Application of Estrogen for the Treatment of Stress Urinary Incontinence in Mice

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Abstract

Background The etiology of stress urinary incontinence (SUI) is complex and diverse among women, with past studies having demonstrated that estrogen deficiency is an important cause of pelvic floor muscle atrophy and urethral degeneration. We comprehensively investigated the effects of estrogen in the treatment of SUI in female mice at cellular and animal levels.

Methods L929 cell mechanical injury model was established by four point bending device, and SUI mouse model was established by vaginal dilation method commonly used to simulate labor injury. After treatment, the expressions of collagen I, collagen III, elastin, TIMP-1, TIMP-2, MMP-2 and MMP-9 were detected, the leak point pressure (LPP) and abdominal leak point pressure (ALPP) of mice in each group were detected, and the effect of estrogen on extracellular matrix remodeling of mouse urethra and anterior vaginal wall was observed from the histological level.

Results These results revealed that an appropriate amount of estrogen can promote the expression of collagen I, collagen III, elastin, TIMP-1, and TIMP-2, decrease the expression of MMP-2 and MMP-9, and maintain the dynamic balance of MMPs/TIMPs at both cellular and animal levels. Meanwhile, we determined that estrogen can increase the LPP and ALPP values of SUI mice. The collagen fibers' content in the mice treated with estrogen was significantly greater than in the control group mice.

Conclusions The estrogen may alleviate the symptoms of SUI by reconstituting ECM, toward laying a solid foundation for the further exploration of estrogen therapy.

1. Introduction

Stress urinary incontinence (SUI) is a usual disease affecting women, which refers to a type of pelvic floor dysfunction disease wherein urine leaks involuntarily from the external urethra at enhanced abdominal pressure due to activities such as coughing, exercising, laughing, or sneezing^{1,2}. SUI can lead to physical, psychic, and social adverse effects, thereby seriously affecting the quality of life³. The etiology of SUI is complex and diverse for women, including obesity, vaginal delivery, aging, decreased ovarian function, and estrogen deficiency⁴⁻⁷. Previous studies have indicated that estrogen deficiency is an important cause of pelvic floor muscle atrophy and urethral degeneration⁸. The Phase III clinical trial of the estrogen receptor blocker levormeloxifene revealed that its main side effect was to induce SUI⁹. In addition, the incidence rate of postmenopausal SUI was significantly higher¹⁰.

In recent years, in parallel with the development of the social economy, the diagnosis and treatment of SUI have attracted more and more attention from the medical profession, with the development of newer diagnoses and treatment methods. Presently, the treatment of mild to moderate SUI often uses conservative therapies, such as biofeedback therapy¹¹, pelvic floor muscle training¹², and drug therapy such as topical estrogen therapy¹³. Severe SUI treatment is usually based on surgical methods, including mesh vaginal surgery¹⁴, placement of synthetic slings¹⁵, local injection of bulking agents, and

implantation of artificial urinary sphincters^{16,17}. For patients with mild to moderate symptoms, topical estrogen therapy is the preferred approach owing to its convenience and fewer side effects. However, for a long time, the basic research of estrogen in the treatment of SUI is relatively lacking.

Extracellular matrix (ECM) is an important portion of the connective tissues of the pelvic floor. It is a complex long-chain protein mixture, composed of collagen, elastin, and proteoglycan¹⁸. Previous studies have confirmed that ECM remodeling is an important association in the pathogenesis of SUI¹⁹. It has been found that estrogen affects the transcription and expression of collagen, which are the main component of ECM²⁰. Estrogen replacement therapy (ERT) has been reported to reshape collagen in the urogenital tissues of postmenopausal women by changing its quantity and quality²¹. Animal experiments have proven that estrogen can increase the composition of loose connective tissues rich in blood vessels in the urethra²², and, at the same time, increase the distribution density of sympathetic nerves in the pelvic floor tissues and regulate neurotrophyl²³. Therefore, the supplementation of exogenous estrogen in postmenopausal SUI patients can effectively alleviate the symptoms of SUI²⁴.

