

Intratunical Injection of Human Adipose Tissue–Derived Stem Cells Restores Collagen III/I Ratio in a Rat Model of Chronic Peyronie’s Disease

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ABSTRACT

Introduction: Previous studies have shown that the injection of adipose tissue–derived stem cells (ADSCs) into the tunica albuginea (TA) during the active phase of Peyronie’s disease (PD) prevents the development of fibrosis.

Aim: To investigate, using an animal model, whether local injection of human ADSCs (hADSCs) can alter the degree of fibrosis in the chronic phase of PD.

Methods: 27 male, 12-week-old rats were divided into 3 equal groups: sham, PD without treatment, and PD treated with hADSCs 1 month after disease induction. Sham rats underwent 2 injections of vehicle into the TA 1 month apart. PD rats underwent transforming growth factor β 1 (TGF β 1) injection and injection of vehicle 1 month later. PD-hADSC rats underwent TGF β 1 injection followed by 1 million hADSCs 1 month later. 1 week after treatment, $n = 3$ animals/group were euthanized, and the penises were harvested for quantitative polymerase chain reaction. 1 month after treatment, the other animals, $n = 6$ per group, underwent measurement of intracavernous pressure (ICP) and mean arterial pressure (MAP) during electrostimulation of the cavernous nerve. After euthanasia, penises were again harvested for histology and Western blot.

Main Outcome Measure: The primary outcome measures included (a) gene expression at one week post-injection; (b) measurement of ICP/MAP upon cavernous nerve stimulation as a measure of erectile function; (c) elastin, collagen I and III protein expression; and (d) Histomorphometric analysis of the penis. Means were compared by analysis of variance (ANOVA) followed by a Student-Newman-Keuls test for post hoc comparisons or Mann-Whitney test when applicable.

Results: No significant difference was noted in ICP or ICP/MAP in response to cavernous nerve electrostimulation between the 3 groups at 2.5, 5, and 7.5 V ($P > .05$ for all voltages). PD animals developed tunical and subtunical areas of fibrosis with a significant upregulation of collagen III protein. The collagen III/I ratio was higher in the PD (4.6 ± 0.92) group compared with sham (0.66 ± 0.18) and PD-hADSC (0.86 ± 0.06) groups ($P < .05$). These fibrotic changes were prevented when treated with hADSCs. Compared with PD rats, PD-hADSC rats demonstrated a decreased expression of several fibrosis-related genes.

Conclusion: Injection of hADSCs reduces collagen III expression in a rat model of chronic PD. **Castiglione F, Hedlund P, Weyne E, et al. Intratunical Injection of Human Adipose Tissue–Derived Stem Cells Restores Collagen III/I Ratio in a Rat Model of Chronic Peyronie’s Disease. Sex Med 2018;XX:XX–XX.**

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Key Words: Peyronie’s Disease; Stem Cell; Adipose Stem Cell; Fibrosis

Received July 24, 2018. Accepted September 30, 2018.

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<https://doi.org/10.1016/j.esxm.2018.09.003>

INTRODUCTION

Peyronie's disease (PD) is a sexually debilitating fibrotic disease of the penis that results in penile deformity, impaired penetrative intercourse, and significant psychological stress for patients and their partners.¹ The prevalence of PD is estimated at 3.2% in the general male population, with rising incidence with age and up to 9% in men with erectile dysfunction (ED).² The disease is characterized by the formation of a fibrous plaque within the tunica albuginea (TA) containing disarranged depositions of collagen and elastin, which form during a painful phase of inflammation (the acute phase).³ Ongoing inflammation during the acute phase results in aberrant wound healing with the formation of a Peyronie's plaque, leading to a progressive penile curvature over a 12- to 18-month period.⁴ After this period, the scar tissue retracts, calcifies, and occasionally ossifies, resulting in a permanent and painless deformity (the chronic phase).⁴ The penile deformity may present as a curvature, waist deformity, or complex deformity with a combination of waisting, rotation, and curvature, which result in impaired penetrative intercourse. It is frequently accompanied by severe and difficult-to-treat ED.⁵ Currently there are no evidence-based treatment options to halt the disease in the acute phase.⁶ Therefore, patients have to undergo corrective penile surgery, which is associated with penile shortening and development of ED. The alternative option for less severe deformities is injection of collagenase.⁵

The multipotent stromal cell (MSC) is characterized by its ability to divide into a copy of itself and more terminally differentiated daughter cells within the mesodermal lineage (multipotency). However, this ability is not the only feature that makes these cells appealing for therapeutic use. The secretion of a broad range of paracrine factors, such as growth factors, cytokines, and chemokines, makes MSCs able to influence and modify their biological environment, specifically following tissue injury.⁷ In this regard, MSCs have been attributed immunomodulatory, antifibrotic, trophic, and free radical-scavenging capabilities.⁸ Researchers have therefore used MSCs in various fibrotic conditions, in both the animal and the human setting, and it is increasingly being recognized that MSCs may represent a promising avenue of research in the prevention and treatment of fibrosis.^{9,10} The exact mechanisms of the antifibrotic effects of stem cell therapy still remain to be understood.¹¹ One theory is that stem cells act as a "drugstore," influencing simultaneously various fibrogenic pathways.¹² However, definitive answers have not been given, and further research focusing on the mechanisms of action are still ongoing.

