].

Importantly, evidence has accumulated over the past decade to suggest that endogenous MSCs have a crucial role in the regulation of the normal wound-healing process. Specifically, MSCs can accelerate wound contraction and increase the quality of the tissue, counteract healing anomalies and reduce the amount of ECM produced (which contributes to an improved functional and cosmetic result of the scar)[51,61–69]. However, evolutionary natural selection has led to rapid, rather than perfect, healing processes; inefficient endogenous regulation of this process can result in the formation of hypertrophic and debilitating scar tissue (for example, hypertrophic scar formation (keloid), loss of hair follicles after burn wounds or cardiac fibrosis resulting from an ischaemic cardiac event)[1,37,70]. In such instances, preclinical studies have shown that administration of autologous MSCs can shift the balance towards normal wound-healing, indicating that exogenous administration of the body's own MSCs in high concentrations, and at the correct site, can support the endogenous healing process[71–75]. Supported by these observations, researchers have initiated clinical trials in the past decade to exploit the immune-regulatory and paracrine properties of MSCs

a Haemostasis: platelet aggregation, degranulation and fibrin formation

Epithelial damage

Epithelial cell　Damaged cell

Triggers
- Infections　• Trauma
- Toxins　• Inflammation

Platelet aggregation and fibrin clot formation

Epithelial layer

Sub-epithelial layer

Collagen I fibre

Fibroblast

Fibrin
Platelet

IL-1
PDGF
Fibrin
CCL2

TGFβ1
PDGF

b Inflammation: activation of the innate and adaptive immune system

Adaptive immune system activation

T_H1: IL-12 and IFNγ
T_H2: IL-25, IL-35 and TSLP
T_H17: IL-6 and TGFβ1
T_{reg}: TGFβ1

Innate immune system activation

Myofibroblast activation

T_H1　T_{reg}　T_H17　T_H2

Neutrophils

IL-1
TGFβ1
ROS

B cell　Antibody　Plasma cell

Mast cell　Basophils　Macrophages and antigen presenting dendritic cells

Myofibroblast

MMPs
VEGF
ROS

c Proliferation: angiogenesis, collagen III synthesis and ECM formation

ECM:
Collagen II
Fibronectin

Inflammation

Apoptosis

New blood vessels

Blood vessel

Normal wound healing
- TIMPs balance MMP production
- Activation of regulatory macrophages and T_{reg} cells
- Clearance of inflammatory cells
- Inhibition of myofibroblast activity (apoptosis)
- Re-epithelialization

Fibrosis
- Persistent trigger
- Deregulation of immune system
- Persistent myofibroblast activation
- Chronic inflammation
- Imbalanced MMP production

d Remodelling: collagen remodelling, vascular maturation and regression

Normal wound healing

New epithelial cells

Normal scar

Fibrosis

Fibrotic plaque

◀ Fig. 1 | **Overview of the wound-healing process and fibrosis. a** | During an acute tissue insult, innate immune cells are recruited to restore homeostasis via normal wound healing. During the haemostasis phase, tissue damage activates the coagulation cascade and induces platelet activation and blood clot formation. When the epithelium is damaged, prestored interleukin-1 (IL-1) is released. Moreover, circulating platelets release coagulation factors for haemostasis (fibrin activation); and growth factors, such as platelet-derived growth factor (PDGF), a potent chemoattractant for inflammatory cells and mitogenic factor for myofibroblasts, and transforming growth factor-β1 (TGFβ1), which stimulates extracellular matrix (ECM) production and fibroblast-to-myofibroblast transformation[1,38,165]. Aside from its ubiquity in the coagulation cascade, thrombin also activates C-C motif chemokine 2 (CCL2)[4], and myeloid cells (macrophages) migrate from the bone marrow to the site of injury in response to the CCL2 gradient. Together with neutrophils, these cells release a wide array of toxic mediators — reactive oxygen species (ROS) and reactive nitrogen species (RNS) — that exacerbate tissue damage if they are not removed in a timely manner[166]. **b** | The inflammation phase is characterized by the chemotaxis and activation of innate immune cells by CCL2, IL-1, PDGF and TGFβ1. The classic inflammatory macrophage exhibits a strong microbicidal or tumoricidal activity through expression of IL-1β, tumour necrosis factor (TNF) and IL-6 (REFS[70,167]). Additionally, macrophages also produce large quantities of TGFβ1, which has an important role in myofibroblast generation[87]. Local inflammation activates naive (unactivated) T cells of the adaptive immune system towards different phenotypes via various cytokines[2]. Precursor B cells are activated mainly by T cell-dependent cytokines and antigen presentation (although T cell-independent pathways also exist). A fully matured B cell becomes a nondividing plasma cell, producing large quantities of antibodies directed against a specific antigen[168]. **c** | During the proliferation phase, the immune system — via secretion of cytokines, chemokines and free radicals — attempts to remove the triggering factor while activating the myofibroblasts that regulate angiogenesis and ECM production in normal wound healing[169]. In fibrotic conditions, dysregulation of genes involved in ECM remodelling occurs; for example, downregulation of matrix metalloproteinases (MMPs; which remove excessive collagen fibres) and upregulation of tissue-inhibitors of metalloproteinases (TIMPs; produced by myofibroblasts and macrophages)[170–172]. **d** | During the remodelling phase, activated myofibroblasts promote wound contraction. Different collagen types (switch from type III to type I collagen) are produced, blood vessels repaired, excessive scar tissue removed, and epithelial cells divide and migrate over the basal layers to regenerate the epithelium, restoring the damaged tissue to its normal appearance with minimal scar tissue[2,37]. Failure to effectively eliminate the triggering factors can induce persistent inflammation of the tissue, which leads to fibrosis. A few crucial concepts are generally applicable for the progression of fibrotic disease: persistent chronic tissue injury owing to chronic exposure and/or inflammation; ongoing recruitment of innate and adaptive immune cells to create a profibrogenic environment favouring chronic inflammation; and inefficient remodelling modulated by tissue hypoxia and subsequent neoangiogenesis[173,174]. TSLP, thymic stromal lymphopoietin. T_H cell, T helper cell; T_{reg} cell, regulatory T cell; VEGF, vascular endothelial growth factor.

in several therapeutic scenarios, including fibrotic disease[56]. Notable examples include the attenuation of graft-versus-host disease[76], immunomodulation in multiple sclerosis[77], arthritis[78] and Crohn's disease[79], and the induction of trophic effects in brain and spinal cord injury[80] and myocardial infarction[81].

The precise antifibrotic properties of exogenously administered MSCs are not yet properly understood and are a topic of intensive research[82,83]. A growing body of evidence suggests that MSCs function through a plethora of mechanisms including, but not limited to, immunomodulation of the innate and adaptive immune system, reactive oxygen species (ROS) neutralization and/or scavenging, promotion of angiogenesis, and interaction with the tissues and cells responsible for the fibrotic process (for example, by blocking myofibroblast activation)[84]. Nonetheless, MSCs have been described to exert both profibrotic and antifibrotic effects, depending on the inflammatory setting, dosing and time interval of repeated MSC administration[17,35]. This paradox can be

in part explained by the broad immunoregulatory capabilities of MSCs, whereby they can influence both the adaptive and innate immune systems[85] (FIG. 3).

In the presence of inflammation (high levels of tumour necrosis factor (TNF) and interferon (IFN)-γ), MSCs adopt an immunosuppressive phenotype and secrete high levels of anti-inflammatory and antifibrotic soluble factors, including nitric oxide (NO), prostaglandin E2 (PGE2), interleukin-10 (IL-10), hepatocyte growth factor (HGF), indoleamine 2,3-dioxygenase (IDO) and haem oxygenase (HO)[17].

NO is one of the most important antifibrotic agents and functions through the direct inhibition of ECM and transforming growth factor-β1 (TGFβ1) production, induction of myofibroblast apoptosis and stimulation of MMP secretion[86]. NO released from MSCs can also scavenge ROS through the formation of less-toxic reactive nitrogen species (RNS), such as peroxynitrite[12,13,47]. Prolonged ROS exposure during wound-healing induces fibrosis through a mechanism involving membrane lipid oxidation and induction of TGFβ1 expression[87].

PGE2 from endothelial cells, fibroblasts and/or myofibroblasts, and cells of the innate immune system, inhibits the release of IL-2 from T cells and dendritic cells, thereby reducing T cell proliferation at the wound site[88]. PGE2 is also involved in shifting the balance from a T helper 1 (T_H1) cell-response into a T_H2 cell-response by inhibition of IFNγ production and upregulation of IL-4 expression, respectively. Low IFNγ and high IL-4 levels lead to polarization towards the M2 macrophage phenotype[88–90]. M2 macrophages have a complex role in inflammation, and are generally accepted to be mainly anti-inflammatory, regulate wound healing, suppress T cell responses and suppress the host defence system[91,92].

