Acceleration of Pelvic Tissue Construction by Overexpression of Basic Fibroblast Growth Factor in the Stem Cells

Xiaotong Wu
Peking University People's Hospital https://orcid.org/0000-0001-7567-8674
Yuanyuan Jia
Peking University People's Hospital
Shiyan Wang
Peking University People's Hospital
Jiaqi Wang
Peking University People's Hospital
Xiuli Sun (sunxiuli918@126.com)
Peking University People's Hospital
Jianliu Wang
Peking University People's Hospital

Research

Keywords: Pelvic organ prolapse, adipose-derived stem cells, tissue engineering, gene therapy

Posted Date: September 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-70178/v1

Abstract

Background Pelvic organ prolapse (POP) is a common debilitating condition affecting about 30–40% of women. The application of stem cells therapy and growth factor has greatly promoted the development of pelvic tissue engineering, which remains a promising approach, but there is no consensus on the therapeutic mechanism of stem cells and the application of growth factors. Stem cells were mainly used as seed cells to differentiate into target tissue cells, fuse with target tissue after transplantation and paracrine effect to play a therapeutic role in pelvic tissue engineering. However, whether stem cells can be differentiated into target tissue cells is still to be a question,in this regard, the contemporary trend is to investigated the effect of adipose-derived stem cells (ADSCs) as the seed cells of pelvic tissue engineering on the repair of POP and the underlying mechanisms.

Methods In the present study,we evaluated the therapeutic potential of gene-modified ADSC that overexpress basic fibroblast growth factor（bFGF）and evaluated its effects on paracrine function and directional differentiation ability.

Results The results showed that following ADSCs are designed to continuously release controllable levels of growth factors during the control period of repair, taking advantage of the paracrine function of stem cells to accelerate cell growth and extracellular matrix (ECM) reconstruction during the early stage of stem cell implantation, and then stem cells are differentiated into target tissues-fibroblasts to accelerate the reconstruction of pelvic floor tissues.

Conclusions We suggest that the observed effects are determined by pleiotropic effects of bFGF, along with the multifactorial paracrine action of ADSC which remain viable and functionally active within the engineered cell construct.Thus, we demonstrated the high therapeutic potential of the utilized approach for pelvic tissue engineering.

1. Introduction

POP is the herniation of pelvic organs into the vagina with symptoms of bladder, bowel, and sexual dysfunction[1].For women who suffering from POP, surgery is the primary treatment option and recurrence is common with ungrafted methods[2].Several complications such as foreign body reactions, excessive inflammatory responses, and vaginal erosion affect the use of synthetic mesh or biological grafts in clinical practice[3].Therefore, tissue engineering methods using stem cells for POP are rapidly emerging as potential strategies to treat patients the application of stem cells has greatly promoted the development of tissue engineering,which has been successfully applied to the regeneration of bone, cartilage, skin, muscle and other organs[4, 5].A variety of studies have reported differences between various types of stem cells, including bone marrow mesenchymal stem cells (BMSCs), endometrial mesenchymal stem cells (EMSCs), and ADSCs, which showed promising safety and efficacy for POP in vitro and in vivo[6].Adipose tissue is easily obtained by liposuction which has been shown to have more stem cells/progenitor cells than bone marrow tissue, and ADSC has a faster expansion capacity than

stem cells derived from other tissues,A recent study compared the differences of ADSCs and BMSCs, they found ADSCs as a more stable and controllable stem cell source, was more adaptable and exhibited superiority in regulating inflammation [7]. ADSCs are more active than BMSCs, secrete more autocrine cytokines and immunoregulatory factors, and exhibit lower immunogenicity, which is ideal for cell therapy[8–10].The main therapy mechanism of stem cell is directional differentiation and self-renewal ability, which can be used as seed cells to differentiate into target tissue cells or fuse with target cells after transplantation. With proper conditions, they can display strong tissue and organ repair capabilities. However, if stem cell are not properly controlled, they may not achieve the goals, and may even cause safety hazards such as tumors. Therefore, how to induce stem cells in a direction which suitable for pelvic floor supporting tissues—fibroblast,is also the current study bottleneck.

Research has demonstrated that ADSCs can be induced into fibroblasts by growth factors in vitro[11–12].Growth factor is an important factor that constitutes the cell micro-environment. FGF is one of the most widely studied among many cell growth factors. It is one of the important wound healing factors in the body, has a wide range of biological activities and can affect early inflammation, granulation and the entire wound repair process such as tissue regeneration, epithelialization, and tissue remodeling,thus, has an important application value[13].Our data indicate successfully induced the ADSCs into fibroblasts by bFGF in vitro, and confirmed that bFGF can significantly increase the proliferation of differentiated fibroblasts, moreover the expression of collagen.However, the growth factor half-life is short and the biological permeability is poor, so it is very easy to be inactivated, and repeated dosing is required to maintain the local concentration [13]. Additionally, studies have shown that bFGF injection is associated with severe anemia, which limits the systemic application of bFGF [14–15]. Therefore, it is necessary to achieve continuous release and an effective concentration of bFGF, in order to provide a suitable micro-environment for the directed differentiation of stem cells.

Therefore, we hypothesized that the use of ADSC to overexpress the bFGF gene and provide a reasonable microenvironment of bFGF which can accelerate the repair and reconstruction of pelvic floor tissue. When ADSCs are designed to continuously release controllable levels of growth factors during the control period of injury repair, take advantage of the paracrine function of stem cells to accelerate cell growth and extracellular matrix reconstruction during the early stage after stem cell implantation, and later stem cells are differentiated into target tissues-fibroblasts under the continuous stimulation of bFGF to accelerate the reconstruction of pelvic floor tissues. The combination of gene and cell therapy will accelerate the reconstruction of pelvic floor tissue through a combination of paracrine and directed differentiation mechanisms, and may be more effective than systematically giving individual growth factors.The results of this basic study may indicate a new strategy to speedily ameliorate POP.