Recently, several studies have suggested a possible role of MSCs in the treatment of corpus cavernosum fibrosis^{13,14} and spongiofibrosis.^{15,16} In a previous study,¹⁷ our group showed the efficacy of human adipose tissue-derived MSCs (ADSCs) in preventing fibrosis in a rat model of acute-phase PD. The rat is most commonly used for the study of PD. This species exhibits morphological and biological penile characteristics similar to

those of humans, has low costs for purchase and maintenance, and offers excellent possibilities for experimental turnover and multimethodological investigative approaches. Bivalacqua et al¹⁸ reported that injection of the recombinant transforming growth factor b1 (TGF β 1) protein produced similar effects but that a combined intervention of surgical trauma and TGF β 1 injection caused more profound PD-like changes. Furthermore, either procedure, alone or in combination, induced erectile dysfunction.¹⁸

Clinically, the majority of patients present either late in the acute phase or in the chronic phase, when the fibrosis is established.¹⁹ The aim of this study was to investigate the effects of a local injection of ADSCs after establishment of TA fibrosis in rat model for chronic PD based on TGF β 1 injection in TA.

METHODS

Ethical Approval

All experiments on animals and human tissues were approved by the ethics committee of the University Hospitals (registration number: ML7263), Leuven, Belgium, and the Institutional Ethical Committee for Animal Experimentation, KU, Leuven, Belgium (Internal Review Board number P 272/2014). Informed consent for adipose tissue processing was obtained (B322201110944). We calculated a sample size of 18 considering 3 groups (6 animals for each group), a statistical power of 0.9, effect size d : 2, alpha level 0.05 (G*Power 3.1, University of Düsseldorf, Germany). We included 9 other rats (3 for each group) for gene expression investigation.

Animals

Male Sprague Dawley rats ($n = 27$; 12 weeks old; 300–350 g; Charles River Laboratories, Wilmington, MA) were used. Rats were housed in pairs under 12-hour reversed cycle lighting with ad libitum access to food and water. Intraperitoneal ketamine (75 mg/kg) and xylazine (50 mg/kg) were used for anesthesia for the surgical procedures.²⁰ Amoxicillin (50 mg/kg intraperitoneally) was administered 1 hour before the surgical procedures as prophylaxis. Rats were euthanized using carbon dioxide asphyxia.

Adipose Tissue-Derived Stem Cell Isolation

Subcutaneous human adipose tissue was harvested from a consenting female adult patient undergoing surgery for a benign condition; the tissue was deemed surplus. ADSCs were isolated as previously described.¹⁷ Briefly, adipose tissue was minced and rinsed with phosphate-buffered saline (PBS) and incubated in a solution containing 0.075% collagenase type IA (Sigma-Aldrich, St. Louis, MO) for 1 hour at 37°C. This was shaken for 15–20 seconds every 20 minutes. The superficial lipid layer was removed, and the solution was centrifuged for 10 minutes at 1,000 $\times g$. The pellet was treated with 160 mM NH₄Cl for 10 minutes to lyse red blood cells. The remaining cells were suspended in 10 mL Dulbecco's modified Eagle medium

supplemented with streptomycin, fungizone, penicillin, and 10% fetal bovine serum. The suspension was filtered through a 70- μ m cell strainer, plated at a density of 1×10^6 cells in a 10-cm dish, and cultured at 37°C in 5% CO₂. After 24 hours, the cells were rinsed with PBS. Cells were cultured until passage 5, when they were used for treatment. The cells were characterized using flow cytometry and tested for multiple lineage differentiation¹⁷ as required by the International Society for Cellular Therapy.²¹

Study Design

Rats were randomly divided into 3 equal groups. The sham group (n = 9) underwent injection of 50- μ L vehicle (citrate buffer) in the dorsomedial aspect of the right midshaft TA with a microliter syringe after opening the buck fascia as previously described.¹⁷ The remaining 18 animals were injected with 0.5 μ g recombinant TGF β 1 in 50 μ L vehicle.¹⁷ After 1 month, rats received a second identical TA injection with either PBS (sham and PD group) or 1 million ADSCs in PBS (PD-ADSC group). 1 week after the second treatment, 3 animals per group were euthanized, and the penises were directly harvested during anesthesia and snap frozen for gene expression investigation.²² 4 weeks after the second treatment, 6 rats per group underwent in vivo erectile function evaluation, after which the animals were euthanized, and the penises were harvested for histological analysis and protein extraction.

Erectile Function Measurement

Intracavernous pressure (ICP) response to electrostimulation of the cavernous nerve (CN) was used to evaluate erectile function.¹⁷ Briefly, under anesthesia, the right CN was exposed, and the right crus of the corpus cavernosum was identified and cannulated with a heparinized (200 U/ml) 25-G needle connected to a pressure transducer. The CN was activated (2.5, 5, and 7.5 V) by platinum electrodes connected to a stimulator at 20 Hz for 60 seconds.¹⁷ The nerve was stimulated once per voltage, and a resting period of 2 minutes was allowed for nerve recovery between stimulations. Mean arterial pressure (MAP) was recorded by carotid artery cannulation.¹⁷

Histological Analysis of Tissue

The penile midshaft at the level of the injection site was harvested, fixed, and further processed for histology. Hematoxylin and eosin and Masson's trichrome staining procedures were performed according to a standard protocol previously described.^{20,22,23}