IL-10 is a powerful inhibitor of neutrophil invasion and, therefore, prevents the further release of ROS during inflammation[93]. IL-10 might also reduce the expression of TGFβ1 and collagens and, additionally, increase the release of MMPs by myofibroblasts[93]. HGF reduces fibrosis through several mechanisms, the most notable of which being the downregulation of TGFβ1 and collagen production[1,4,36].

Angiogenesis is an indispensible process during the proliferation phase of wound healing and provides a source of nutrients for the production of ECM components. The absence of adequate angiogenesis leads to deficiencies in wound closure and might lead to the development of a chronic non-healing wound[1,4,36]. Importantly, stem cells release basic fibroblast growth factor (bFGF) and vascular endothelial growth factor A (VEGFA), which provide powerful mitogenic stimuli to promote the proliferation of endothelial cells[12,13,15].

Thus, the ability of MSCs to adapt according to their environmental conditions is crucial for understanding their therapeutic potential in fibrotic diseases.

Stem cell therapy for penile fibrosis

Penile fibrosis, an umbrella term for fibrotic disorders affecting different components of the penis, can be localized either on the tunica albuginea, the corpora cavernosa or the urethral corpus spongiosum (FIG. 2). Owing to a lack of available medical treatment options,
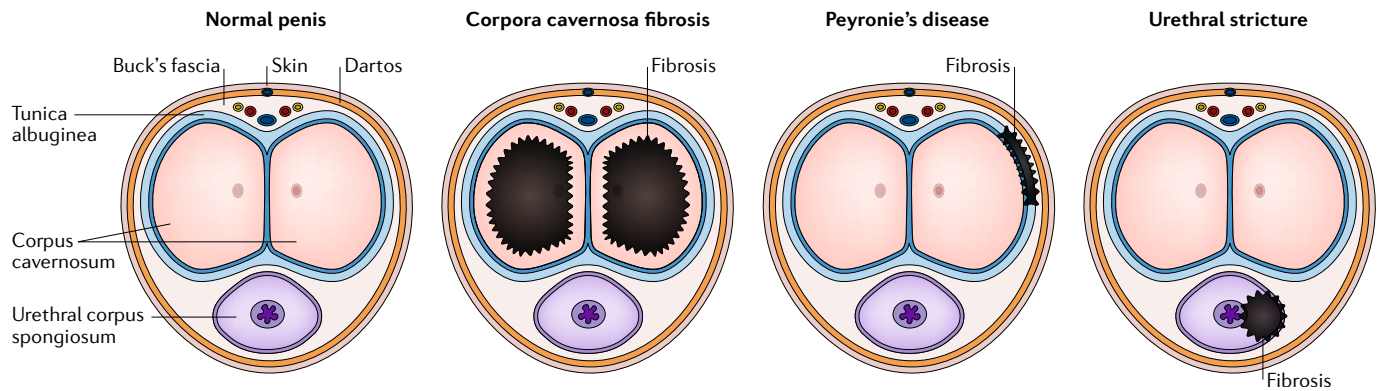
Fig. 2 | **Penile fibrosis.** Peyronie's disease, urethral stricture and corpora cavernosa fibrosis are fibrotic disorders of the penile connective tissues that are characterized by changes in the collagen composition (formation of a fibrous plaque) of the tunica albuginea, the urethral corpus spongiosum and the penile corpora cavernosa, respectively[8,11]. Corpora cavernosa fibrosis is characterized by a generalized fibrosis in the erectile tissue of the penis. When the expansion of corporal spongy tissue is compromised as a result of fibrosis, rapid filling of the sinusoids will no longer occur, leading to a subsequent lack of compression of the subtunical venules[94], rendering the penis incapable of becoming completely rigid (corporal veno-occlusive dysfunction). The most important aetiological factors for corpora cavernosa fibrosis include atherosclerosis (owing to chronic penile hypoxia), diabetes mellitus (owing to endothelial dysfunction, nerve damage, impaired arterial flow, reactive oxygen species and advanced glycation end-products (AGEs)), and iatrogenic causes (damage of the neurovascular bundle during prostatectomy in patients with prostate cancer)[94–96]. Peyronie's disease is a localized fibrotic disorder situated on the tunica albuginea of the penis. Peyronie's disease is associated with erectile dysfunction (veno-occlusive dysfunction), curvatures of up to 90° or more (which impair sexual intercourse) and up to 50% of patients experience clinically significant depression and/or relationship problems[175]. The leading hypothesis states that Peyronie's disease is caused by repetitive microtrauma as a result of buckling of the penis during intercourse[176]. Patients experience an acute phase with a painful penis (usually during erections), no palpable plaque and a progressive curvature. After 12–24 months, the disease evolves into a chronic, stable plaque and curvature that is painless. Currently available treatment options include surgery and intratunical injections of collagenase in stable disease. Other (medical) treatment options have proven unsuccessful[177]. Urethral strictures are characterized by an abnormal narrowing of the urethra that functionally causes a bladder outlet disorder, which can negatively affect a patient's quality of life by leading to genitourinary infection (epididymitis, prostatitis, cystitis or pyelonephritis), impairing voiding and causing secondary bladder overactivity (with complaints of urgency and urgency incontinence)[9]. Several aetiologies have been proposed and are categorized as iatrogenic, traumatic, inflammatory and idiopathic[9]. Since the advent of endourological surgical techniques and urine catheters, the iatrogenic factors have been the most important causes of urethral strictures.

stem cells — specifically MSCs — have been investigated in both preclinical and clinical studies to inhibit the fibrotic process.

Corpora cavernosa fibrosis

Historically, the concept of penile fibrosis has almost exclusively been associated with fibrosis of the tunica albuginea and urethral stricture. Conversely, fibrosis of the corpora cavernosa was considered to be a rare condition that was only seen after trauma or following priapism[10]. However, in the past two decades, several studies have shown that corpora cavernosa fibrosis is a common pathological disorder underlying most cases of vasculogenic and/or neurogenic ED[8]. Penile erection is controlled by a sophisticated mechanism that requires the cooperation of the nitrergic and adrenergic nerves, endothelium and smooth muscle cells of the corpora cavernosa[94]. Diseases and conditions that influence one or more of these elements might cause corporal fibrosis and, therefore, lead to ED[8]. When the elasticity and compliance of the corporal spongy tissue is reduced (for example, when the corpora cavernosa become fibrotic), filling of the sinusoids with blood will no longer result in the effective engorgement of the corpora cavernosa and will subsequently lead to the insufficient compression of the subtunical venules[94]. This lack of compression will

result in leakage of blood from the corporal tissue (corporal veno-occlusive dysfunction; also called 'venous leak') during erection, which renders the penis incapable of becoming completely rigid. ED is caused by several common medical disorders and is defined as the persistent inability to attain and/or maintain an erection that is sufficient for satisfactory sexual performance[94]. For example, diabetes mellitus results in endothelial dysfunction, impaired nitrergic nerve function and the overproduction of ROS and advanced glycation end-products (AGEs) in the corpora cavernosa[95]. These events, in turn, lead to the reduced bioavailability of NO and, consequently, impairment of smooth muscle relaxation. In advanced diabetic disease, apoptosis and impaired regeneration of vital cells in the corpora cavernosa, such as endothelial and smooth muscle cells, occurs[8]. The combination of these factors, in addition to chronic relative hypoxia caused by the absence of regular tumescence, render a chronic inflammatory state in the corpora cavernosa and induce the transition of healthy smooth muscle cells into myofibroblasts, which, in combination with infiltrating inflammatory cells, deposit ECM proteins and cause fibrosis[96]. Thus, corporal fibrosis should be considered as a potential cause of treatment failure in ED, particularly because it leads to the sustained impairment of the erectile mechanism, even
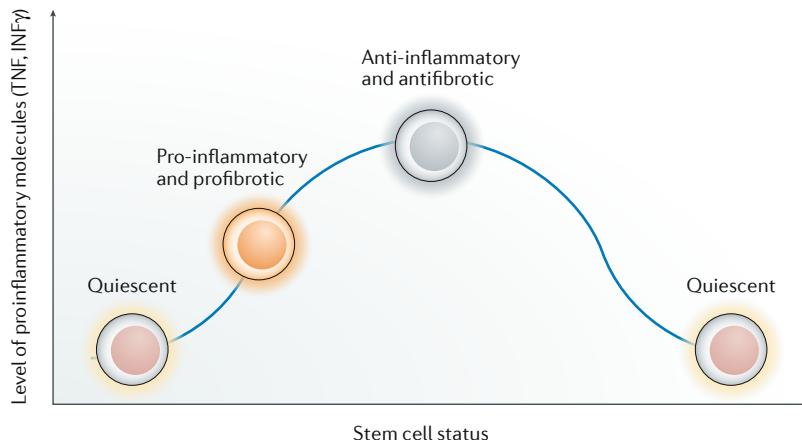
Fig. 3 | **Pro-inflammatory and anti-inflammatory MSC phenotypes.** Mesenchymal stem cells (MSCs) can both promote and inhibit fibrosis, depending on the inflammatory context in which they function[35]. This paradox is in part explained by their broad immunoregulatory capacities with respect to both the adaptive and innate immune system[85]. By interacting with cells of the innate immune system, MSCs can elicit both anti-inflammatory and pro-inflammatory effects[35]. MSCs are predominantly in a state of quiescence and reduced cellular metabolism[47]. This quiescent state seems to be necessary for the long-term preservation of the reconstituting capacity of stem cells. Stem cells can exit quiescence and rapidly expand and differentiate in response to stress[35]. In the absence of inflammation (low tumour necrosis factor (TNF) and interferon (IFN)-γ levels), MSCs can adopt a pro-inflammatory phenotype and enhance M1 macrophage polarization and T cell response by secreting pro-inflammatory cytokines (IL-1, IL-2, TNF and IFNγ)[35,85]. Secretion of TGFβ1 induces the myofibroblast phenotype, further establishing the inflammatory and profibrotic milieu. In the presence of inflammation (high TNF and IFNγ levels), MSCs are activated and adopt an immunosuppressive phenotype[35]. Activated MSCs can also skew the differentiation of monocytes towards an anti-inflammatory and antifibrotic profile, therefore, shifting the balance towards anti-inflammatory M2 macrophage polarization rather than pro-inflammatory M1 macrophage polarization.