2. Materials And Methods

2.1 Isolation and culture of rat ADSCs

SD rats (8weeks old) were sacrificed with 3% sodium pentobarbital. The bilateral inguinal fat pads were harvested and digested at 37℃ with mild agitation for 1 hour using 0.1% collagenase type I (Sigma-Aldrich, St. Louis, MO, USA). Digestion was terminated by adding an equal volume of Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12; GIBCO Cell Culture, Carlsbad,CA, USA) containing 10% fetal bovine serum (FBS; GIBCO Cell Culture).Then, the suspension was centrifuged at 500 g for 5 minutes. Next, the cell pellet was resuspended and cultured in DMEM/ F12 and 10% FBS and 1% penicillin/streptomycin (GIBCO Cell Culture). Cultures were maintained at 37℃ in a humidified incubator containing 5% CO2,These cells were 99.99% CD45 negative and positive for CD105 (> 70%), CD29 (> 99%) and CD44 (> 98%) [13].

2.2 Construction lentivirus and transfection

Lentiviral constructs expressing rat bFGF were prepared by Hanbio(shanghai)using HBLV-r-Fgf2-Null-ZsGreen-PURO.ADSCs at passage 2 were co-transduced or single-transduced with lentiviral constructs at a multiplicity of infection (MOI) of 100 and were screened with 4 μg/mL puromycin. After 72 h of screening, green fluorescence were observed using an immunofluorescence microscope to confirm successful transfection of ADSCs. In addition, the expression of bFGF was detected using Real time RT-PCR and western blot. Real time RT-PCR was carried out by isolation of the total RNA cultured on the all groups using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). For synthesize of the cDNA, random hexamer primer and M-MuLV Reverse Transcriptase kit (Fermentas, Helsingborg, Sweden) was applied based on the manufactured protocol. Finally, real time RT-PCR was carried out using SYBR premix ExTaq (Takara Bio, Shiga, Japan) and an ABI StepOne system. All primers were used in this study was bFGF:Forward5'-ACGCCTGGAGTCCAATAACTACAAC-3'Reverse5'-AGGCCCCGTTTTGGATCCGAGTTTA – 3';β-Actin:Forward5'-TGGGTATGGAATCCTGTGGCA – 3',Reverse5'- TGTTGGCATAGAGGTCTTTACGG – 3')

2.3 Preparation of cell culture media(CM)

ADSC, ADSC^{bFGF} were cultured in 6-well plates (105 cells/well). When the cells reached 90% confluence, the medium was replaced with 1 mL of serum-free medium, and the cells were incubated for 24 hours. The supernatant was collected after centrifugation at 12 000 g for 10 minutes and stored at − 80 °C.

2.4 The Paracrine of ADSCs and ADSC^{bFGF}

2.4.1 CCK8 assay.

When the confluency of the ADSCs and ADSC^{bFGF} reached ~ 80%, the cells were washed with PBS twice and treated with 0.25% trypsin, followed by preparation of a single cell suspension. After counting, the cells were seeded in a 96-well plate(Costar) with a cell density of 2×10^3 cells per well (0.2 ml, with 6 replicate wells), which were placed in a 5% CO2 cell culture medium and kept at 37 °C and saturated humidity; 10 ul CCK-8 was added to each well followed by a further incubation at 37 °C for 2 h. The medium was then removed, and the dye intensity was then read on a micro-plate reader (Bio-Rad) at 450 nm.

2.4.2 Co-culture assay

To confirm the paracrine effect of ADSCs and ADSC^{bFGF} for fibroblasts, we used the co-culture system (Fig. 1).The co-culture groups were established between ADSCs (5 × 103cells/well) in Cell culture inserts (Cell Culture inserts, 0.4 μm pores, BD Falcon™) and fibroblasts (5 × 103cells/well) in twelve-well plates (Cell Culture Insert Companion Plates, BD Falcon™) in DMEM.The control group was established between no cells in Cell culture inserts and fibroblasts only in twelve-well plates (5 × 103cells/well) in DMEM.

2.4.3 Wound healing assay

For the chemotaxis of ADSCs and ADSC^{bFGF} ,migration assay of fibroblasts, the fibroblasts were prepared by Yubio(shanghai)in ADSCs-CM treated and ADSC^{bFGF}-CM for 24 h, perspectively.The fibroblast monolayers were carefully scratched using a 10-μl pipette tip. After 24 h, the wounded area was photographed. The empty area which indicates the wound region was calculated.

2.4.4 Transwell assy

Cell transwell was assayed using transwell chambers (Costar, Cambridge, MA) with 8-mm-pore polycarbonate filters. Fibroblasts(10,000 cells/chamber) were seeded in the top of the cylindrical cell culture inserted in the DMEM without FBS. ADSCs-CM and ADSC^{bFGF}-CM was placed in the wells to allow the cells to migrate through the filter for 36 h at 37 °C in 5% CO2,perspectively. Cells that did not migrate were removed from the upper surfaces of the chambers by scrubbing with a cotton swab. Cells that migrated to the lower membrane were fixed in 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet (Invitrogen) for 30 min. Invasion cells were counted under a microscope.

2.4.5 Human umbilical vein endothelial cell tube formation assay

The human umbilical vein cell line, EA. hy926 (HUVEC), was were prepared by Yubio(shanghai).Tube formation was evaluated by culturing HUVEC on BD Matrigel (BD Biosciences). After incubating the wells with 80 μL of Matrigel for 1 hour, the HUVEC were resuspended in theADSCs-CM and ADSC^{bFGF}-CM and DMEM medium alone as negative control (NC) into 96-well plates (5000 cells per well), then the number of tube-like structures were analysed 4 hours later. Quantitative analysis based on the number of lumens in each high-power field.

2.5 Western blot analysis

Total protein was extracted from cells with a radio-immunoprecipitation assay buffer (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) containing PMSF, incubated on ice for 30 min and centrifuged at 4˚C for 10 min (12,000 x g). The supernatant was obtained. The protein concentration of the supernatant of each sample was determined using a bicinchoninic acid kit (cat. no. 23225; Thermo Fisher Scientific, Inc.), and deionized water was then used to adjust the amount of protein. Next, a 10% SDS-PAGE gel (cat. no. P0012A; Beyotime Institute of Biotechnology, Shanghai,

China) was prepared and 50 μg of the protein sample was added to each well. Electrophoresis was conducted at a constant voltage of 80 V for 2 h. The proteins were transferred onto polyvinylidene fluoride (PVDF) membrane (cat. no. ISEQ00010; EMD Millipore, Billerica, MA, USA) with a voltage of 110 V for 2 h. The PVDF membrane was blocked with TBST buffer containing 5% skimmed milk powder for 2 h. Then, the membrane was washed with TBST and incubated with rabbit polyclonal anti-body ColIA1 (1:100; cst), rabbit polyclonal antibody Col III A1(1:100; NOVUS), and GAPDH antibody (1:100;Abcam) The gray intensity of protein bands was quantitated with Image J and normalized to GAPDH. The p-Akt (Thr308), Akt, p-PI3K (Tyr458),PI3K, antibodies were purchased from Cell Signaling. bFGF growth factor antibodies were purchased from peprotech, at 4°Covernight, followed by three washed with TBST, 10 min each time for 3 times. The membrane was then washed with 0.1% PBS/Tween-20 (PBST) 3 times at room temperature, 10 min each time, immersed in enhanced chemiluminescence reaction solution (cat. no. WBKLS0100; EMD Millipore), and developed GAPDH was used as the internal reference, and the ratio of the gray values of the target protein band to the internal reference band was used as the relative protein expression.