Western Blot Analysis

Western blot was performed as previously described^{20,22,23} for the detection of collagen I, collagen III, and elastin proteins at the level of the penile midshaft. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal standard. Primary antibodies were rabbit anti-collagen III (1:1,000; Abcam Inc, Cambridge, MA), mouse anti-elastin (1:500,

Abcam), rabbit controls against GADPH (1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA), and rabbit anti-collagen I (1:500; Abcam).^{20,22,23}

Gene Expression Evaluation Using Fibrosis-Focused Quantitative Polymerase Chain Reaction Array

The expression of 84 genes associated with fibrosis was evaluated using reverse transcription-2 polymerase chain reaction (PCR) array system (PAMM-120; SA Biosciences, Antwerp, Belgium).^{22,24} The urethra and dorsal neurovascular compartment were stripped from the corpora cavernosa, and the remaining corporal tissue and TA were homogenized in Trizol reagent followed by purification with the RNEasy system (Qiagen, Valencia, CA). RNA was reverse-transcribed and amplified using PCR with the reagents recommended by the manufacturer. Normalized gene expression data were derived by the $2^{-\Delta\Delta CT}$ method. Gene expression values were normalized to the reference genes β -actin, hypoxanthine phosphoribosyltransferase1, β -2-microglobulin, and GADPH.^{22,24} The expression of each gene in the PD and PD-ADSC groups was reported as fold increase of the mean expression of the same gene in the sham group. Differences in gene expression^{22,24} were considered significant with $P < .05$ using analysis of variance (ANOVA).

Statistical Analysis

The results were analyzed using Prism v.4 (GraphPad Software, San Diego, CA) and expressed as mean and standard deviation of the mean. Multiple groups were compared using 1-way ANOVA followed by the Student-Newman-Keuls test for post hoc comparisons. Statistical significance was set at $P < .05$.^{22,24}

RESULTS

Erectile Function

No significant difference was noted in the ICP and ICP/MAP in response to cavernous nerve electrostimulation between the 3 groups (n = 6 per group) at 2.5, 5, and 7.5 volts ($P > .05$ for all voltage) 4 weeks after vehicle or hADSC injection (Figure 1, Supplemental Figure 1).

Histological and Western Blot Analysis

Rats injected with TGF β 1 (PD group) displayed a deposition of amorphous matrix and a haphazard organization of collagen fibers in the TA which extended into the subtunical corpus cavernosum (Figure 2). These morphologic results were corroborated by quantitative Western blot analysis, which revealed an increased protein content of collagen III and elastin compared to the sham group ($P < .05$ for both) (Figure 3 and Supplemental Figure 2). In the PD-hADSC group, the overall structure of the TA and collagen III expression of the penile shafts were comparable to those of sham rats (Figure 3). Penile shafts from PD-hADSC rats showed more elastin expression than the sham group ($P < .02$) and had slightly increased expression of collagen

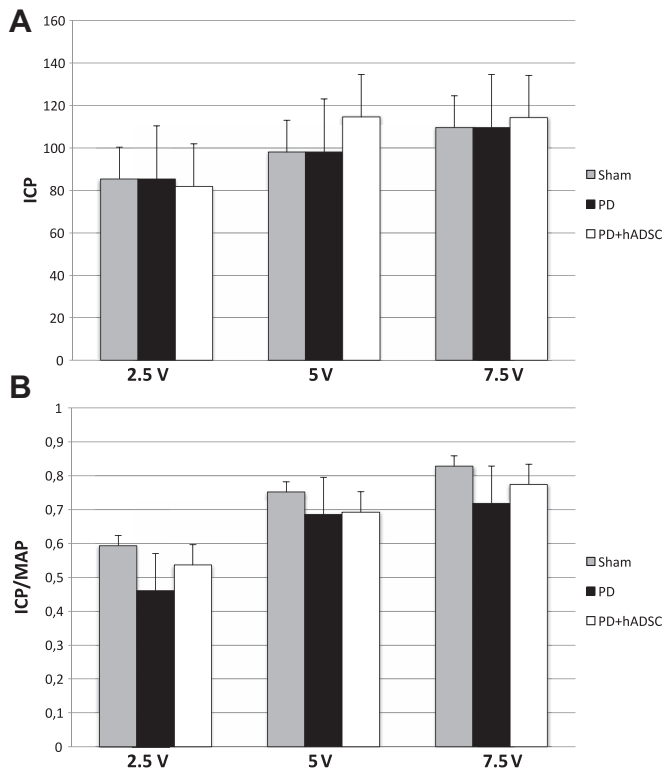


Figure 1. Erectile function measurement. Summarized data comparing erectile function measurements in sham PD rats and rats treated with adipose tissue–derived stem cells at various voltages during cavernous nerve electrostimulation in analysis of variance with post hoc Student–Newman–Keuls analysis. A, ICP. B, ICP normalized over mean arterial pressure. hADSC = human adipose tissue–derived stem cell; ICP = intracavernous pressure; PD = Peyronie’s disease.

I compared with the PD and sham groups ($P < .05$) (Figure 3). Interestingly, the collagen III/I ratio was higher in the PD (4.6 ± 0.92) group compared with sham (0.66 ± 0.18) and PD-hADSC groups (0.86 ± 0.06 ; $P = .01$; $n = 6$ per group).