Neuropraxia
Temporary loss of motor and/or sensory function in a nerve from the peripheral nervous system as a result of impaired nerve conduction. Neuropraxia usually recovers fully after 6–8 weeks.

Wallerian degeneration
Active nerve degeneration resulting from a nerve being cut or crushed, whereby the axonal tail distal to the damage (furthest from the neuronal body) degenerates.

when the primary cause has been resolved (for example, after nerve regeneration in iatrogenic neuropraxia of the cavernous nerves)[8].

Iatrogenic ED, including erectile impairment following the surgical treatment of prostate cancer (radical prostatectomy), is a well-characterized example of the relationship between ED and corpora cavernosa fibrosis. Studies have shown that only 23% of men aged <60 years regain their baseline potency after bilateral nerve-sparing radical prostatectomy (NSRP)[97]. During NSRP surgery, neuropraxia occurs owing to manipulation of the cavernous nerves followed by Wallerian degeneration and loss of conductivity, leading to functional denervation of the smooth muscle in the corpora cavernosa[98]. Similar to noniatrogenic ED, this process results in decreased NO bioavailability and the absence of nocturnal and ergogenic tumescence. The leading hypothesis for this phenomenon states that oxygenated blood is mainly supplied to the penis during erectile activity and, therefore, that the absence of spontaneous tumescence leads to a state of persistent cavernous hypoxia[98]. Both in vitro and in vivo studies support the hypothesis that penile hypoxia results in collagen accumulation, smooth muscle cell apoptosis, and, ultimately, fibrosis[96,99–101]. These changes again seem to be coordinated by TGFβ1, as multiple studies in rats have illustrated that TGFβ1 is upregulated at both

the gene and protein levels following cavernous nerve crush or transection injury[102,103]. Rho-associated coiled-coil protein kinase (ROCK) isoforms are upregulated and activated in the cavernous tissue following nitrergic denervation, as is the case in many other fibrotic disorders[104–106]. Pharmacological inhibition of ROCK has been shown to ameliorate a variety of fibrotic disorders, which probably occurs through inhibition of myofibroblast proliferation[104–106]. Thus, ROCK upregulation probably has an important role in corpora cavernosa fibrosis. Unsurprisingly, TGFβ1 is a potential driver of ROCK activation[104–106].

A final key factor explaining the emergenece of ED after NSRP is the imbalance of the parasympathetic and orthosympathetic nervous system. As a result of neuropraxia of the parasympathetic nerves, an enhanced adrenergic tonus occurs in the corpora cavernosa, which impedes the relaxation of the smooth muscle[107]. Accordingly, long-term administration of α-adrenergic antagonists (α-blockers) was shown to improve erections, as well as potentiate the effect of the phosphodiesterase type 5 (PDE5) inhibitor tadalafil in a bilateral cavernous nerve injury (bCNI) rat model and in human corpus cavernosum strips from patients with vasculogenic ED[107].

When venous leak occurs, oral medication for ED (PDE5 inhibitors), as well as intracavernosal injections (for example, alprostadil), will have suboptimal or completely absent effects given that the fibrotic penis cannot trap the incoming blood resulting from the pharmacologically induced vasodilation. This observation implies that these patients must resort to penile prosthesis implantation, which is notoriously difficult in the context of fibrotic corporal tissue[108]. Thus, novel therapies that halt the progression of corpora cavernosa fibrosis are urgently needed.

Stem cell therapy for corpora cavernosa fibrosis. In the context of corpora cavernosa fibrosis, the application of stem and progenitor cells from a broad range of sources has been described, including adipose-derived stem cells (ADSCs), bone marrow stem cells (BMSCs), ESCs, endothelial progenitor cells (EPCs), urine-derived stem cells (USCs) and skeletal muscle-derived stem cells (SkMSCs)[32,33]. In the past decade, several studies have evaluated the effect of stem cell therapy on the recovery of erectile function in several animal models of ageing, diabetes mellitus and cavernous nerve injury[32,33]. This Review evaluates select studies that focused on the effect of stem cell therapy on corpora cavernosa fibrosis; studies in which the fibrotic status of the corpora cavernosa was not evaluated (those that only assessed functional erectile function changes) were not considered (TABLE 1). These select studies focused mainly on two aetologies of corpora cavernosa fibrosis using rat models — the diabetic rat model and the bCNI rat model — that mimic ED after radical prostatectomy[109–141].

In diabetic rat models, BMSCs, USCs and ADSCs have been investigated as treatments for ED (TABLE 1). In studies that evaluated administration of ADSCs and BMSCs, the authors reported contrasting results. One study that used a genetic rat model of diabetes mellitus

Table 1 | **Preclinical studies of stem cell therapy for penile fibrosis**

Study	Species	Disease model	Stem cell type	Characterization	Delivery and dose	Timing of treatment	Timing of evaluation	Method for fibrosis evaluation	Effect on fibrosis	Refs
Corpora cavernosa fibrosis										
Yang 2018	SD rat	Aged rats	alADSCs	CD29$^+$, CD90$^+$, CD31$^-$, CD34$^-$, CD45$^-$, CD106$^-$	i.c. (5×10^5 cells)	Age 24 months	2 weeks after alADSC treatment	Histology (MT and IHC); in vitro culture of corporal cells; immunofluorescence	Improved	[144]
Fang 2018	SD rat	NSRP-bCNI	xBMSCs, xUCBMSCs (human)	CD11b$^-$, CD31$^-$, CD45$^-$, CD73$^+$, CD90$^+$, CD103$^-$, CD105$^+$	Fibrin scaffold with SCs	During bCNI	2 weeks after treatment	Histology (MT and IHC); WB; qPCR	Improved	[146]
Matsuda 2017	SD rat	NSRP-bCNI	alBMSCs	CD45$^-$, CD90$^+$, CD106$^-$, CD73$^+$	i.v. (1×10^6 cells)	i.v. immediately after bCNI	After 2 days of treatment for qPCR; after 4 weeks of treatment for histology	Histology (MT and IHC); qPCR	Improved	[143]
Wang 2015	SD rat	Diabetes (STZ, 60 mg/kg)	alADSCs or alADSCs pretreated to induce sub-lethal hypoxia	CD90$^+$, CD29$^+$, CD45$^-$, CD34$^-$; adipogenic and osteogenic lineage differentiation in vitro	i.c. (1×10^6 cells)	8 weeks after STZ injection	4 weeks after alADSC treatment	Histology (MT and IHC)	Improved	[109]
Liu 2015	SD rat	Diabetes (STZ, 60 mg/kg)	alADSCs or alADSCs expressing HGF	CD90$^+$, CD105$^+$, CD45$^-$, CD13$^-$; no differentiation assay	i.c. (2×10^6 cells)	12 weeks after STZ injection	4 weeks after alADSC treatment	Histology (MT and IHC)	Improved	[110]
Liu 2013	SD rat	Diabetes (STZ, 40 mg/kg)	alADSCs or alADSCs expressing VEGF	CD73$^+$ (99.8%), CD90$^+$ (99.7%), CD105$^+$ (98.0%), CD146$^+$ (7.12%), CD31$^-$ (0.81%), CD34$^-$ (0.92%), CD45$^-$ (1.05%), CD117$^-$(0.86%); adipogenic and osteogenic lineage differentiation in vitro	i.c. (2×10^6 cells)	8 weeks after STZ injection	4 weeks after alADSC treatment	Histology (MT and IHC)	Improved	[139]
Ryu 2016	C57 BL/6 J mice	Diabetes (STZ, 50 mg/kg)	alBMSCs	CD44$^+$, SCA1$^+$, CD34$^-$, CD45$^-$, MHC II$^-$; adipogenic, chondrogenic and osteogenic lineage differ-entiation in vitro	i.c. (3×10^6 cells)	8 weeks after STZ injection	2 weeks after alBMSC treatment	Histology (MT and IHC)	Improved	[119]
Ouyang 2014	SD rat	Diabetes (STZ, 35 mg/kg plus high-fat diet for 4 weeks)	xUSCs (human) or xUSCs (human) expressing FGF2	CD24$^+$ (100%), CD29$^+$ (99.9%), CD44$^+$ (98.7%), CD73$^+$ (98.6%), CD90$^+$ (99.9%), CD146$^+$ (99.3%), SSEA-4$^+$ (98.4%), CD105$^+$ (8.06%), STRO-1$^+$ (14.03%), CD31$^-$ (2.1%), CD34$^-$ (2.75%), CD45$^-$ (0.18%); no differentiation assay	i.c. (1×10^6 cells)	8 weeks after STZ injection	4 weeks after xUSC treatment	Histology (MT and IHC)	Improved	[132]