2.6 The ADSCs differentiate into fibroblasts

ADSCs and ADSC^{bFGF} from the third generation were selected and divided into three groups, with the cell density adjusted to 1×10^5/ml: Control group (cells inoculated and cultured in DMEM-F12 containing 5% FBS),ADSCs group (cells inoculated and cultured in DMEM-F12 containing 5% FBS and 20 ng/ml bFGF) and ADSC^{bFGF} group (cells inoculated and cultured in DMEM-F12 containing 5% FBS). Three parallel samples were used for each group. The medium was replaced every day. The survival condition of cells was observed after 1–4 weeks under an inverted microscope, followed by subsequent studies.The cells were then identified by immunohistochemistry of fibroblastspecific protein1 (FSP1).

2.7 Statistical analysis

SPSS 21.0 statistical software (IBM Corp. Armonk, NY, USA) was used to analyze the data, which were processed with normal distribution and homogeneity of variance tests. Measurement data are expressed as the mean ± standard deviation. Comparisons between two groups were conducted by t-test. Comparisons among multiple groups were assessed by one-way analysis of variance. The pairwise comparisons among groups were assessed by Tukey post hoc test. Enumeration data are presented as % and were assessed by the chi-square test. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

1. ADSC^{bFGF} exhibited a higher bFGF level and cell viability

Primary ADSCs were isolated from adipose tissue of 2-week-old Sprague-Dawley rats cultured in vitro. Spindle morphology and fibroblast-like morphology were observed at passage 1, and these characteristics were more pronounced at passage 3 (Fig. 1a). Fluorescence microscopy showed that most cells expressed green fluorescent protein, which indicated successful transduction. Furthermore,

qPCR and western blot analysis showed that the expression of bFGF was significantly higher in ADSCs transfected with bFGF than that in vehicle-treated ADSCs (Fig. 1F,G,H). Western blot and qPCR results confirmed that bFGF were expressed at higher levels in $ADSC^{bFGF}$ than in vehicle (Fig. 1L,M,N).CCK8 assay demonstrate that after transfected with bFGF,the proliferation of $ADSC^{bFGF}$ correspondingly increased.

2. ADSC paracrine ability and function was further increased after bFGF transfected

To confirm the paracrine effect of ADSCs on fibroblasts, their cell viability was evaluated using a co-culture system (Fig. 2a).The cell viability of fibroblasts co-cultured with $ADSC^{bFGF}$ was higher than that of fibroblasts cultured with ADSCs and alone(Fig. 2b). ADSCs made tubule formation of HUVECs and chemotaxis of fibroblasts(Fig. 2c-g).Then we examined the effect of ADSC on the fibroblast collagen synthesis ability and found both collagen I and III expression levels were increased ,collagen synthesis ability were further increased after ADSC transfected with bFGF（Fig. 2 h-i).

3. $ADSC^{bFGF}$ differentiate into fibroblasts with a higher efficiency

In order to further verify the differentiation efficiency of ADSCs differentiate into fibroblasts after transfection, The three group differentiation following treatment with or without bFGF for 4 weeks. The control group and $ADSC^{bFGF}$ group vehicle-treated ,bFGF group treated with 20 ng/ml stimulation.The results showed that that the $ADSC^{bFGF}$ cell morphology gradually becomes long spindle after 2 weeks of culture,present a radial growth，with compact cytoplasm that was different from the loose cytoplasm of ADSCs under a light microscope,the ADSC group have a large and loose cytoplasm, short protrusions and without morphological change until 4 weeks culture.Most cells have the typical fibroblast cell morphology on the 4 weeks, indicating that the cells gradually differentiate into fibroblasts cells (Fig. 3a).After 4 weeks, immunocytochemical staining was performed on the three groups of cells，$ADSC^{bFGF}$ were positive for FSP1 and FGF group FSP-1 positive about 50%,the control group FSP-1 were negative(Fig. 3b). The collagen expression ability was tested on the 2th and 4th week，immunofluorescence and WB showed that the collagen expression ability of $ADSC^{bFGF}$ increased from the 2nd week and reached the highest in the 4th week, which was significantly higher than the bFGF stimulation group(Fig. 3c-f).Together，these results suggest that ADSCs overexpress bFGF may induce ADSC differentiation in vitro more effectively than with bFGF or alone.

4. $ADSC^{bFGF}$ activated PI3K/Akt signaling pathway to promote ADSC differentiate into fibroblast

PI3K phosphorylase is a class of enzymes that phosphorylate phosphatidylinositol and can mediate intracellular signal transduction. The study found that growth factors can mediate stem cell differentiation by activating PI3K /Akt signaling and regulating Akt phosphorylation levels.Thus, we examined if the PI3K/Akt signaling pathway was involved in the process of $ADSC^{bFGF}$ mediate differentiation. We detected the changes in this pathway via Western blotting. Consistent with our hypothesis,we found in $ADSC^{bFGF}$ further increased the phosphorylation of PI3K, and Akt proteins compared with the ADSCs group (Fig. 5a,b,c). Then to elucidate if the PI3K/Akt signaling pathway is necessary for the differentiation process, we treated the two groups with or without PI3K inhibitor, LY294002.We found that LY294002 decreased the collagen expression which was consistent with our data (Fig. 4d,e), LY294002 reversed the increased expression of collagen.These data demonstrated that $ADSC^{bFGF}$ accelerated the differentiation process via increasing the activity of PI3K/Akt signaling pathway in a time-dependent manner.