Gene Expression

In an exploratory experiment ($n = 3$ per group), 32 genes were differentially expressed in PD and PD-hADSC groups compared with the sham group ($P < .05$) (Figure 4). 6 genes were differentially expressed in PD and PD-hADSC groups: C-C motif chemokine ligand 13 (CCL13); C-X-C motif chemokine receptor 4 (CXCR4); plasminogen activator, tissue type (PLAT); serpin family H member 1 (serpinh1); TGF β 1; and tumor necrosis factor (TNF) ($P < .05$).

DISCUSSION

We provide novel evidence that xenotransplantation of hADSCs reduces TA fibrosis in a rat model representing the chronic phase of PD. PD is a fibrotic disorder of the penis characterized by the development of a plaque in the tunica albuginea.⁴ Fibrosis itself can be considered a result of abnormal wound

healing.²⁵ Wound healing is an intricate pathophysiological process, involving a coordinated production of growth factors, cytokines, and extracellular matrix (ECM) and crosstalk between many cell types. The fact that diverse diseases in different organ systems are associated with fibrotic changes implies common intricate pathogenic pathways.²⁶ The aforementioned intricate pathway likely suggests the complexity of fibrosis development, and it partly explains why an efficacious antifibrotic treatment has yet to be established.²⁷ As stated by El Agha et al,²⁸ “it might be that a single anti-fibrotic magic bullet is simply unable to override such multifactorial and complex diseases.”

Fibrotic disease embodies a varied spectrum of disorders and is characterized by a disproportionate accumulation of ECM elements, comprising interstitial collagens (types I and III), cellular fibronectin, and basal membrane proteins such as laminin.²⁶ Collagens are primarily structural proteins composed of 3 procollagen chains configured in a classic triple helical pattern. Early in the course of wound healing, myofibroblasts deposit type III collagen.²⁹ Type III collagen belongs to the fibrillary collagen group and is the predominating tensile ECM until the later phase of wound healing, when it is replaced by the stronger type I collagen.³⁰ Most flexible tissues (skin, intestine, blood vessels, lung, TA) have a III/I ratio of 1 to 2–3.³¹ Fibrotic tissues are associated with a shift in the normal III/I ratio toward an increase in the content of collagen III.³¹

The rapidly expanding and highly promising body of pre-clinical work in stem cell medicine provides a potential cure for fibrotic diseases such as lung, kidney, and heart fibrosis.^{32–34} All the available antifibrotic drugs act against 1 step of the redundant and intricate fibrotic pathway. Conversely, stem cells are able to counteract fibrosis acting in multiple steps and not as a single weapon.³⁵ This characteristic makes stem cells potentially superior to the currently available treatments.

In our previous study,¹⁷ we showed that in the acute or inflammatory phase of the disease, injection of hADSCs into the affected area prevents formation of fibrosis and elastosis in the tunica and corpus cavernosum and restores erectile function. In this study, we injected the hADSCs 1 day after the TGF β 1 treatment, aiming to mimic the early phase of the disease, which is characterized by inflammation, penile pain, curvature progression, and no stable identifiable fibrotic plaque.¹⁷ After 1 month, rats injected with TGF β 1 (PD group) displayed extensive TA and corporal fibrosis and elastosis at the injection site together with impaired erectile function.¹⁷ Although this study provided a proof of principle for the efficacy of stem cells in treating PD, most patients present to their healthcare provider with later stages of PD, and thus these results cannot be directly translated into clinical application.¹⁹

In the present study, we injected the hADSCs 1 month after TGF β 1 injection, trying to replicate a condition similar to the chronic phase of PD. During the chronic phase, since the inflammatory process has settled, pain is absent and the penile curvature is stable. Interestingly, in our study, 2 months after