Table 1 (cont.) | **Preclinical studies of stem cell therapy for penile fibrosis**

Study	Species	Disease model	Stem cell type	Characterization	Delivery and dose	Timing of treatment	Timing of evaluation	Method for fibrosis evaluation	Effect on fibrosis	Refs
Corpora cavernosa fibrosis (cont.)										
Cengiz 2017	SD rat	Diabetes (STZ, 35 mg/kg)	xUCBMSCs (human)	Not reported	i.c. (1×10^6 cells)	4 weeks after STZ injection	4 weeks after xUCBMSC treatment	Histology (MT)	Improved	[136]
Shan 2017	SD rat	Diabetes (STZ, 65 mg/kg)	alBMSCs	CD44+, CD90+, CD29+, CD34−, CD45−; no differentiation assay	i.c. (1×10^6 cells)	Not reported	4 weeks after alBMSC injection	Histology (MT)	Improved	[137]
Wang 2017	SD rat	Diabetes (STZ, 60 mg/kg)	alADSCs	CD90+, CD29+, CD45−, CD34−; adipogenic and osteogenic lineage differentiation in vitro	i.c. (1×10^6 cells)	Not reported	4 weeks after alADSC injection	Histology (MT)	Improved	[138]
Kovanecz 2016	ZDF rats	Diabetes (genetic)	alMDSCs	Not reported	i.c. (1×10^6 cells)	Not reported	8 weeks after alMDSC injection	Histology (MT); hydroxyproline assay	Improved	[138]
Garcia 2010	ZDF rats	Diabetes (genetic)	alADSCs	Not reported	i.c. (1×10^6 cells)	Not reported	5 weeks after alADSC injection	Histology (MT)	Worsened	[141]
Huang 2016	SD rat	Tobacco-associated-ED (2 hours of continuous cigarette smoke exposure per day, 5 days per week for 12 weeks)	aADSCs	Not reported	i.c. (1×10^6 cells)	12 weeks after tobacco exposure	4 weeks after aADSC treatment	Histology (MT)	Improved	[111]
Kovanecez 2012	Fisher rat	bCNR	xMDSCs (mouse)	SCA1+, CD34+, CD44+; no differentiation assay	i.c. (1×10^6 cells)	Immediately after bCNR	6 weeks after xMDSC treatment	Histology (MT)	Improved	[112]
Ying 2014	SD rat	bCNR	alADSCs	Not reported	i.v. (2×10^6 cells) plus microsurgical repair of the resected CN by autologous saphenous vein graft	Immediately after bCNR	12 weeks after alADSC treatment	Histology (MT)	Improved	[113]
Chen 2016	SD rat	bCNI	alADSCs	CD73+ (99.2%), CD90+ (94.5%), CD105+ (58.6%), CD146− (1.87%), CD31− (1.55%), CD34− (1.92%), CD45− (1.31), CD117− (1.28); adipogenic and osteogenic lineage differentiation in vitro	i.c. (1×10^6 cells)	Immediately after bCNI	4 weeks after alADSC treatment	Histology (MT)	Improved	[114]

Table 1 (cont.) | **Preclinical studies of stem cell therapy for penile fibrosis**

Study	Species	Disease model	Stem cell type	Characterization	Delivery and dose	Timing of treatment	Timing of evaluation	Method for fibrosis evaluation	Effect on fibrosis	Refs
Corpora cavernosa fibrosis (cont.)										
Albersen 2010	SD rat	bCNI	alADSC-derived or alADSC-derived lysate	Not reported	i.c. (1×10^6 cells) or lysate from 1×10^6 cells	Immediately after bCNI	4 weeks after treatment	Histology (MT)	Improved	[115]
Kim 2013	SD rat	bCNI	alADSCs or alADSCs covered with NGF-hydrogel	Not reported	Periprostatic implantation (1×10^6 cells)	Immediately after bCNI	4 weeks after treatment	Histology (MT, IHC)	Improved	[116]
You 2015	SD rat	bCNI	alADSCs or aSVF	• aSVF: CD45$^+$ (33%), CD452/CD31$^+$ (6%); 57% of cells expressed neither CD45 nor CD31; CD73 and CD90 were expressed by almost two-fifths of CD452$^+$ cells. • alADSCs: CD29$^+$ (99.8%), CD44$^+$ (99.9%), CD73$^+$ (99.9%), CD90$^+$ (99.9%), CD34$^-$ (0.2)%, CD45$^-$ (0.1%); no differentiation assay	i.c. (1×10^6 cells)	Immediately after bCNI	4 weeks after treatment	Histology (MT)	Improved	[117]
Martinez Salamanca 2016	WK rat	bCNI	alBMSCs	CD73$^+$, CD105$^+$, SH-4$^+$, vimentin$^+$, CD34$^-$, CD45$^-$, CD3$^-$, CD14$^-$, CD19$^-$, CD38$^-$, glycophorin A$^-$, HLA II$^-$; no differentiation assay	i.c. (1×10^6 cells) or oral tadalafil (5 mg/kg, for 4 weeks) plus i.c. (1×10^6 cells)	Immediately after bCNI	4 weeks after alBMSC treatment	Histology (MT)	Improved	[118]
Ryu 2014	C57 BL/6 J mice	bCNI	alBMSCs	CD44$^+$, SCA1$^+$ CD34$^-$, CD45$^-$, MHC II$^-$; no differentiation assay	i.c. or i.p. (5×10^5 cells)	Immediately after bCNI	2 weeks after alBMSC treatment	Histology (MT)	Improved	[121]
Qui 2012	SD rat	bCNI	aSVF	Not reported	i.c. of 400 ml SVF solution (5000 cells per μL)	The same day or 4 weeks after bCNI	2 weeks after aSVF treatment	Histology (MT)	Improved	[120]
Jeong 2013	SD rat	bCNI	xADSCs (human) covered with a BDNF-PLGA membrane	Not reported	Periprostatic implantation (1×10^6 cells)	Immediately after bCNI	4 weeks after xADSC treatment	Histology (MT)	Improved	[122]
Fandel 2012	SD rat	bCNI	alADSCs	Not reported	i.c. (2×10^6 cells)	Immediately after bCNI	4 weeks after alADSC treatment	Histology (MT)	Improved	[123]
Ying 2013	SD rat	bCNI	aADSCs	Not reported	i.c. (1×10^6 cells)	Immediately after bCNI	4 weeks after aADSC treatment	Histology (MT)	Improved	[124]

Table 1 (cont.) | **Preclinical studies of stem cell therapy for penile fibrosis**