5. The $ADSC^{bFGF}$ decreased the inflammation response in the injured fibroblasts

ADSC has been widely used for the anti-inflammatory therapy and wound healing and bFGF is an important damage repair factor. And it has been demonstrated that immune response plays important roles in the process of wound healing. Thus, we wanted to know if the immune response was also regulated by $ADSC^{bFGF}$ during the promotion of wound healing. We treated the injured fibroblasts isolated for 0,3,and 5 days post injury and treated them with LPS, which could induce the immune response ,together co-culture with or without $ADSC^{bFGF}$.After 1 day, we examined the expression of inflammation factors, TNF-αandIL-6, by CBA.Indeed, we found that LPS significantly induced the immune response ,with the up-regulation of IL-1,TNF-αand IL-6,while $ADSC^{bFGF}$ dramatically repressd the increase in IL-1, IL-6 and TNF-α (Fig. 5). These data demonstrated that $ADSC^{bFGF}$ could suppress the inflammation in vitro.The present study demonstrated that $ADSC^{bFGF}$ provided strong pleiotropic effects (Fig. 6), contributing to more effective morphological and functional recovery. This therapeutic strategy that could be applied as seed cells which holds great promise for the pelvic floor tissue engineering.

4. Discussion

In view of the current status of mesh application in pelvic floor reconstruction surgery, the ability to induce autologous tissue regeneration is the study and development direction of pelvic floor tissue engineering mesh. Considering the role of bFGF in the repair of pelvic floor tissue is obvious but uncontrollable, the study transduced the bFGF gene into ADSCs to produce sustained, stable, and long-term expression of bFGF, moreover avoiding some adverse reactions.When ADSCs is designed to overexpress growth factors such as bFGF, this paracrine effect may be amplified [16]. We observed that $ADSC^{bFGF}$ increased its own proliferation and the ability to induce angiogenesis also increased, which is consistent with the results of our observation of increased levels of bFGF after transfection. BFGF

stimulates blood vessel growth and has a synergistic effect with VEGF and platelet-derived growth factor (PDGF) during angiogenesis, which is important for wound healing [17–18].Moreover, prolonged exposure to protein mitogens, such as FGFs, is associated with increased risk for cancer [19–21] and induces pro-inflammatory responses in vitro[22].The oncogenic and proinflammatory effects of bFGF were observed with continuous exposure to bFGF at the dose of 10 ng/mL or larger, which were approximately at least 1,000-fold higher than the level of bFGF being released from MSCs[23] .ADSCs play an important role in the activation and migration of fibroblasts in tissue repair,we found that the chemotaxis ability of $ADSC^{bFGF}$ to fibroblasts did not increase, which may be related to the expression of chemokines didn't increase.

We found $ADSC^{FGF}$ has increased fibroblast proliferation and collagen expression. Fibroblasts can synthesize many ECM proteins, such as collagen, remodeling enzymes and their inhibitors. Fibrous collagen I and III are the main components of the vaginal and pelvic floor supporting tissues.Collagen I forms thick collagen fibers that provide continuous tension to the pelvic floor tissue. Collagen III mainly affects the flexibility and expansibility of tissues to overcome cyclical stress. Therefore, Col-I and III play an important role in repairing POP [24]. Studies have shown that abnormal metabolism of collagen may be one of the reasons that cause pelvic floor dysfunction disease in women [25], we observed that the $ADSC^{FGF}$ can be induced into fibroblasts and enhance the synthesize collagen capability,the microenvironment of the implantation site is improved，which may mediates the reparation of pelvic floor tissue. Through the activation of fibroblasts, collagen synthesis is promoted, the collagen content of local tissues is improved,the ECM is reconstructed，thereby，The repair efficiency of tissue engineering has been improved [26].Increasingly evidence shows that during wound healing, collagen mediates the ECM to promote tissue regeneration[27]. Fibroblasts play a key role in wound healing by precisely regulating their function [28].

A key mechanism for ADSCs to promote tissue repair is the ability to differentiation. With appropriate conditions, they can display strong tissue and organ repair capabilities. However, if they are not properly controlled, we can not reach the goal, and may even cause safety hazards such as tumors. Therefore, how to induce stem cells to a directional differentiation which suitable for the repair of pelvic floor supporting tissues is a key for pelvic tissue engineering mesh, and is also the current research bottleneck. In this study have shown that indirect co-culture can promote the synthesis of type Ⅰ and Ⅲ collagen fibers by $ADSC^{bFGF}$, and successfully induce themselves differentiate into fibroblasts [29–30]. Lee et al [31] added connective tissue growth factor to the culture medium to differentiate bone marrow umbilical cord mesenchymal stem cells into fibroblasts. Yin et al [32] colonized mesenchymal stem cells on interlaced scaffold materials. The cells could differentiate into ligament-like fibroblasts and express ligament-related collagens such as type Ⅰ, type Ⅲ collagen, and Tenascin-C gene. The study by Xiong et al [33] showed that bFGF can induce bone marrow mesenchymal stem cells differentiate into fibroblasts, and there is no significant statistical difference in cell morphology and collagen synthesis ability from cells derived from veins.We successfully induced the differentiation of ADSCs into fibroblasts through bFGF[13],and found that the $ADSC^{bFGF}$ gradually differentiated into fibroblasts and more efficient: the

expression of the fibroblast surface marker fsp-1 has been observed in about two weeks, most of the cells expressed fsp-1in fourth week.Then we found the production ratio and efficiency of collagen are higher. This provides a basis for the directed induction of differentiated stem cell in the direction of suitable pelvic floor tissue repair.

PI3-K/Akt is one of the important signaling pathways involved in the regulation of cell proliferation, self-renewal and multi-directional differentiation potential[34]. PI3-K mainly are activated by platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), insulin, FGF, guinea pig hepatocyte growth factor (HGF), recombinant human epidermal growth factor (EGF), bone morphogenetic protein 2 (BMP-2) and other receptors by binding to their receptors. Activated PI3-K phosphorylates the 3rd hydroxyl group of the inositol ring to generate three 3-phospholipid inositol phospholipids. The product of PI3-K finally phosphorylates Ser473 and Thr308 on the Akt protein sequence to make it fully activated, which in turn activates the Akt downstream signaling pathway, mediating cell proliferation, migration, differentiation and survival[35]. Recently, the role of PI3-K/Akt signaling pathway in stem cell differentiation has received attention. Whether the PI3-K/Akt signaling pathway plays a regulatory role in the induction of ADSC differentiation into fibroblasts after bFGF transduction is unclear. We observed an increase in p-Akt levels of $ADSC^{bFGF}$, indicating that $ADSC^{bFGF}$ can activate the PI3-K/Akt signaling pathway. In addition, the collagen expression of the cells after induction was significantly increased.Importantly, we found inhibition of PI3K with LY294002 reversed the effect of $ADSC^{bFGF}$ on the differentiation process,p-Akt levels decreased significantly, $ADSC^{bFGF}$ were negative for FSP1, after Ly294002 used to suppress the PI3-K/Akt signal pathway, the collagen expression was significantly reduced. The results of this experiment indicate that after ADSC overexpresses bFGF gene, it activates PI3-K /Akt signaling pathway and promotes the differentiation of ADSCs into fibroblasts. Therefore, we believe that by regulating the expression of bFGF and the activation of the PI3-K/Akt pathway, it promotes the induction and differentiation of ADSCs, thereby speeding up the process of tissue repair and provides a new treatment strategy for pelvic floor tissue engineering.