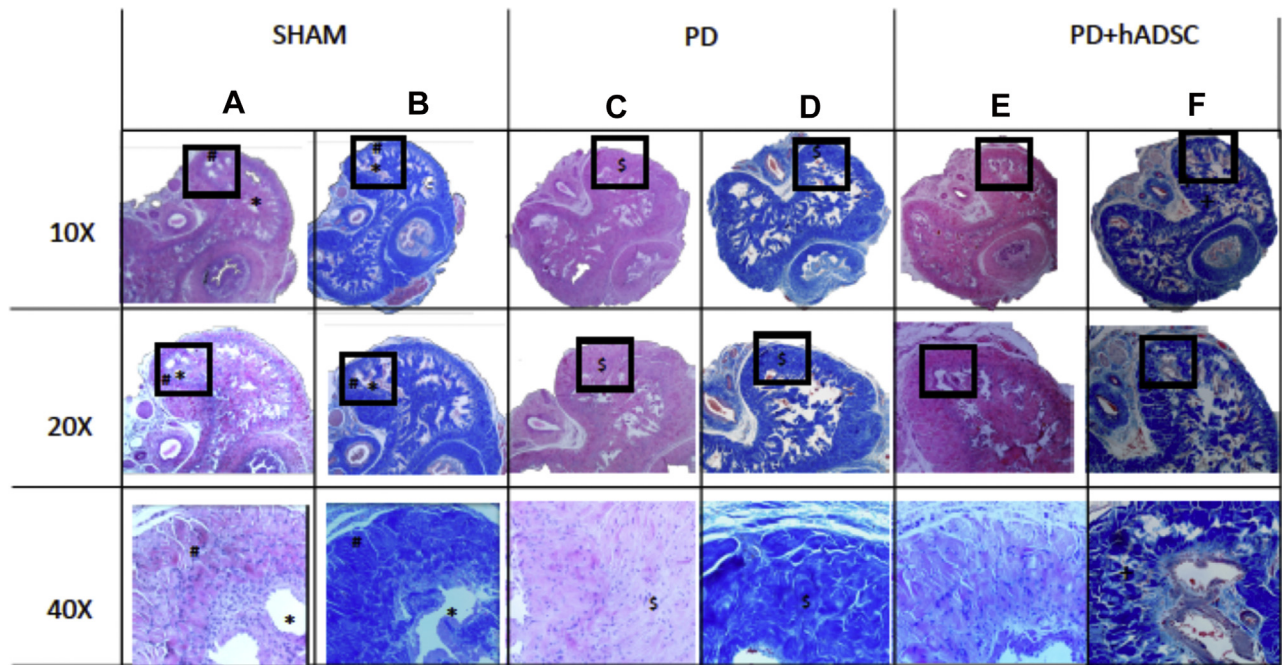


Figure 2. Histology. Representative photomicrographs of Masson's trichrome and H&E staining in midshaft sections of rat penises at magnification 10 \times , 20 \times , and 40 \times . A and B, H&E staining on sections from a sham rat (A) and corresponding Masson's trichrome staining on an adjacent section from the same rat (B). C and D, H&E on sections from a PD rat (C) and corresponding Masson's trichrome staining on an adjacent section from the same rat (D). E and F, H&E sections from a hADSC-treated PD rat (E) and corresponding Masson's trichrome staining on an adjacent section from the same rat. (F) Note the open cavernous sinusoids in the sham rats (*) and the surrounding normal bilayer structure of the tunica albuginea (#). In PD rats, there is deposition of amorphous extracellular matrix material (\$). In the PD+hADSC group, there is an increase in extracellular matrix deposition (+); however, collagen fibers seem better organized and sinusoid structure is largely preserved. hADSC = human adipose tissue-derived stem cell; H&E = hematoxylin and eosin; PD = Peyronie's disease.

TGF β 1 injection, the PD rats showed less fibrosis on histological analysis than detected after 1 month in the previous study.¹⁷ Based on these data, it appears that the fibrotic plaques in the TA tend to partially regress spontaneously after 60 days in the TGF β 1 rat model of PD. This regression may be a limitation of the TGF β 1 PD model for the study of the condition in the long term. Furthermore, in contrast to the previous study, we did not detect any significant corporal fibrosis, and plaques were limited to the tunica and the immediate subtunica area. This may explain the lack of erectile function impairment in the PD group compared with the sham rats, although it should be noted that differences were significant but small in the acute study.¹⁷ In the present study, we showed that late ADSC therapy was able to reduce the expression of collagen III but had no effect on collagen I and elastin expression. Conversely, in our previous study we showed that early hADSC treatment was able to prevent elastosis; however, in both studies hADSC treatment was able to restore the collagen III/I ratio.

Our results are in line with the 2 preclinical studies performed by Gokce et al.^{36,37} Those studies had evaluated the efficacy of allogeneic ADSCs and genetically modified allogeneic ADSCs expressing human interferon A-2b for the prevention and, more importantly, the treatment of TA fibrotic plaques. In the first study,³⁷ the allogeneic ADSC-treated groups received tunica

albuginea injections with 0.5 million rat ADSCs immediately after (early phase) or 1 month after (late phase) the TGF β 1 injection. 6 weeks after TGF β 1 injection, in both prevention and treatment groups, TA injection of ADSCs resulted in significantly lower tunica albuginea fibrosis and a better erectile function response compared with the rats treated only with TGF β 1. In the second study, Gokce et al.³⁶ compared the efficacy of ADSCs expressing human interferon A-2b and normal allogeneic ADSC in the prevention and treatment of PD using a similar design to the previous study. The results of that study showed that both types of cells are effective in preventing and treating Peyronie's-like changes, but interestingly, ADSCs expressing human interferon A-2b induced a better recovery of erectile function.³⁶

To further preliminarily identify potential mechanisms that may be involved in the antifibrotic effect by hADSCs, we profiled expressions of fibrosis-associated genes in the 3 groups. We found that 32 genes involved in different pathways and steps of the wound healing process were differentially expressed by PD and PD-hADSC groups compared with the sham group. 6 genes were differentially expressed in PD and PD-hADSC groups: CCL13, CXCR4, PLAT, serpinh1, TGF β 1, and TNF. These preliminary genomic data, despite requiring a confirmation with far more in-depth experiments using protein expression analysis,