Study	Species	Disease model	Stem cell type	Characterization	Delivery and dose	Timing of treatment	Timing of evaluation	Method for fibrosis evaluation	Effect on fibrosis	Refs
Corpora cavernosa fibrosis (cont.)										
You 2013	SD rat	bCNI	xBMSCs (human)	Not reported	i.c. (1×10^6 cells) or periprostatic implantation (1×10^6 cells)	Immediately after bCNI	4 weeks after alADSC treatment	Histology (MT)	Improved	[125]
You 2016	SD rat	bCNI	xBMSCs (human)	CD105$^+$ (99.74 ± 0.15%), CD73$^+$ (99.86 ± 0.08%), CD14$^-$ (0.85 ± 0.06%), CD34$^-$ (0.78 ± 0.05%), CD45$^-$ (0.64 ± 0.05%); no differentiation assay	i.c. (1×10^6 cells)	Immediately after bCNI; immediately after bCNI plus second injection after 14 days; 4 weeks after bCNI; 4 weeks after bCNI plus second injection after 14 days	4 weeks after xBMSC treatment	Histology (MT)	Improved	[126]
Zhu 2015	SD rat	bCNI	xUCBMSCs (human)	CD29$^+$ (99.95%), CD44$^+$ (97.2%), CD105$^+$ (90%), CD4$^-$ (0.05%), CD34$^-$ (0.73%); no differentiation assay	i.c. (2×10^6 cells)	Immediately after bCNI	4 weeks after xUCBMSC treatment	Histology (MT)	Improved	[127]
Song 2016	SD rat	bCNI	xUCBMSCs (human) or xUCBMSCs (human) expressing BDNF	Not reported	i.c. (2×10^6 cells)	Immediately after bCNI	4 weeks after xUCBMSC treatment	Histology (MT)	Improved	[128]
Yang 2016	SD rat	bCNI	xUSCs (human) or xUSCs-PEDF (human)	CD24$^+$, CD44$^+$, CD73$^+$, CD90$^+$, CD133$^+$, CD146$^+$, CD31$^-$, CD34$^-$, CD45$^-$; adipogenic and osteogenic lineage differentiation in vitro	i.c. (1×10^6 cells)	Immediately after bCNI	4 weeks after xUSC treatment	Histology (MT)	Improved	[135]
Tunica albuginea fibrosis (Peyronie's disease)										
Goktce 2014	SD rat	PD (TGFβ1 TA injection)	alADSCs	CD29$^+$ (97.98%), CD90$^+$ (90.02%), CD105$^+$ (22.4%), CD45$^-$ (1.69%), CD90$^-$ (2.16%), CD105$^-$ (2.66%); no differentiation assay	TA injection (5×10^5 cells)	The same day or 30 days after the TGFβ1 injection	5 weeks after alADSC treatment	Histology (MT)	Improved	[129]
Goktce 2015	SD rat	PD (TGFβ1 TA injection)	alADSCs or alADSCs expressing IFNα2b	CD29$^+$ (97.98%), CD90$^+$ (90.02%), CD105$^+$ (22.4%), CD45$^-$ (1.69%); no differentiation assay	TA injection (5×10^5 cells)	The same day or 4 weeks after TGFβ1 injection	5 weeks after alADSC treatment	Histology (MT and VVG)	Improved	[130]
Castiglione 2013	SD rat	PD (TGFβ1 TA injection)	xADSCs (human)	CD14$^-$, CD34$^-$, CD45$^-$, HLA-DR$^-$, CD29$^+$, CD90$^+$, CD166$^+$, CD73$^+$, CD105$^+$, CD44$^+$, HLA-ABC$^+$; adipogenic and osteogenic lineage differentiation in vitro	TA injection (1×10^6 cells)	The day after TGFβ1 injection	5 weeks after xADSC treatment	Histology (IHC); WB	Improved	[131]

Table 1 (cont.) | **Preclinical studies of stem cell therapy for penile fibrosis**

Study	Species	Disease model	Stem cell type	Characterization	Delivery and dose	Timing of treatment	Timing of evaluation	Method for fibrosis evaluation	Effect on fibrosis	Refs
Tunica albuginea fibrosis (Peyronie's disease) (cont.)										
Levy 2016	Human	Patients with PD	alPMDMSCs	Not reported	TA and CC injection (cell count not reported)	Not reported	6 months after alPMDMSC injection	Erectile function evaluation by IIEF and Doppler ultrasound measurements	Improved	134
Jiang 2017	SD rat	TA-derived fibroblasts; stimulation with TGFβ1 until myo-fibroblasts	alADSCs	CD90+, CD31−, CD34−, CD45−, CD44+	Co-culture of myofi-broblasts and alADSCs	After transfor-mation; co-culture	After 24, 48 and 72 hours of incubation	Hydroxyproline concentration; collagen contraction assay; WB	Improved	157
Urethral stricture										
Castiglione 2016	SD rat	US (TGFβ1 urethral injection)	xADSCs (human)	CD14−, CD34−, CD45−, HLA-DR−, CD29+, CD90+, CD166+, CD73+, CD105+, CD44+, HLA-ABC+; adipogenic and osteogenic lineage differentiation in vitro	Urethral injection (1×10^6 cells)	The day after TGFβ1 injection	4 weeks after xADSC treatment	Histology (MT); WB	Improved	31
Sangkum 2016	SD rat	US (TGFβ1 urethral injection)	alADSCs	CD29+ (97.98%), CD90+ (90.02%), CD105+ (22.4%), CD45− (1.69%); no differentiation assay	TA injection (2×10^5 cells	The same day of TGFβ1 injection	2 weeks after alADSC treatment	Histology (MT) WB	Improved	133

aADSCs, autologous adipose-derived stem cells; alADSCs, allogeneic adipose-derived stem cells; alBMSCs, allogeneic bone marrow-derived stem cells; alMDSCs, allogeneic muscle-derived stem cells; alPMDMSCs, allogeneic placental matrix–derived mesenchymal stem cells; aSVF, autologous stromal vascular fraction; bCNI, bilateral cavernous nerve crush injury; bCNR, bilateral cavernous nerve resection; BDNF, brain-derived neurotrophic factor; CC, corpora cavernosa; CN, cavernous nerve; ED, erectile dysfunction; FGF2, fibroblast growth factor 2; HGF, hepatocyte growth factor; HLA-ABC, MHC class I antigen; HLA-DR, human leukocyte antigen DR isotype; i.c., intracavernosal injection; IFNα2b, interferon-α2b; IHC, immunohistochemistry; IIEF, International Index of Erectile Function; i.p., intraperitoneal injection; i.v., intravenous injection; MHC II, class II major histocompatibility complex; MT, Masson's trichrome; NGF, nerve growth factor; NSRP, nerve-sparing radical prostatectomy; PD, Peyronie's disease; PLGA, poly-lactic-co-glycolic; qPCR, quantitative PCR; SCA1, ataxin-1; SD, Sprague-Dawley; SSEA-4, stage-specific embryonic antigen-4; STZ, streptozotocin; TA, tunica albuginea; TGFβ1, transforming growth factor-β1; US, urethral stricture; VEGF, vascular endothelial growth factor; VVG, Verhoeff-van Gieson; WB, western blot; WK, Wistar Kyoto; xADSCs, xenogeneic adipose-derived stem cells; xBMSCs, xenogeneic bone marrow-derived stem cells; xMDSC, xenogeneic muscle-derived stem cells; xUCBMSCs, xenogeneic umbilical cord blood mesenchymal stem cells; xUSCs, xenogeneic urine-derived stem cells; xUSCs-PEDF, xenogeneic urine-derived stem cells genetically modified with pigment epithelium-derived factor; ZDF, Zucker diabetic fatty.

reported that the ratio of intracavernosal collagen to smooth muscle was not significantly different between autologous ADSC treatment and control groups[141]. However, an increase in corpora cavernosa innervation and endothelial cell number was observed in the treatment group. Conversely, another study reported that allogeneic ADSC treatment enhanced the recovery of the smooth muscle and reduced fibrosis in corpus cavernosum of a streptozotocin-induced model of diabetes mellitus in rats[109]. Two studies found that the injection of BMSCs markedly decreased cavernous fibrosis and improved smooth muscle content in mice with chemically-induced diabetes after only 2 weeks[119,137]. In addition, these studies confirmed the improvements in nitrergic innervation and endothelial cell content with stem cell therapy.

Other studies have modified the stem cells before injection to increase their efficacy. The ability of allogeneic ADSCs modified to express VEGF — a signalling protein normally produced by endothelial and inflammatory cells that stimulates angiogenesis and has possible immunomodulatory effects — to reverse diabetic ED in rats was evaluated in one study[139]. This study reported an increase in the intracavernosal smooth muscle:collagen ratio and endothelial nitric oxide synthase (eNOS) expression in the group treated with *VEGF*-transfected ADSCs compared with those that received unmodified ADSCs. Another group evaluated the efficacy of ADSCs expressing HGF in a diabetic rat model[110]; ADSCs improved erectile function and decreased fibrosis (increased content of smooth muscle, improved smooth muscle:collagen ratio and lower apoptotic index), but *HGF*-transfected ADSC had superior effects with respect to almost all parameters. Additionally, *HGF*-transfected ADSCs increased platelet and endothelial cell adhesion molecule 1 (PECAM1) and eNOS expression levels, further suggesting attenuated fibrosis.