Although the application of estrogen as a conservative treatment is clinically effective in the treatment of SUI²⁴, the basic research of estrogen in the treatment of SUI is relatively lacking. Meanwhile, reports on the remodeling of ECM caused by estrogen are not systematic. Therefore, in this article, according to our previous experience²⁵, we constructed the animal model of stress urinary incontinence by vaginal dilation, and correspondingly, we constructed the mechanical injury model of L929 fibroblasts²⁶. We have described the application of topical estrogen to treat SUI from the animal and cellular level to investigate the reasons affecting the efficacy of topical estrogen for SUI, the mechanism by which estrogen affects the expression of ECM, and then pave the way for the mechanism of treatment effects.

2. Materials And Methods

2.1 Cell Experimental Design and Cell Culture

Mouse L929 fibroblasts were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). Fibroblasts were fostered in Dulbecco's modified Eagle's medium (DMEM) (Procell Life Science & Technology Co., Ltd.), (supplemented with 4.5 g/L D-glucose, without Phenol red indicator) with 10% fetal bovine serum (FBS; Gemini Bio-Products, California, USA) and 1% antibiotics (100 KU/mL penicillin G, 100 mg/mL streptomycin; Genom Biotech Ltd.) in an incubator at 37°C under 5% CO₂ atmosphere.

In order to explore the expression of ECM without or with estrogen therapy before and after cell stress loading, we categorized the cells with the same growth status into four groups: normal cells without estrogen therapy (Group C), normal cells with estrogen therapy (Group E2), cells that had undergone cyclic mechanical strain with estrogen therapy (Group S+E2), and cells that had undergone cyclic mechanical strain without estrogen therapy (Group S). For cells treated with estrogen, the concentration of estrogen was 10⁻⁵ nmol/L, which was based on cell proliferation and apoptosis experiments. These

four groups of cells were cultured in an incubator for 4 h after their respective treatments, followed by collection in 6-well plates for subsequent experiments.

2.2 Cyclic Mechanical Strain

The L929 cells were collected on four-point bending cell strain gauges (Miracle Technology Co. Ltd., Chengdu, China) at a density of 1×10^5 cells/mL, and 2-mL cell suspension from this was uniformly spread on each of the strain gauges. Next, 4 pieces of stress gauges were laid each time, and only 2 of them were subjected to mechanical stress loading. The cells were continuously cultured in an incubator with 5% CO₂ at the temperature of 37°C for 24 h to prepare the cell slide. While the cells adhered firmly and grew up to a sub-fusion state, the slide was placed in the strain state containing 65-mL of complete culture medium and then stretched for mechanical strain loading, as shown in Fig. 1(b). The parameters of the four-point bending facility parameters (Miracle Technology Co. Ltd., Chengdu, China) were adjusted to a frequency of 1 Hz for 4 h and the cells were subjected to 5333 μ as described in our previous research, using 0 μ strain as the control strain²⁷. At the end of the cyclic mechanical strain, the cells on the slide were cultured for another 4 h, as shown in Fig. 1(c). Then, all the cells were collected to detect the corresponding indicators.

2.3 Cell Proliferation

β -Estradiol (E8875; Sigma) was dissolved in anhydrous ethanol to a concentration of 1 M, which was further diluted in DMEM (4.5 g/L D-glucose, without Phenol red indicator) to make the final concentration of β -estradiol to 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , and 10^{-8} nmol/L, respectively. The L929 cells were uniformly cultured in 96-well plates at the density of 5000 cells per well and fostered in a medium containing different concentrations of estradiol (100 μ L medium per well). The proliferation of L929 cells was investigated by using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc., Shanghai, China) for 24 h. The medium of each well was discarded, replaced with a mixture containing 90 μ L serum-free medium and 10 μ L of CCK-8 reagent to each well. After 2 h, the absorbance was measured by spectrophotometer at 450 nm (5 holes in each group).