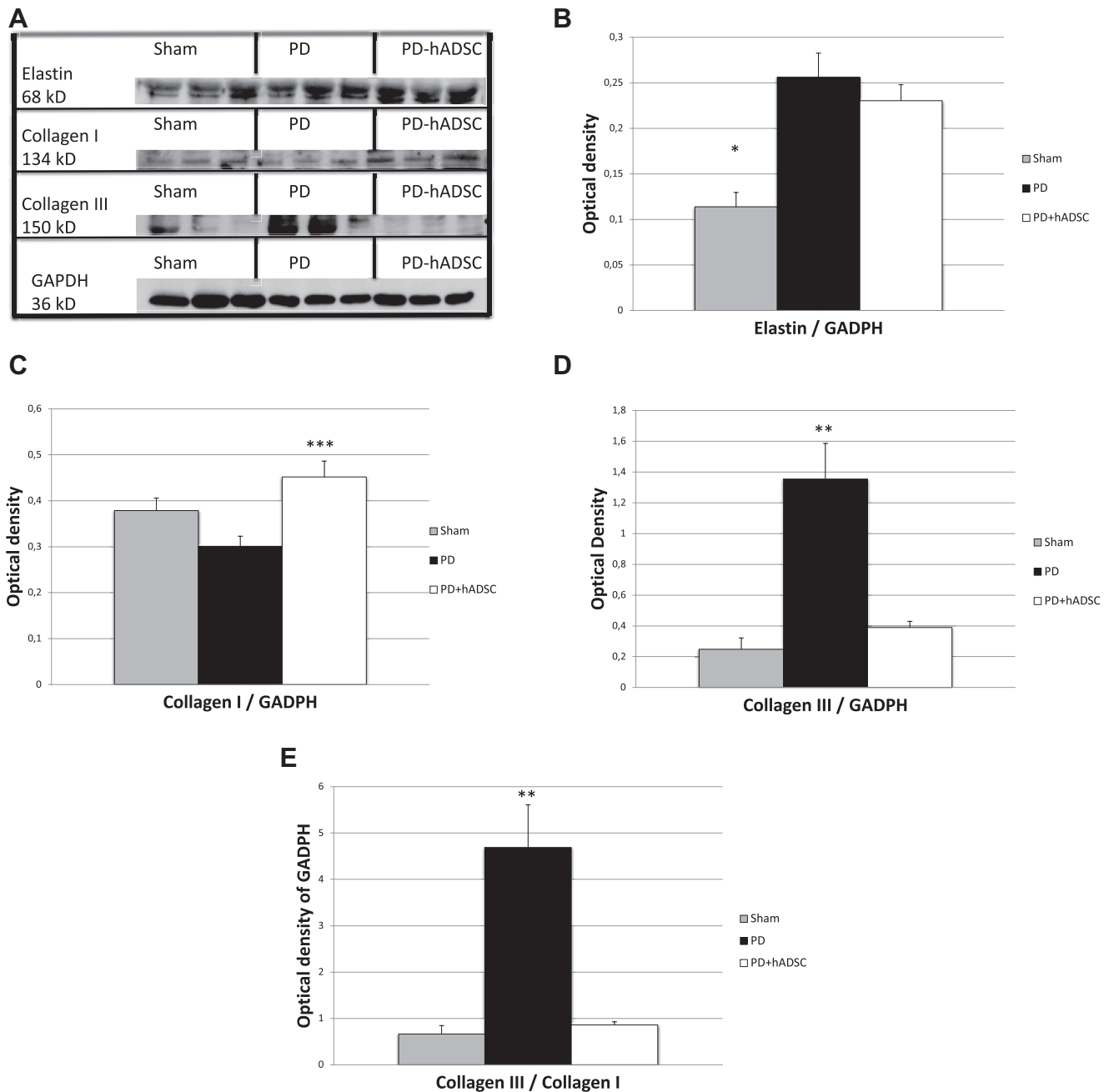


Figure 3. Western blot analysis for collagen III, collagen I, and elastin. A, Representative chemiluminescence images of blotted membranes containing protein extracts of all 3 groups. Double bands are caused by binding of antibodies to glycosylated and nonglycosylated forms of these molecules. B, Summarized protein expression levels for elastin; * $P < .05$ vs both PD and PD-hADSC in ANOVA with post hoc Student–Newman–Keuls analysis. C, Summarized protein expression levels for collagen I; *** $P < .05$ vs both PD and sham in ANOVA with post hoc Student–Newman–Keuls analysis. D, Summarized protein expression levels for collagen III; ** $P < .05$ vs both sham and PD-hADSC in ANOVA with post hoc Student–Newman–Keuls analysis. E, Collagen III and I expression ratio; ** $P < .05$ vs both sham and PD-hADSC in ANOVA with post hoc Student–Newman–Keuls analysis. ANOVA = analysis of variance; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; hADSC = human adipose tissue–derived stem cell; PD = Peyronie’s disease.

are in line with the current idea that stem cells do not act on a single target but by altering the local inflammatory environment.^{8,38,39} Indeed, a growing body of evidence suggests that MSCs act via a plethora of effects including, but not limited to, immunomodulation, reactive oxygen species neutralization, and angiogenesis.^{8,38,39}

Even if the current approach to using xenogeneic stem cells offers a novel option for the management of PD, it may be regarded as a limitation compared with autologous grafting, which may be a more attractive procedure from a therapeutic perspective. However, MSCs, including ADSCs, have been shown to be immunomodulatory and immunosuppressive, and