Fibroblast growth factor 2 (FGF2) has an important role in the regulation of cell survival, cell division,

angiogenesis, differentiation and cell migration[142]. In a diabetic rat model of ED and penile fibrosis, intracavernous injection of human USCs transfected with *FGF2* markedly improved the intracavernosal smooth muscle:collagen ratio and increased the expression of VEGF and eNOS compared with other treatments (vehicle, USC only and *FGF2* delivered to the tissue via lentivirus)[132].

Several studies have evaluated the effect of stem cell therapy in rat models of radical prostatectomy (modelled by severing the cavernous nerves, either by crushing or transection). The first use of allogeneic ADSCs in the bCNI rat model was reported in 2010 (REF.[115]). In this study, erectile function was improved at 4 weeks after allogeneic ADSC injection. Moreover, smooth muscle content was partially restored, corpora cavernosa fibrosis was prevented and neuronal nitric oxide synthase (nNOS; encoded by *Nos1*) expression was restored in the dorsal penile nerves. The findings of many other studies that used similar methodologies support these results and confirmed that prevention of corpora cavernosa fibrosis in the bCNI rat model was possible through intracavernous injection of ADSCs[113,114,123,124]. Furthermore, these studies investigated whether injected ADSCs remained at the site of injection (suggesting engraftment) or whether they were transported elsewhere. Surprisingly, they found that ADSCs dispersed to a niche resembling their usual site (for example, the perivascular space of the bone marrow) or the major pelvic ganglion (MPG). Two studies also reported that periprostatic application of human ADSCs incorporated in hydrogels and/or membranes containing neurotrophic factors prevented corpora cavernosa fibrosis and increased eNOS protein expression after 4 weeks in a bCNI rat model[116,122].

Intravenous infusion of green fluorescent protein (GFP)-labelled allogeneic BMSCs was also effective in preventing corpora cavernosa fibrosis in a bCNI rat model[143]. Interestingly, following infusion, BMSC homing to the MPGs and injured cavernous nerves was observed. Additionally, higher mRNA expression of brain-derived neurotrophic factor (*Bdnf*) and glial cell line-derived neurotrophic factor (*Gdnf*) was seen in the MPGs of BMSC-treated rats compared to untreated rats, indicative of improved neuronal repair mechanisms.

Allogeneic ADSCs have been shown to secrete greater levels of bFGF, insulin-like growth factor (IGF) and VEGF than resident corporal cells, and allogeneic ADSC treatment was shown to increase the concentration of these molecules in the penile tissues of aged rats, which had the beneficial effect of lowering oxidative stress[144]. Allogeneic ADSCs also increased the endothelial and cavernous smooth muscle content in the corpora cavernosa, which led to improved erectile function.

Stromal vascular fraction (SVF) is a component of the lipoaspirate obtained from liposuction of excess adipose tissue, which contains a large population of ADSCs[145]. In two studies that used the same animal model of NSRP[117,120], SVF treatment — either immediately after or 4 weeks after CNI injury — markedly prevented and reduced corpora cavernosa fibrosis and increased the expression of nNOS and neurofilament in the dorsal penile nerves, even in comparison with allogeneic ADSC injection.

The most commonly used stem cells in basic research have been ADSCs, but a few studies have evaluated the effect of xenogeneic BMSCs and human umbilical cord blood MSCs (UCBMSCs) (TABLE 1). BMSCs were equally efficient as daily administration of tadalafil in preventing fibrosis in a bCNI rat model[118]. Both intracavernosal and intraperitoneal injections of BMSCs markedly restored endothelial and smooth muscle content in the corpora cavernosa, as well as penile levels of nNOS and neurofilament[121].

An additive effect of using a fibrin scaffold containing xenogeneic BMSCs and UCBMSCs in rats during bCNI surgery was observed with respect to improved erectile function, increased nNOS and PECAM1 mRNA expression and decreased mRNA expression of α-actin expression and caspase 3 (REF.[146]); histological assessment and immunostaining corroborated these results.

Overall, a large amount of preclinical data suggests the benefit of stem cells (predominantly ADSCs) as an antifibrotic treatment strategy for corpora cavernosa fibrosis. However, further characterisation of the route of administration, timing and the molecular effectors of stem cells will be required to translate these findings into the clinical setting.

Peyronie's disease

Peyronie's disease is a chronic fibrotic disease of the penile shaft, the course of which occurs over 12–24 months and is divided into the acute (exacerbating curvature; painful) and chronic (stabilization of deformation; painless) phases[11]. Peyronie's disease is commonly believed to occur as a result of repetitive trauma and buckling of the tunica albuginea during intercourse[96]. Two leading theories concerning the onset of Peyronie's disease are commonly referred to in the scientific literature[6,96,147]. The first theory suggests that trauma separates the inner and outer layer of tunica albuginea, preferentially at the dorsomedial aspect of the penis where the inner layer forms the intercavernous septum. The second hypothesis suggests that trauma separates the sinusoidal tissue from the inner layer of the tunica albuginea, causing haematoma formation in the space of Smith. The first hypothesis is evidenced by the fact that Peyronie's disease most often occurs at the dorsomedial aspect of the penis, whereas the most convincing argument for the second theory is the finding that fibrotic plaques in Peyronie's disease frequently extend into the corpus cavernosum and are not limited to the tunica albuginea.

Independent of the exact pathophysiological mechanism, these virtual spaces (either between the inner and outer layer of the tunica albuginea or between the inner layer and the space of Smith) represent planes in which endothelial damage can occur resulting from rupture of the small vessels during trauma or friction. The role of trauma in the pathophysiology of Peyronie's disease is supported by findings from studies in various rat models, in which injection of TGFβ1, surgical trauma and suturing of the rat tunica albuginea caused an increase in the thickness of the tunica albuginea and fragmentation of the elastic fibres of the tunical tissue[148–150].

Space of Smith
A vascular, loose, areolar connective tissue sleeve that separates the corpus cavernosum from the tunica albuginea.
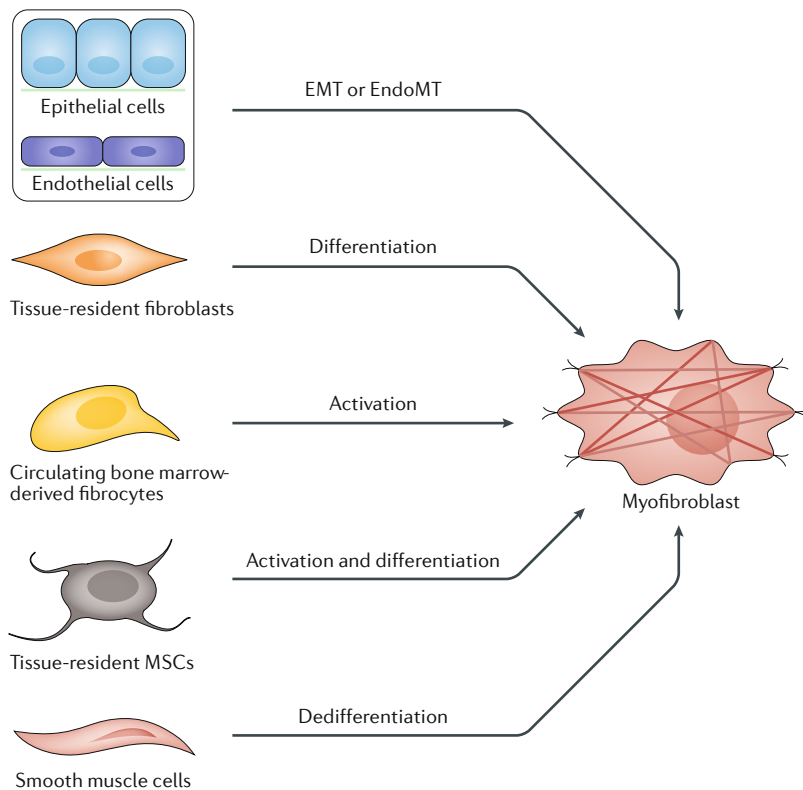
Fig. 4 | **The origin of myofibroblasts.** Myofibroblasts are possibly the most unique cells involved in the wound-healing process; they actively regulate the extracellular matrix (ECM) while simultaneously possessing a cytoskeletal contractile apparatus similar to that of smooth muscle cells[178,179]. Local cells undergo a phenotypic switch into myofibroblasts, which is indicated by the production of the myofibroblast marker α-smooth muscle actin (αSMA) and ECM components such as collagens (mostly type I and III collagen), laminin and fibronectin, a process that is mainly driven by transforming growth factor-β1 (TGFβ1)[170–172]. The principal myofibroblast progenitor after the tissue injury seems to vary, and includes epithelial or endothelial cells, which undergo epithelial-to-mesenchymal transition (EMT) or endothelial-to-mesenchymal transition (EndoMT), respectively; local tissue-residing fibroblasts (within the tissue where the fibrosis occurs); circulating bone marrow-derived fibrocytes; tissue-resident mesenchymal stem cells (MSCs); and smooth muscle cells. However, the relative contribution of each cell type to the formation of myofibroblasts in a fibrotic scar is still under debate[180]. For EMT and EndoMT, the organized dedifferentiation of epithelial cells occurs with a loss of polarity, adherence, and tight junctions through a process that is mainly driven by TGFβ1, fibroblast growth factor 2 (FGF2), insulin-like growth factor 2 (IGF2), and epidermal growth factor (EGF)[181–183]. Almost 0.5% of the leukocytes in peripheral blood are fibrocytes, which can be identified by the expression of the haemotpoietic cell marker CD34, leukocyte markers (CD11b, CD13 and CD45), and fibroblast products (such as type I and III collagen and fibronectin); however, fibrocytes lack specific macrophage, dendritic cell or B cell markers. TGFβ1 and endothelin-1 stimulate the activation and differentiation of fibrocytes into myofibroblast-like cells (αSMA-positive)[180]. Tissue-resident MSCs can be distinguished from fibrocytes by their lack of haemotpoeitic and leukocyte marker expression and αSMA positivity[15,180]. During atheromatous plaque formation, smooth muscle cells have been shown to dedifferentiate into myofibroblasts and are considered to be an important contributor to the associated fibrosis[154].