2.4 Western Blotting

After applying the design-based treatment, the proteins were successfully obtained from L929 cells, the urethra, and the anterior vaginal wall of mice with RIPA lysate supplemented with phenylmethanesulfonyl fluoride (PMSF, 1 mM; Servicebio, Wuhan, China). Western blotting was performed as described previously²⁷. The antibodies used are listed below: anti-Collagen type I antibody (1:1000, Abcam, ab88147), anti-collagen type III antibody (1:500, Abcam, ab7778), anti-Elastin antibody (1:1000, Abcam, ab2173560), anti-MMP-2 antibody (1:1000, Abcam, ab86607), anti-MMP-9 antibody (1:1000, Abcam, ab228402), anti-TIMP-1 antibody (1:500, Santa, sc-21734), anti-TIMP-2 antibody (1:500, Santa, sc-21735), and anti-GAPDH antibody (1:1000, Abcam, ab8245).

2.5 Quantitative Real-Time Polymerase Chain Reaction (Q-PCR)

Q-PCR was performed to investigate the expression of related genes in this experimental design. The reaction was performed as described elsewhere²⁷. All the primers used in the study were obtained from Sangon Biotech (Shanghai, China; listed in Table 1).

Table 1
A list of primers used for the quantitative real-time polymerase chain reaction in this study.

Gene name	Gene ID	Primer sequence (5'-3')	Amplicon size (bp)
M-GAPDH	NM_008084.3	F: TGAAGGGTGGAGCCAAAAG R: AGTCTTCTGGGTGGCAGTGAT	227
M-COL1A1	NM_007742.4	F: CTGACTGGAAGAGCGGAGAG R: CGGCTGAGTAGGGAACACAC	116
M-Col3a1	NM_009930	F: CTCAAGAGTGGAGAATACTGGGTT R: GGTATGTAATGTTCTGGGAGGC	298
M-MMP2	NM_008610.2	F: GAATGCCATCCCTGATAACCT R: GCTTCCAACTTCACGCTCTT	127
M-MMP9	NM_013599.3	F: AAGGGTACAGCCTGTTCTGGT R: CTGGATGCCGTCTATGTCGTCT	149
M-TIMP-1	NM_053819.1	F: CCAGAAATCATCGAGACCACC R: ATTTCCGTTCTTAAACGGC	201
M-TIMP-2	NM_011594.3	F: GACACGCTTAGCATCACCCA R: TCTGTGACCCAGTCCATCCAG	140
M-Elastin	NM_007925.4	F: ACCCACAGGACAAGGAAATCA R: CCCACAAAGAAGAAGCACCA	177

2.6 Animal Experiment Design and Treatment

All our animal experimental protocols and implementations were conducted based on the institutional guidelines of the Institutional Review Committee and authorized by the Ethics Committee of the Institutional Animal Care and Use Committee of Wuhan University People's Hospital (20210306).

We purchased wild-type C57BL/6 female mice (8–9-week-old) from the Center for Animal Experiment of Wuhan University. A total of 60 wild-type C57BL/6 female mice were stochastically separated into four groups, as detailed here: control group (Group C), estrogen-alone group (Group E2), vaginal dilatation group (Group VD), and vaginal dilation with estrogen group (Group VD+E2). There were 15 mice in each

group. The groups VD and VD+E2 were subjected to vaginal dilatation on the same day. From the second day of vaginal dilation, conjugated estrogen cream (CEEs; Xinjiang Xinziyuan Bio-Pharmaceutical Co. Ltd, Yili, China) was applied to the vagina of the mice in groups E2 and VD + E2 using a 1-mL syringe. The calculation of vaginal CEE dose was based on the comparison of body weight and human dose, as mentioned in a previous study²⁸. The usual vaginal maintenance dose (1 g cream) for a 60-kg woman could produce 625 µg of CEEs. By weight comparison, the CEE dose for a 20-g animal was found to be 0.21 µg.

After 7 days of continuous administration of CEE to the mice in the two groups, the suprapubic canal was implanted in all mice belonging to the four groups, and the leakage point pressure (LPP) and abdominal pressure LPP (ALPP) were measured on the same day. Then, the anterior vaginal wall and the urethra of inanimate mice were obtained for western blotting and section staining.