The inflammatory response is the first step after trauma, and plays an important role in the wound healing process, and the continuous inflammatory response will damage the wound healing process. Therefore, it is also crucial to suppress inflammation during wound healing.In recent years, adipose tissue has become an attractive source of MSCs for cell-based therapies and regenerative medicine. ADSCs can be harvested from an ever increasing number of liposuction procedures.ADSCs have similar properties to BMSCs but do not decline with the age of the donor and are an alternative source of MSCs in regenerative medicine [36].Regardless of their origin, MSCs are usually defined by their trophic, paracrine and immunomodulatory functions[37]. These non-stem cell properties appear to have the greatest therapeutic impact, evidenced by the large number of MSC-based clinical trials conducted for several life-threatening inflammatory or immune-related diseases [38]. A large body of medical literature indicates that MSCs repair damaged tissues because they respond to inflammation and migrate to injured sites and influence the microenvironment through the release of molecules involved in reparative processes and tissue regeneration [39]. Biomaterial-based delivery of MSCs may benefit organ and tissue repair through

paracrine effects.These properties make MSCs an attractive source of cells for seeding on the engineered biomaterials to influence the foreign body reaction following implantation [40].We found that ADSC^{bFGF} significantly inhibited the inflammatory response of damaged fibroblasts treated with lipopolysaccharide. The results show that ADSC^{bFGF} can reduce inflammatory.

5. Conclusion

There were multiple beneficial effects for use of ADSC^{bGF} as seed cells in pelvic tissue engineering.In first,as a direct ADSCs supplement;secondly,ADSC^{bGF} stimulated trophic factors such as bFGF that stimulate angiogenesis, fibroblast differentiation; and thirdly,the early stage after ADSC^{bGF} implantation, the paracrine function was used to accelerate cell growth and extracellular matrix reconstructon,in the late stage, ADSCs were differentiated into fibroblasts to accelerate the reconstruction of pelvic floor tissue. The combination of gene and cell therapy will accelerate the repair of pelvic floor tissue through a combination of paracrine and directed differentiation mechanisms, and may be more effective than systematically giving individual growth factors.Taken together, ADSC^{bGF} may serve as a potential cell-based treatment for POP repair as it can provide an environment rich in stem cells,growth factors, and matrix proteins over a short time period, which can promote pelvic floor regeneration.

Abbreviations

POP: Pelvic organ prolapse; ADSCs: Adipose-derived stem cells; bFGF: Fibroblast growth factor; BMSCs: marrow mesenchymal stem cells; EMSCs: Endometrial mesenchymal stem cells; VEGF: Vascular endothelial growth factor; PDGF: platelet-derived growth factor; ECM: Extracellular matrix; IGF: insulin-like growth factor; HGF: hepatocyte growth factor; EGF: Epidermal growth factor; BMP-2: bone morphogenetic protein 2.

Declarations

Acknowledgments

None.

Authors' contributions

All authors contributed to the experimental design. WXT wrote the manuscript. WXT and JYY acquired, analysed, and interpreted the data. WSY and WJQ instruct the experiments technology. SXL and WJL supervised the study, interpreted the data, and revised the manuscript. All authors approved the final version of the manuscript.

Funding

This work was funded by National key research and development project, Grant/Award Number: 2018YFC2002204.