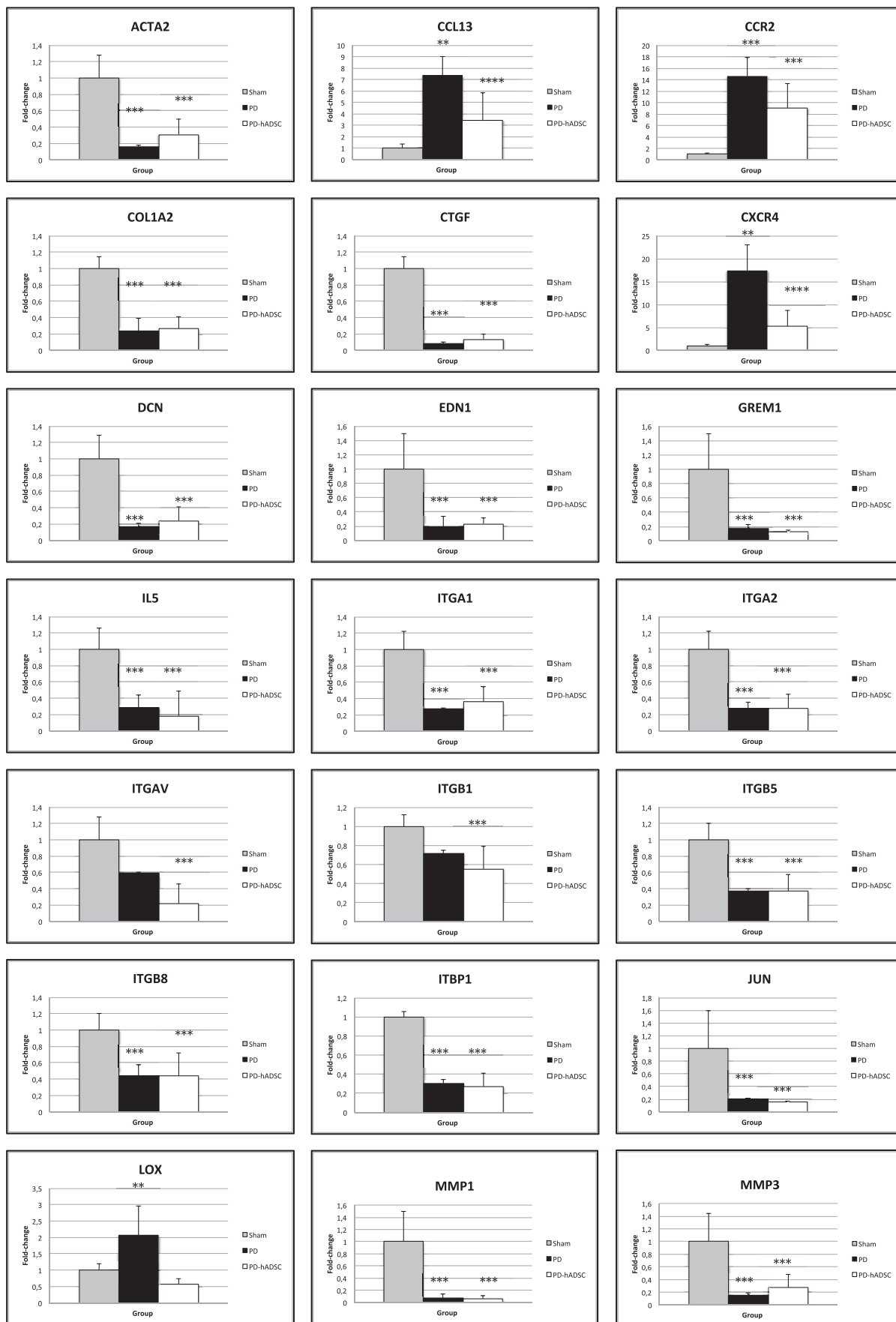


Figure 4. Continued.