2.7 Masson trichrome staining

A portion of the animal samples was intercalated into paraffin, sliced into transverse sections of thickness 4 µm, and stained with the Masson Kit HT15 (Sigma, USA) following the manufacturer's protocol. The mean optical density (MOD) value gathered by the ImageJ software was considered as the quantitative index to assess the content of collagen fibers.

2.8 Statistical analysis

All data were expressed as mean ± standard deviation. Statistical analysis was conducted with the GraphPad Prism software version 8 (San Diego, CA, USA). One-way analysis of variance (ANOVA) was employed for several comparisons in three or more groups. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1 Estradiol promotes the proliferation of L929 fibroblasts

The proliferation effect of estradiol at different concentrations on L929 cells was detected by the CCK8 assay. As shown in Fig. 1(a), β-estradiol had an increasingly significant proliferation effect on the L929 cells with increasing concentration. Specifically, at the concentration of 10^{-5} nmol/L, the L929 cells showed the maximum proliferation effect. These data suggest that 10^{-5} nmol/L concentration is the optimal concentration for L929 fibroblasts proliferation. Therefore, we selected 10^{-5} nmol/L as the optimal concentration of β-estradiol to treat L929 fibroblast for the subsequent experiments.

3.2 The promoting effect of estradiol on the expression of ECM for L929 fibroblasts

ECM is a complex long-chain protein mixture composed of collagen, elastin, and proteoglycan. Among these constituents, collagen I and collagen III are the main collagen types in the pelvic floor. They are of

vital importance for maintaining the elasticity and toughness of the pelvic floor support system. To reveal the effect of estradiol on ECM, we treated L929 fibroblasts according to the cell experiment design, after which the ECM production of L929 fibroblast was investigated by Western blotting and Q-PCR. As can be seen from Fig. 2 (a–c) and Fig. 3 (a–c), both the protein and mRNA expressions of COL1A1, COL3A1, and elastin were dramatically improved in the group E2 cells when compared with those in group C cells. Similarly, both the protein and mRNA expressions of COL1A1, COL3A1, and elastin were also dramatically improved in the group S+E2 cells when compared with those in the group S cells. These data together suggest that moderate β -estradiol has a positive effect on the expression of ECM.

3.3 Estradiol induces TIMP/MMP imbalance on L929 fibroblasts

Under normal physiological conditions, MMPs and TIMPs maintain a state of dynamic balance, which is essential for maintaining ECM homeostasis. MMPs mainly hydrolyze collagen and the basement membrane, while TIMPs inhibit the breakdown of ECM and the basement membrane. We conducted western blotting and Q-PCR to investigate the expression of MMP-2, MMP-9, TIMP-1, and TIMP-2 in the L929 fibroblasts among the four groups. As shown in Fig. 2 (d–g) and Fig. 3 (d–g), both the protein and mRNA expressions of MMP-2 and MMP-9 were significantly reduced in the group E2 cells when compared with that in the group C cells. Both the protein and mRNA expressions of TIMP-1 and MMP-9 showed dramatic improvement in the group S+E2 cells relative to that in the group S cells. These data together suggest that moderate β -estradiol can increase the expression of MMPs while decreasing the expression of TIMPs, thereby maintaining a balance of TIMP/MMP to stabilize the ECM homeostasis.

3.4 Estradiol improved the urodynamic parameters of LPP and ALPP in female SUI mice

To further corroborate the results of the above-mentioned *in vitro* studies, we employed the vaginal dilation method for establishing a mouse model of SUI disease. The computer, physiological recorder, pressure sensor, micro-injection pump, and other devices were used to ascertain that the urodynamic parameters were connected to obtain a urodynamic curve. For instance, the LPPS and ALPPs of mice in groups C and E2 are shown in Table 2. The LPPS and ALPPs of mice in groups VD and VD+E2 are shown in Table 3. The quantitative analyses of the parameters are indicated in Tables 2 and 3. As can be seen from these tables, the levels of LPPS and ALPPs were almost identical between groups C and E2. However, the LPPs and ALPPs in the group VD+E2 were significantly higher than those in the group VD. These data suggest that, for female mice with SUI, vaginal topical application of estrogen can improve the symptoms of urinary incontinence, although, for normal mice, the vaginal topical application of estrogen showed no significant effect on the assessed urodynamic parameters.