Availability of data and materials

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

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

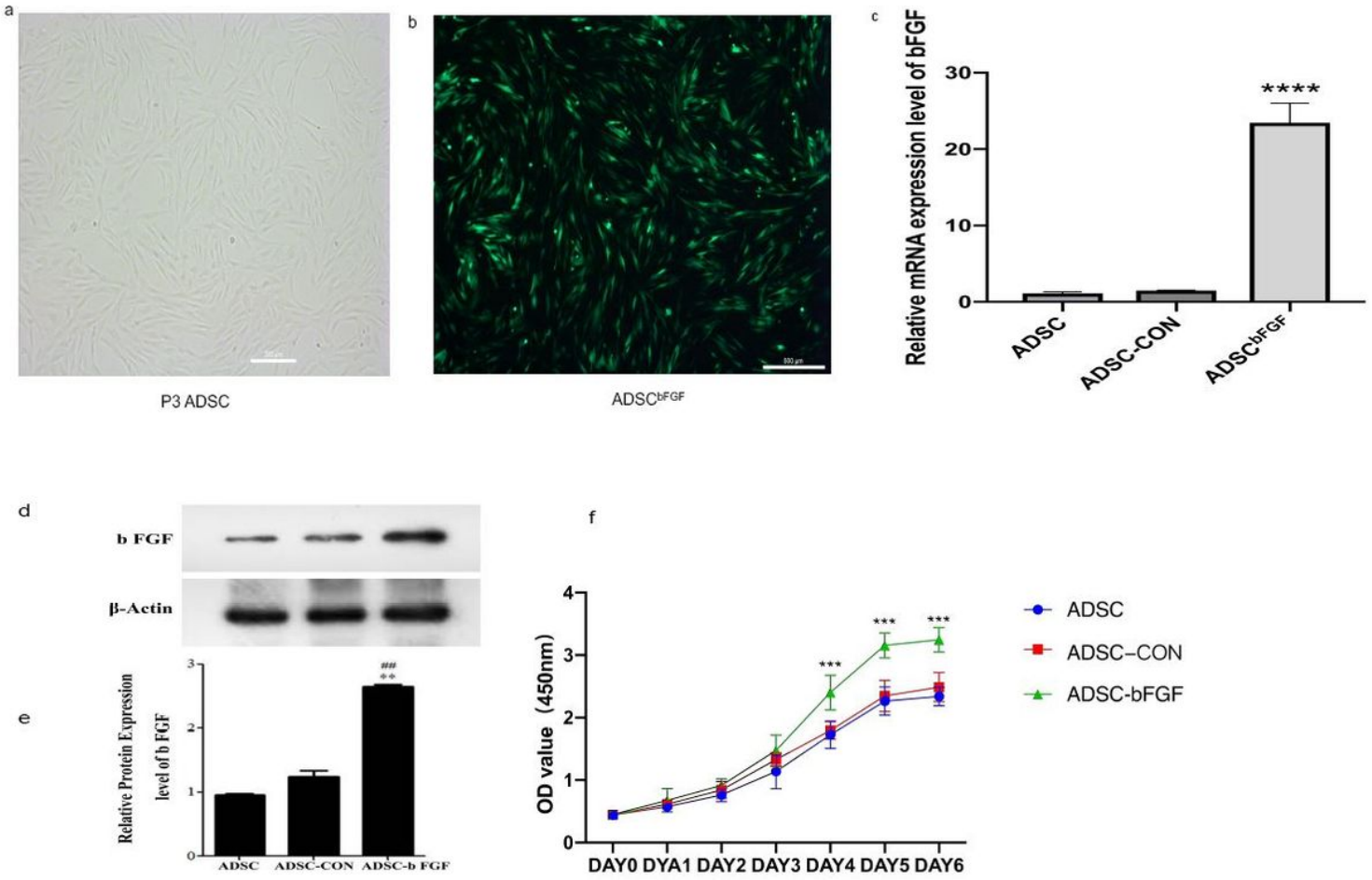

Figure 1

After transfected with bFGF,ADSC exhibited a higher bFGF level and cell viability a:Morphology of ADSCs in passage 3. b□Immunofluorescence analysis of the expression of GFP in transfected ADSCs. C,d,e:Levels of mRNA and protein of bFGF in ADSCs were examined48h,72h after transduction□ respectively. The value is shown in each graph as mean ± SD. *p < 0.05, **p < 0.01□***p < 0.001 and ****p < 0.0001.f:the proliferation after ADSCs transfection.

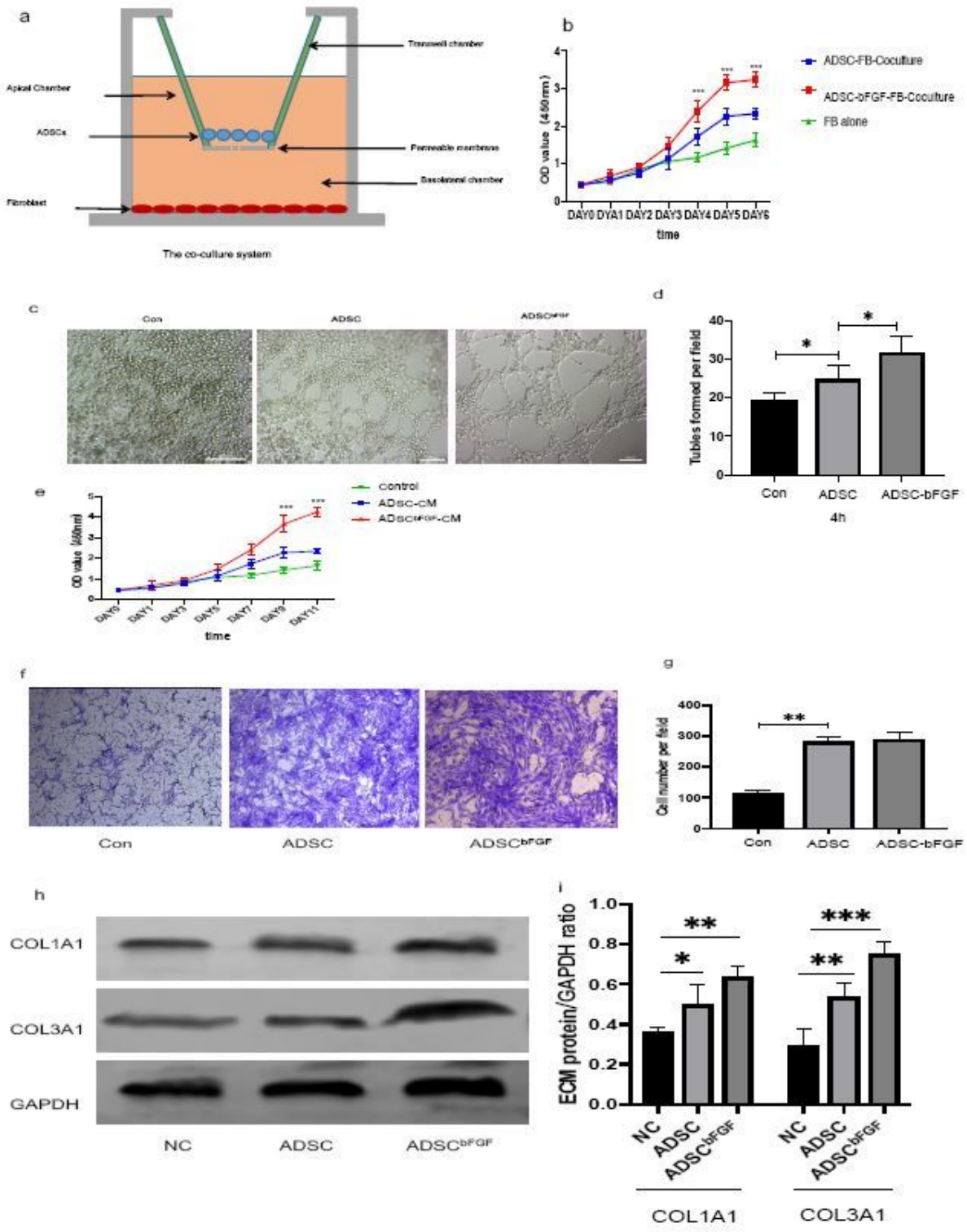

Figure 2

The ADSCbFGF paracrine after bFGF transfected ADSCs transfected with bFGF enhanced the tubule formation of HUVEC both upregulated the fibroblast proliferation and collagen expression.a:The co-culture system is comprised of ADSCs in transwell inserts and fibroblasts in six-well plates. ADSCs itself cannot pass cell culture chamber, but can secreted factors and affect fibroblasts. b:The fibroblast proliferation ratio with ADSC,ADSCbFGF or alone. c:Tubule formation of HUVECs cultured with different

supernatants.d: Quantitative analysis of tubular formation of HUVECs. e:The HUVECs proliferation ratio in response to different ADSC cell culture supernatants. f,g: Quantification of migrated cells. h,i:collagen I and III expression levels .Each bar depicts the mean ± SD (***P < 0.001, **P < 0.01 and *P < 0.05, n = 3); one-way ANOVA followed by the S-N-K test.