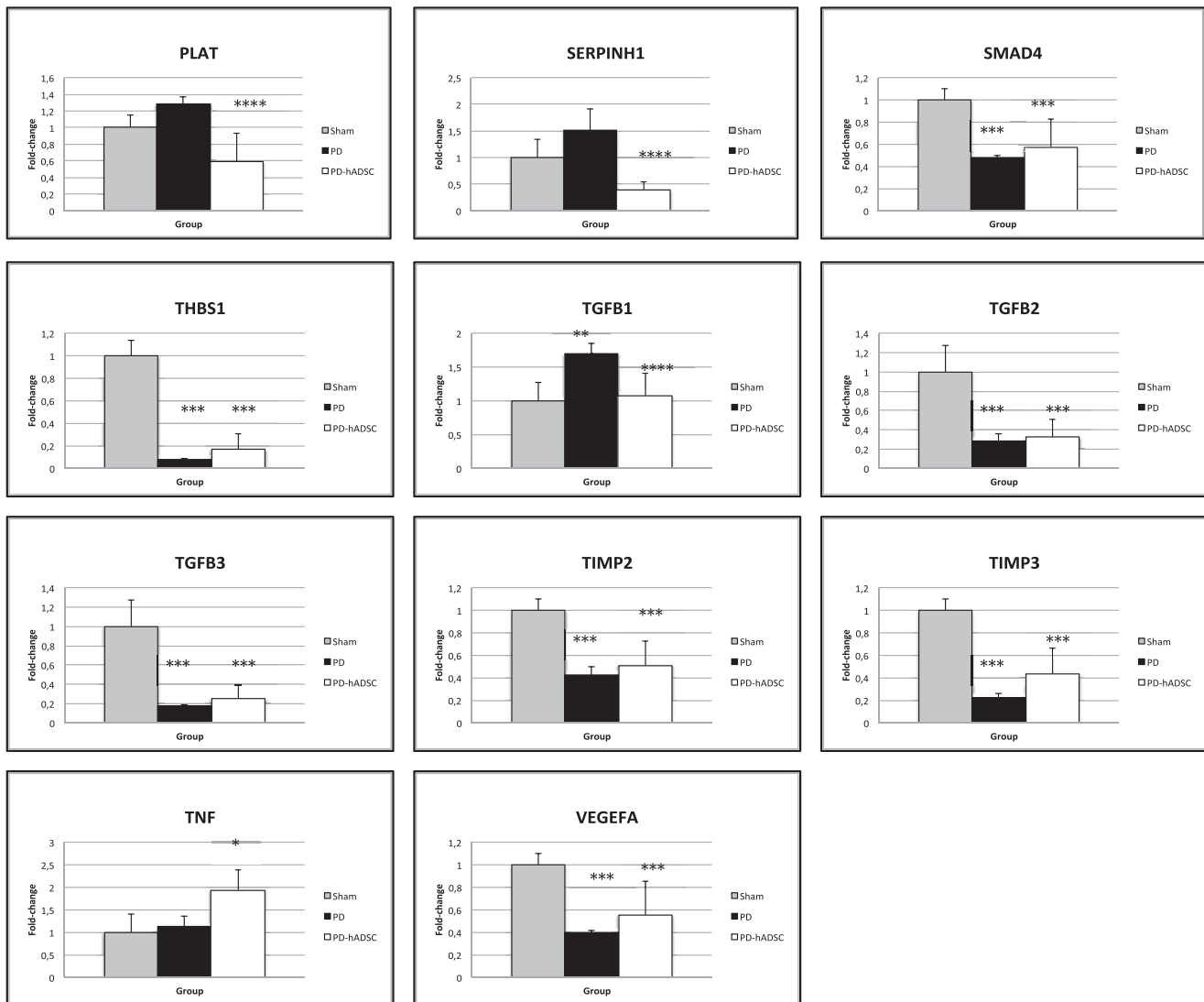


Figure 4. Fibrosis-associated gene expression (3 rats per group). The expression of each gene in the PD rat and PD-hADSC groups was reported as a fold increase of the mean expression of the same gene of the sham group. Differences in gene expression were considered significant when $P < .05$ by analysis of variance. $*P < .05$ versus sham and PD. $**P < .05$ versus both Sham and PD-hADSC groups. $***P < .05$ versus Sham. $****P < .05$ versus PD group. ACTA2 = alpha-actin-2; CCL13 = chemokine (C-C motif) ligand 13; CCR2 = C-C chemokine receptor type 2; COL1A = collagen, type I, alpha 1; CTGF = connective tissue growth factor; CXCR4 = C-X-C chemokine receptor type 4; DCN = decorin; EDN1 = endothelin 1; GREM1 = gremlin 1; IL5 = interleukin 5; ITGA1 = integrin subunit alpha 1; ITGA2 = integrin subunit alpha 2; ITGAV = integrin subunit alpha V; ITGB1 = integrin subunit beta 1; ITGB5 = integrin subunit beta 5; ITGB8 = integrin subunit beta 8; ITBP1 = integrin beta-1-binding protein 1; JUN = un proto-oncogene; LOX = lysyl oxidase; MMP1 = matrix metalloproteinase 1; MMP3 = matrix metalloproteinase 3; PLAT = plasminogen activator, tissue type; SERPINH1 = serpin family H member 1; SMAD4 = mothers against decapentaplegic homolog 4; THBS1 = thrombospondin-1; TGFB1 = transforming growth factor beta-1 proprotein; TGFB2 = transforming growth factor beta-2 proprotein; TGFB3 = transforming growth factor beta-3 proprotein; TIMP2 = metalloproteinase inhibitor 2; TIMP3 = metalloproteinase inhibitor 2; TNF = tumor necrosis factor; VEGEFA = vascular endothelial growth factor.

their xenogeneic transplantation in immunocompetent animals was extensively evaluated. Indeed, ADSCs have been shown to lack major histocompatibility complex II expression and its immunosuppressive effects mediated by prostaglandin E2.^{40–42} Furthermore, the genetic expression results need to be validated with protein expression analysis.

More importantly, in the TGF β 1 rat chronic model of PD, the absence of erectile dysfunction, together with the evidence

that the fibrotic plaques of TA tend to partially regress spontaneously after 60 days, represent important limits of the study.

CONCLUSION

Local injection of hADSCs in a rat model of chronic PD significantly decreased the collagen III/I ratio in the TA. Further

animal and clinical studies are needed to confirm the promising translational potential of this treatment strategy.

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Conflict of interest: The authors have no conflicts of interest.

Funding: This study was funded by the European Society for Sexual Medicine (ESSM) grant for basic medical research 2011 awarded to F.C. and M.A. and by the European Urological Scholarship Programme (EUSP) awarded to F.C. A.M. is supported by the NIHR Biomedical Research Centre, University College London Hospital.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.esxm.2018.09.003>.