Table 2
Comparison of the urodynamic parameters between Group C and Group E2 (n = 15)

Index	Group C	Group E2	Difference(E2-C)	t	P
LPP (mmHg)	20.04±3.07	21.59±3.22	1.43±1.15	1.26	0.22
ALPP(mmHg)	21.12±2.98	23.23±3.34	2.10±1.16	1.82	0.08

Table 3
Comparison of the urodynamic parameters between Group VD and Group VD+E2 (n = 15)

Index	Group VD	Group VD+E2	Difference(VD+E2-VD)	t	P
LPP(mmHg)	13.68±2.91	18.42±3.21	4.73±1.12	4.24	<0.05
ALPP(mmHg)	15.58±3.14	20.33±2.98	4.84±1.18	4.26	<0.05

3.5 The effect of estrogen on the remodeling of ECM in the anterior vaginal wall and the urethra of SUI mice

To reveal the therapeutic effect of estrogen on female SUI mice, we collected samples from the urethra and anterior vaginal wall of each group of mice to investigate the expression of ECM-related genes by employing western blotting and Q-PCR. As shown in Fig. 4 (a–c) and Fig. 5 (a–c), both the protein and mRNA expressions of COL1A1, COL3A1, and elastin were dramatically enhanced in the group E2 mice when compared with those in the group C mice. Analogously, both the protein and mRNA expressions of COL1A1, COL3A1, and elastin were dramatically enhanced in the group VD+E2 mice relative to those in the group VD mice. As shown in Fig. 4 (d–g) and Fig. 5 (d–g), the protein and mRNA expressions of MMP-2 and MMP-9 in group E2 mice were greatly reduced when compared to those in the group C mice. Analogously, the protein and mRNA expressions of MMP-2 and MMP-9 in the group VD+E2 mice were significantly lower when compared to the group VD mice. As for TIMP-1 and TIMP-2, their protein and mRNA expressions were remarkably higher in group E2 mice than in the group C mice. Finally, both the protein and mRNA expressions of TIMP-1 and TIMP-2 were significantly higher in group VD+E2 mice than in group VD mice. These data together suggest that the effect of estrogen on the remodeling of ECM in the urethra and the anterior vaginal wall of female SUI mice is similar to the effect of estradiol on the remodeling of ECM secreted by L929 fibroblasts.

3.6 Histological observation on the effect of estrogen on ECM remodeling of the urethra and anterior vaginal wall in

SUI mice

In order to further reveal the effect of estrogen on the remodeling of ECM in the pelvic floor of mice with SUI, samples from the anterior vaginal wall and the urethra of inanimate mice were subjected to Masson staining and then photography (images shown in Fig. 6a–d). In these sections, the collagen fibers were blue and the muscle fibers were red. The mean optical density (MOD) value obtained by Image J was regarded as the quantitative index to assess the collagen fibers' content, as shown in Fig. 6(e). The collagen fibers' content in the group E2 mice was significantly greater than in the group C mice. Similarly, the collagen fibers' content in the group VD+E2 mice was also significantly greater than those in the group VD mice. These data together indicated that estrogen could effectively upregulate the expression of collagen fibers, which may explain why estrogen could alleviate the symptoms of mice with SUI.

4. Discussion

SUI is a common disease affecting women. It reportedly results from an imbalance of ECM remodeling in the urethra and anterior vaginal^{26, 29}. ECM reconstruction is mainly regulated by fibroblasts present in the pelvic floor³⁰. Based on the present and previous study results, we selected L929 fibroblasts as our experimental cells²⁶. Collagen I and collagen III are the main types of pelvic floor collagen, while elastin is the key component of connective tissues that provide elasticity and support to the pelvic floor tissues³¹. In this study, we found that the expression of collagen I, collagen III, and elastin were lower in the urethra and anterior vaginal wall of mice with SUI when compared with those in the control group mice. Based on the above results, we obviously speculated that the restoration of the expression of ECM may facilitate SUI treatment.