However, multiple studies in humans investigating the incidence of Peyronie's disease after penile fracture or in patients with a long history of forcefully bending the erect penis (taqaandan) did not show a correlation between trauma and Peyronie's disease[151,152]. This observation might suggest that several unknown genetic factors are involved in the development of Peyronie's disease.

A wound-healing process starts after each trauma as a result of injury of the resident endothelial cells, which in turn results in platelet activation, fibrin accumulation and release of pro-inflammatory molecules (for example, cytokines and chemokines) (FIG. 1). The activation of the coagulation system and release of fibrin is thought to have an important role in plaque formation in human Peyronie's disease[96]. The persistence of fibrin elicits an acute — and subsequent chronic — inflammatory response, and the subsequent production of profibrotic factors such as TGFβ1 and ROS induce the development of the fibrotic plaque. The role of endothelial damage in Peyronie's disease is evidenced by the observation that injection of fibrin into the space of Smith of the rat induces a Peyronie's disease-like lesion[150]. Similarly, injections of thrombin (which catalyses conversion of fibrinogen into fibrin) into the rat penis resulted in the formation of a fibrotic plaque[150].

Activated platelets release a diverse set of growth factors, the most important of which is platelet-derived growth factor (PDGF), a potent profibrotic molecule and chemoattractant for inflammatory cells. Inflammation occurs through the influx of cells from the innate and adaptive immune system; lymphocytes, polymorphonuclear leukocytes, eosinophils, basophils, T_H17 cells, T_H2 cells and macrophages are all attracted to the site of inflammation[4,6,96]. The presence of these immune cells at the site of tissue injury is essential for the normal wound-healing process, but they also cause the release of several toxic factors, including ROS and RNS that, if not cleared, can induce further tissue damage[4].

Another important feature of fibrosis is the formation of myofibroblasts[37]. The cellular origin of myofibroblasts remains a subject of debate. Research over the past few decades has implicated several possible sources, including circulating bone-marrow derived cells, smooth muscle cells, local resident fibroblast pools, and epithelial or endothelial cells[153,154] (FIG. 4). Human tunica albuginea-derived fibroblasts have been shown to undergo a phenotypic switch into myofibroblasts after exposure to TGFβ1, after which they secrete ECM proteins, suggesting that they are suitable candidates for a source of locally-derived myofibroblasts[7,150,155].

Myofibroblasts are the main ECM-producing cells, releasing a plethora of ECM products such as elastins, proteoglycans, collagens, fibronectins, fibrillins and tenascins[153]. After the proliferation phase, remodelling and contraction of the ECM occurs, which results in a curvature of the penis in Peyronie's disease. During the remodelling phase, type III collagen (which is prevalent during the proliferation phase) is replaced by type I collagen, causing a stabilization of the plaque. In some patients, the formation of calcifications or ossifications within the plaque ultimately occurs[7,150,155].

Although innate immune cells secrete a wide range of pro-inflammatory growth factors and cytokines, the role of TGFβ1 in Peyronie's disease has been thoroughly investigated. The TGFβ1 pathway seems to have a crucial profibrotic role that is due, at least in part, to its ability to induce myofibroblast activation[5,156]. Indeed, TGFβ1 production also occurs during the progression

of liver, lung, kidney, skin and cardiac fibrosis[2,4]. By contrast, inhibition of the TGFβ1 signalling pathway has been shown to reduce the development of fibrosis in many experimental models of Peyronie's disease[7,150]. The importance of TGFβ1 in the onset of Peyronie's disease is illustrated by the most commonly used rat model, in which recombinant TGFβ1 is injected into the space of Smith and the tunica albuginea, resulting in the upregulation of several pro-inflammatory factors such as C-C motif chemokine 2 (CCL2; also known as MCP1) and the development of a fibrotic plaque characterized by high levels of elastin and type III collagen within several weeks[96].

Stem cell therapy for Peyronie's disease. Peyronie's disease was first described almost 250 years ago, but no satisfactory medical treatment currently exists in the acute phase before the full-blown chronic plaque is established[11]. However, experimental models have provided new insights into the pathophysiology and aetiology of the disease. To date, only four preclinical studies have evaluated the local injection of MSCs as a therapy for Peyronie's disease[129–131,157] (TABLE 1). These studies tested the in vivo efficacy of local MSC injection using the most common model of Peyronie's disease, which is based on the local injection of TGFβ1 into the tunica albuginea of rats.

The potential therapeutic benefit of ADSCs in Peyronie's disease was first shown in 2013 (REF.[131]). Human ADSCs were injected into the tunica albuginea of rats 1 day after the local injection of TGFβ1 to mimic the acute phase and induce the elevated grade of inflammation that is necessary for the ADSCs to develop an anti-inflammatory phenotype. Treated rats had less elastosis and fibrosis of the tunica albuginea and less ED than untreated rats 5 weeks after local ADSC injection to the site of inflammation. The total elastin content decreased by ~50% in the lesions of treated rats compared with that of untreated rats; in addition, the type III collagen content was reduced by ~70% after ADSC injection. Moreover, corporal fibrosis near the tunica albuginea plaque was completely prevented by the ADSC treatment. This study provided a proof of principle for the efficacy of stem cells in the treatment of Peyronie's disease. However, most patients present to their health-care provider with disease in the chronic stage; thus, these results cannot be directly translated into clinical application.

Two preclinical studies[129,130] have evaluated the efficacy of allogeneic ADSCs and genetically modified allogeneic ADSCs expressing human IFNα2b for the prevention and treatment of tunica albuginea fibrotic plaque tissue. ADSC treatment markedly decreased tunica albuginea fibrosis and improved erectile function at 6 weeks after injection in both prevention (immediate ADSC injection) and treatment (ADSC injection after 4 weeks) groups compared with untreated groups in both studies. Possible antifibrotic mechanisms were also investigated in the former study[129]. Local injection of ADSCs seemed to decrease the expression of TIMPs, promoting the production and activity of MMPs to break down the slowly growing plaque.

Finally, an in vitro study using rat tunica albuginea-derived fibroblasts showed that, after TGFβ1-induced myofibroblast transformation, co-culture with allogeneic ADSCs inhibited their contractile and ECM-producing capabilities through inhibition of the SMAD and RHO/RHOA signalling pathways[157]. Moreover, the production of MMP9, MMP13, MMP2 and MMP3 was upregulated following allogeneic ADSC coculture, indicative of collagen breakdown. As the final outcome parameter, the investigators showed an increase in myofibroblast apoptosis after allogeneic ADSC co-culture, which occurred via the elevated activity of caspase 3 and caspase 9.

Overall, qualitative preclinical data regarding the use of stem cells in Peyronie's disease is severely lacking. Additional knowledge and research on the pathophysiology of Peyronie's disease is urgently needed, which will lead to the development of improved disease models that more closely resemble the human situation.