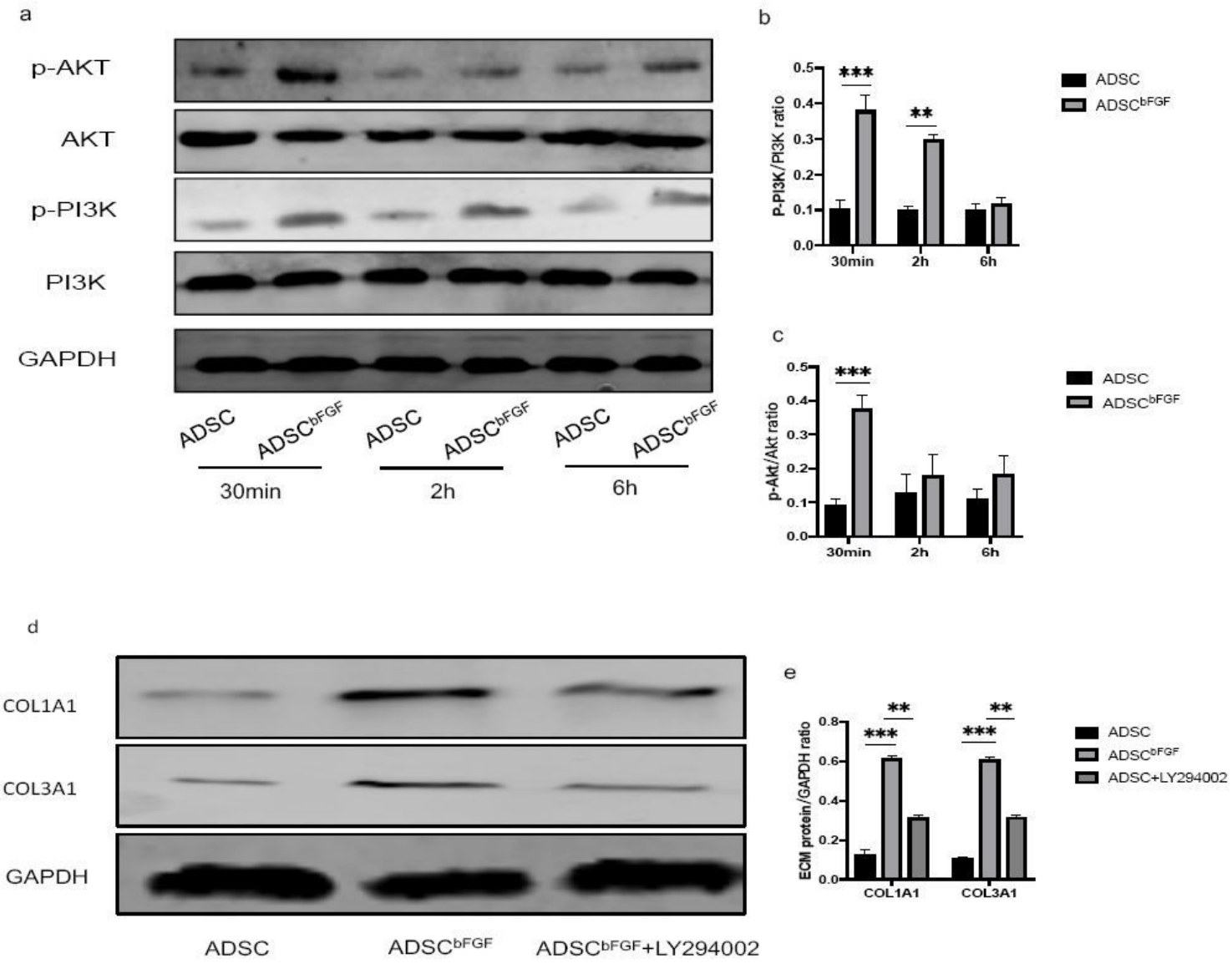

Figure 3

ADSC differentiation ability was further increased following transfection a: Morphology of the three groups of ADSCs after cultured 1-4 weeks.b:FSP-1 immunocytochemical staining after cultured 2 and 4 weeks. c,d: Immunofluorescence analysis of the expression of col1 and col3 in transfected ADSCs. e,f:Western blot analysis showed markedly increased the levels of col1 and col3 in ADSCbFGF. All values are represented as the mean±SD from three independent experiments, each with three replicates. Statistically significant differences from the control group are denoted as follows: ***P < .001, **P < .01 and *P < .05 (independent samples t test)