McKenzie et al. [32] reported that estrogen affects the gene transcription and the expression of collagen, which is the main component of ECM³². ERT has been proven to improve certain aspects of the skin, such as by enhancing the morphology and synthesis of elastic fibers, collagen III, and hyaluronic acid to reduce wrinkles³³. Past studies have demonstrated that conjugated equine estrogen can improve bone quality as it increases the amount of collagen I³⁴. Other past studies have shown that estrogen plays a positive role in protecting glaucoma. For instance, estrogen activates the synthesis of collagen and enhances the flexibility of the eyes, thereby reducing the intraocular pressure³⁵. Past studies reported that, in the menopausal rat model, local estrogen treatment increased the content of total collagen and cross-linked collagen, which significantly stimulated the expression of collagen mRNA in a dose-dependent manner³⁶. Based on the above findings, we attempted to evaluate the efficacy of estrogen for the treatment of SUI mice. The results revealed that the LPP and ALPP of SUI mice treated with estrogen were higher than those of the control mice. Moreover, the expression of the urethra and the anterior vaginal wall in SUI mice treated with estrogen. The urethra and anterior vaginal wall of SUI mice expressed higher levels of collagen I, collagen III, elastin, TIMP-1, and TIMP-2 and lower levels of MMP-2 and MMP-9 relative to those of the control group mice. Our *in vitro* experiments revealed the same outcomes, both L929 fibroblasts after cyclic loading and L929 fibroblasts without cyclic loading expressed higher levels

of collagen I, collagen III, elastin, TIMP-1, and TIMP-2 and lower levels of MMP-2 and MMP-9 in the presence of estrogen when compared with fibroblasts without estrogen treatment. These results together suggest that estrogen may alleviate the symptoms of SUI by reconstituting ECM.

The guidelines toward the diagnosis and treatment of female SUI (2017) in China indicated that vaginal local estrogen therapy can relieve some postmenopausal SUI symptoms and lower urinary tract symptoms. However, the reason why estrogen can be used to treat female SUI continues to remain unclear, and past studies have not been systematic in this regard. Although this study confirmed the effect of estrogen on ECM remodeling for the treatment of female SUI, the specific mechanism remains to be explored. The classic pathway of estrogen action is the estrogen receptor (ER) pathway. Past studies have demonstrated that 17 β -estradiol treatment in cells can activate ER³⁷. The ER subtypes mainly include ER α and ER β , and past studies have indicated that, in cardiac fibroblasts, 17 β -estradiol has a sex-specific activation effect on ER α and ER β ³⁸. Moreover, past studies have demonstrated that, in skin fibroblasts, 17- β estradiol can significantly reduce the stimulation of TGF- β on collagen synthesis as well as reduce the activation of the classic TGF- β /Smad2/3 signaling pathway³⁹. We thus speculated that estrogen may have other pathways to mediate ECM remodeling, and plan to focus on the same in our future work.

Declarations

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Conflict of Interest

The authors have declared that they have no conflict of interest.

Author contributions

LL: study design,, data curation, formal analysis, and manuscript writing; SS: protocol development and data analysis; YL: cell experiment, data collection, and analysis ;XL: mouse modeling, project administration,data collection, and analysis; JF: tissue sampling of mice and data collection; LY:molecular experiment, data collection, and analysis; LH: conceptualization, funding acquisition, and final approval.

Ethics approval

All our animal experimental protocols and implementations were conducted based on the institutional guidelines of the Institutional Review Committee and authorized by the Ethics Committee of the

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

Figure 1

The cyclic mechanical strain on L929 fibroblasts. (a) The proliferation of L929 cells with different concentrations of estradiol was performed with the Cell Counting Kit-8. (b) The strain gauges and four-point bending cell strain gauges. (c) Experimental diagram and the simulation diagram of mechanical strain.