Urethral stricture

Urethral strictures are characterized by an abnormal narrowing of the urethra caused by varying degrees of spongiofibrosis that functionally obstructs the lower urinary tract. Urethral stricture disease can markedly reduce a patient's quality of life by promoting genitourinary infection, impairing voiding and causing secondary bladder dysfunction[9]. Several aetiologies have been proposed and are categorized as iatrogenic, traumatic, inflammatory and idiopathic[9]. Since the advent of endourology and catheter usage, the prevalence of iatrogenic urethral strictures has surpassed that of inflammatory causes[158].

The pathophysiological mechanisms underlying urethral stricture disease are poorly investigated and remain largely obscure, with only a few studies having investigated such mechanisms at the molecular level. Fibrosis in urethral strictures has been shown to be the result of an abnormal wound-healing process similar to that observed in other organs[37]. Moreover, a role for TGFβ1 in urethral fibrosis and stricture formation has been suggested; twofold to threefold higher urine levels of TGFβ1 and increased TGFβ receptor type 2 (TGFβR2) expression have been reported in urethral strictural tissue compared with normal urethral tissue[159].

Stem cell therapy for urethral stricture. The use of stem cells in urethral reconstructive urology has been an ongoing area of study[160]. Research has been focused solely on the regenerative property of stem cells. Various studies have attempted to regenerate lost urethral tissue using tissue engineering and stem cell bioscaffolding[160].

To date, only two studies have described the efficacy of stem cell injection in a rat model of urethral stricture[31,133] (TABLE 1). In 2016, the first evidence that xenotransplantation of human ADSCs prevented urethral stricture formation and voiding impairment in a rat model of urethral stricture was published[31]. This model was first developed in 2015 (REF.[161]) and was established by combining injury of the spongy tissue with the simultaneous local injection of TGFβ1; this method induces fibrosis in the corpus spongiosum with an increase in collagen expression. In the 2016 study[31], the authors

showed that this model of urethral stricture could induce urodynamic changes at 4 weeks after injury similar to those seen in human lower urinary tract obstruction (for example, increased voiding frequency and reduced bladder capacity suggestive of detrusor overactivity caused by bladder obstruction and detrusor hypertrophy). Local injection of human ADSCs 1 day after induction of the fibrotic reaction prevented the formation of spongiofibrosis. Masson's trichrome staining showed that ADSC injection preserved corpus spongiosum tissue architecture and promoted the almost complete normalization of type III collagen and elastin protein content[31]. These findings were further corroborated by the observation of preserved micturition function in treated animals. Moreover, the authors showed that the concentration of inducible nitric oxide synthase (iNOS) and eNOS were increased in the treated group compared with untreated animals. These findings were confirmed by another study[133] using the same rat model of urethral stricture; however, autologous ADSCs were injected the day of TGFβ1 injection. Levels of urethral collagen type I and III collagen were markedly decreased in rats treated with ADSCs compared with untreated rats 2 weeks after induction of the fibrotic reaction.

Overall, urethral stricture research has been limited by the lack of representative in vitro and in vivo models owing to the complexity of the disease. Similar to Peyronie's disease, insufficient preclinical data exist to warrant qualitative clinical trials.

Limitations of preclinical studies

The rapidly expanding and promising body of preclinical research in stem cell medicine can provide a potential cure for fibrotic diseases such as lung, kidney and heart fibrosis. On the basis of the current evidence, stem cells could have a role in the treatment of penile fibrosis. However, the amount of research in this field is still small and is characterized by several limitations that might reduce the translational power of stem cells as treatments for penile fibrotic disorders.

Human wound-healing and repair mechanisms developed early in the evolutionary development of our species. Thus, wound-healing, and, by extension, fibrosis, involves a very complex network of interactions and includes many compensatory pathways. Accordingly, drugs that inhibit and/or influence only one factor in the fibrotic cascade have a small chance of clinical success[2,3,37]. Conversely, stem cells can counteract fibrosis at multiple steps in the fibrotic pathway[84], a characteristic that suggests their potential superiority over currently available treatments[47]

Epigenetic modifications have been increasingly recognized to have a potentially important role in the maintenance of an undifferentiated state in MSCs[59,60]. Further research into the regulation of DNA methylation and its role in stem cell differentiation might provide further insight into improving stem cell treatments[59,60].

Most studies investigating penile fibrotic disorders evaluated fibrosis histopathologically using Masson's trichrome staining followed by quantitative image analysis. However, this method introduces potential biases[162–164]. First, and perhaps most importantly, Masson's trichrome

staining is often evaluated by calculating the ratio of areas stained red to areas stained blue, assuming the red pigment stains only smooth muscle whereas the blue pigment stains only collagen. However, this assumption is erroneous given that all cells contain the intermediate filament keratin — including residual red blood cells, endothelial cells and platelets in the sinusoids — and will, therefore, stain red[162–164]. Ideally, Masson's trichrome staining should be combined with more quantitative techniques to detect fibrosis; depending on the type of tissue, such techniques include either quantitative PCR (qPCR) or western blotting for typical profibrotic genes and proteins, respectively, such as TGFβ1, α-smooth muscle actin (αSMA), collagen (type I and III), elastin and iNOS. Second, image analysis is subject to the selection of slides and areas by the investigator, which can induce some variation between studies; even when automated image analysis is used, this approach should be considered semi-quantitative.

The majority of studies exploring the efficacy of stem cells in the context of corpora cavernosa fibrosis showed that stem cells can prevent the onset of corpora cavernosa fibrosis. Conversely, only a few studies have shown that stem cell treatment was efficacious in reversing corpora cavernosa fibrosis[126,128]. In the clinical setting, most patients present with already established fibrosis, since clinical symptoms only occur when a large part of healthy tissue has been replaced by scar tissue. This point is of utmost importance for the translational application of stem cell treatment[94]; the same holds true for research regarding stem cell therapy for urethral strictures[31,133]. To date, only two preclinical studies[31,133] have explored the efficacy of xenogeneic ADSCs in preventing urethral fibrosis, without validation by other studies.

Only four studies have explored the efficacy of stem cells in Peyronie's disease, and reported similar findings[129–131,157]. Despite these studies showing that ADSCs can prevent and even treat tunica albuginea fibrosis, several limitations jeopardize their translational potential. Of note, the TGFβ1 rat model of Peyronie's disease differs from the human Peyronie's disease in three main pathophysiological aspects, which should be taken into account: rats do not develop a curvature of the penis; the TGFβ1 injection does not induce fibrin accumulation in rats, which occurs in human tunica albuginea plaques, meaning that TGFβ1 is probably not the sole initiator of Peyronie's disease fibrosis; and rat tunica albuginea plaques dissolve spontaneously 60–90 days after induction of injury, which makes it difficult to study the chronic stage of the disease[150]. Importantly, different injection procedures (needle size, injection location and technique, and extravasation of TGFβ1 into the corporal tissue) could induce different degrees of corporal fibrosis and ED that might not be related to fibrosis of the tunica albuginea[150].

In addition, several issues associated with stem cell treatment remain to be explored. Intracavernosal injection is the most frequently used route of stem cell delivery in preclinical studies (TABLE 1); however, intravenous injection has also proved to be effective and should be further examined. Further evaluation into

the comparative superiority of allogeneic versus autologous versus xenogeneic stem cell injection is also warranted. Moreover, ADSCs are the most commonly used type of stem cell, but adipose-derived SVF seems to be a better choice given the lower risk of contamination[82]. However, this observation needs to be clarified through comparative studies. The timing of stem cell injection is another unresolved issue, particularly as stem cells seem to show an antifibrotic phenotype only when they are in an inflammatory environment[35]. Finally, the dosage of stem cells in preclinical studies is arbitrary, and whether the number of stem cells injected can influence the stem cell phenotype and local inflammatory environment is not yet clear.

Conclusions

To date, the clinical application and investigation of conventional antifibrotic therapies has yielded limited results. Conventional approaches focus on the inhibition of one small cog in the large machinery of fibrosis, resulting in the activation of auxiliary pathways that counteract the effects of these antifibrotic drugs. However, the use of stem cells in translational research has the potential to exert antifibrotic functions on several levels by modulating the host response. Despite the amount of research on stem cells and penile fibrosis, the field is still in its infancy and is subject to many limitations. Most preclinical research regarding stem cells in penile fibrosis has focused on corpora cavernosa fibrosis owing to its clear pathophysiology (iatrogenic postprostatectomy ED and corpora cavernosa fibrosis) and representative animal models. Conversely, Peyronie's disease and urethral stricture disease research have been limited by poor disease models and unvalidated findings. Thus, the treatment of corpora cavernosa fibrosis with stem cells seems to be the closest to potential clinical application given additional studies evaluating the efficacy, dosage, timing and route of administration of stem cells or SVF.

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