Figure 2

The promoting effect of estradiol on the ECM expression for L929 fibroblasts. (a–c) Estradiol promoted the expression of collagen I, collagen III, and elastin in L929 cells, which are the main components of ECM. (d–e) Estradiol inhibited the expression of MMP-2 and MMP-9 in L929 cells. (f–g) Estradiol promoted the expression of TIMP-1 and TIMP-2 in L929 cells. (h) Protein electrophoresis bands. (C: normal cells without estrogen therapy; E2: normal cells with estrogen therapy; S: cells undergone cyclic mechanical strain without estrogen therapy; S+E2: cells undergone cyclic mechanical strain with estrogen therapy. *compared with C, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; #compared with E2, $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$; \$compared with S, $p < 0.05$,

$p < 0.01$,

\$ $p < 0.001$,

$p < 0.0001$. Every experiment was repeated thrice).

Figure 3

Estradiol promotes the mRNA expression levels of collagen α 1(I), collagen α 2(I), and elastin related to the ECM in L929 cells. (a–c) Estradiol promoted the mRNA expression levels of collagen α 1(I), collagen α 2(I), and elastin in L929 cells. (d–e) Estradiol inhibited the mRNA expression levels of MMP-2 and MMP-9 in L929 cells. (f–g) Estradiol promoted the mRNA expression levels of TIMP-1 and TIMP-2 in L929 cells. (C: normal cells without estrogen therapy; E2: normal cells with estrogen therapy; S: cells that underwent cyclic mechanical strain without estrogen therapy; S+E2: cells that underwent cyclic mechanical strain with estrogen therapy. *compared with C, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; #compared with E2, $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$; \$compared with S, $p < 0.05$,

$$p < 0.01,$$

$$\$p < 0.001,$$

$p < 0.0001$. Every experiment was repeated thrice).

Figure 4

The effect of estrogen on ECM remodeling in the anterior vaginal wall and the urethra of SUI mice. (a–c) Estradiol promoted the expression of collagen I, collagen III, and elastin in the vaginal wall and the urethra of SUI mice. (d–e) Estradiol inhibited the expression of MMP-2 and MMP-9 in the vaginal wall and the urethra of SUI mice. (f–g) Estradiol promoted the expression of TIMP-1 and TIMP-2 in the vaginal wall and the urethra of SUI mice. (h) Protein electrophoresis bands. (C: control group; E2: estrogen-alone group; VD: vaginal dilatation group; VD+E2: vaginal dilation with the estrogen group. *compared with C, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; #compared with E2, $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$; \$compared with VD, $p < 0.05$,

$$p < 0.01,$$

$$\$p < 0.001,$$

$p < 0.0001$. Every experiment was repeated thrice).

Figure 5

Estradiol promoted the mRNA expression levels of collagen α 1(I), collagen α 2(I), and elastin related to the ECM in the anterior vaginal wall and the urethra of SUI mice. (a–c) Estradiol promoted the mRNA expression levels of collagen α 1(I), collagen α 2(I), and elastin in the anterior vaginal wall and the urethra of SUI mice. (d–e) Estradiol inhibited the mRNA expression levels of MMP-2 and MMP-9 in the anterior vaginal wall and the urethra of SUI mice. (f–g) Estradiol promoted the mRNA expression levels of TIMP-1 and TIMP-2 in the anterior vaginal wall and the urethra of SUI mice. (C: control group; E2: estrogen-alone group; VD: vaginal dilatation group; VD+E2: vaginal dilation with the estrogen group. *compared with C, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; #compared with E2, $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$; \$compared with VD, $p < 0.05$,

$$p < 0.01,$$

$$\$p < 0.001,$$

$p < 0.0001$. Every experiment was repeated thrice).

Figure 6

Histological observation of the effect of estrogen on ECM remodeling of the urethra and the anterior vaginal wall in SUI mice. (a) The collagen fibers' content in group C mice. (b) The collagen fibers' content in group E2 mice. (c) The collagen fibers' content in group VD mice. (d) The collagen fibers' content in group VD+E2 mice. (e) The MODs of collagen fibers after Masson staining. (C: control group; E2: estrogen-alone group; VD: vaginal dilatation group; VD+E2: vaginal dilation with estrogen group. *compared with C, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; #compared with E2, $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$; \$compared with VD, $p < 0.05$,

$$p < 0.01,$$

$$\$p < 0.001,$$

$$p < 0.0001).$$