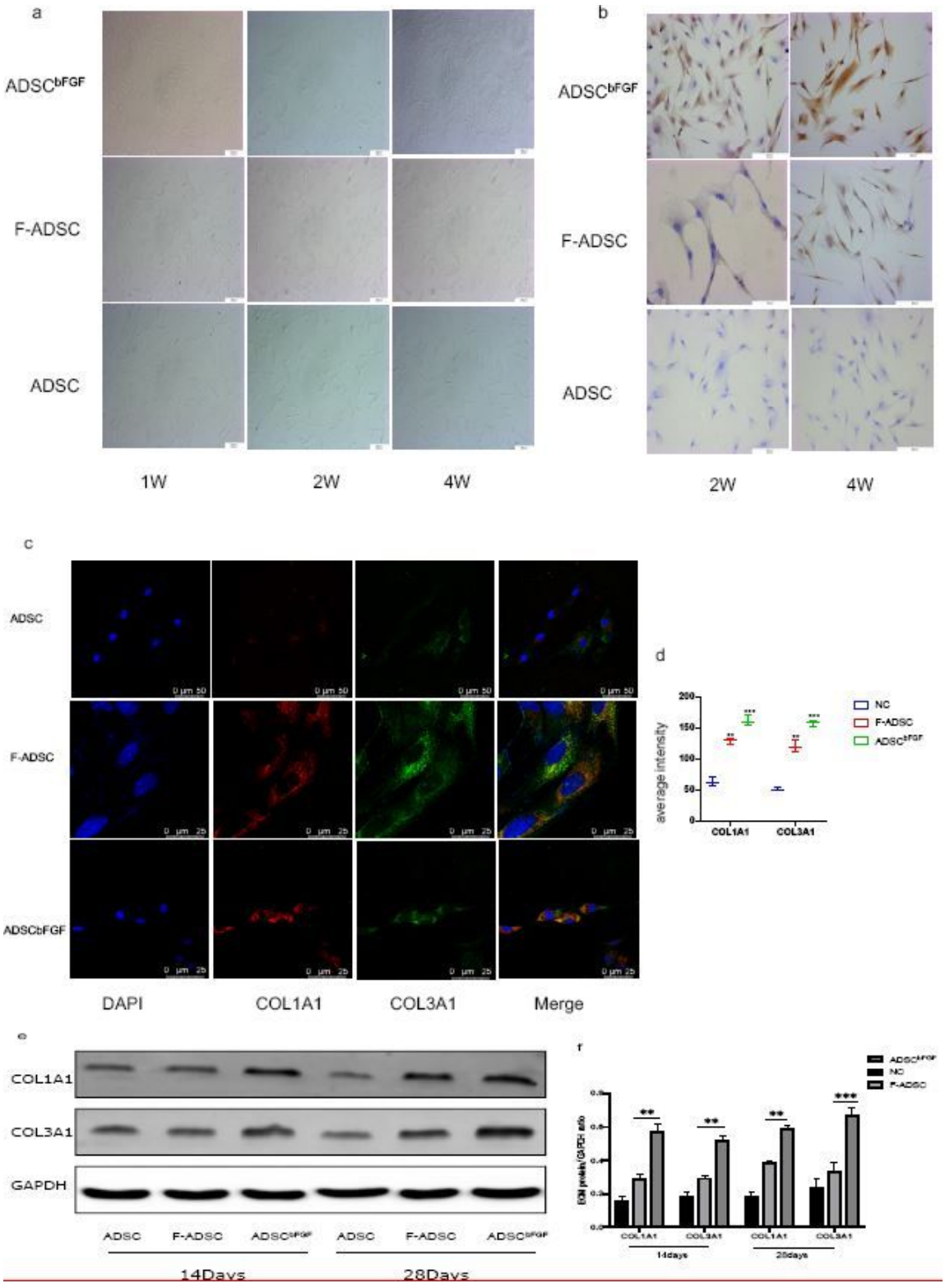

Figure 4

Inhibition of PI3K diminished the effect of ADSCbFGF differentiation and Collagen expression a:Western blotting results show that the expression of important proteins for PI3K/Akt signaling pathway is increased in the ADSCbFGF. b–c:Summary of Western blotting results. Error bars represent the mean±S.D.; *P<0.05 and **P<0.01.) . d–eADSCbFGF were treated with or without PI3K inhibitor, LY294002. The

increased expression of col1 and col3 were reversed by LY294002. f Summary of Western blotting results. Error bars represent the mean± S.D.; *P<0.05, **P<0.01, and ***P<0.001.

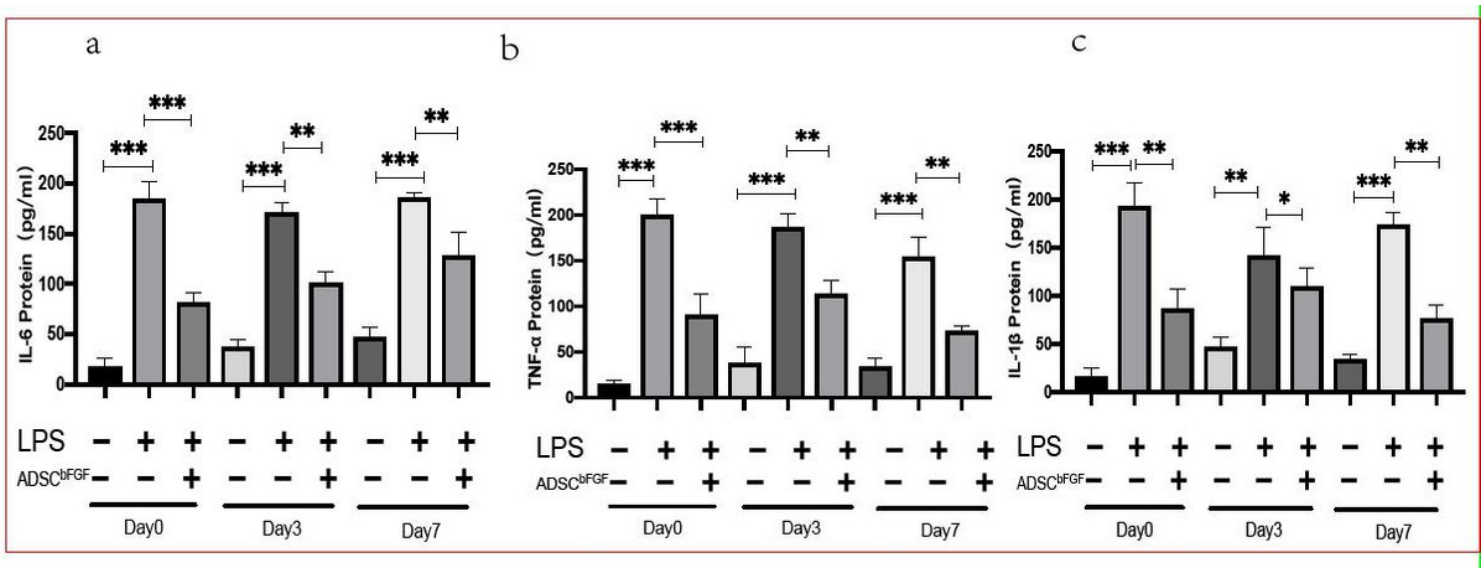

Figure 5

ADSCbFGF reversed the inflammation of the injured fibroblasts a: ADSCbFGF decreased the IL-1 protein level after the injured fibroblasts were treated with LPS b:ADSCbFGF decreased the IL-1 protein level after the injured fibroblasts were treated with LPS c:ADSCbFGF decreased the IL-1 protein level after the injured fibroblasts were treated with LPS Error bars represent the mean±S.D.; *P<0.05 and **P<0.01.

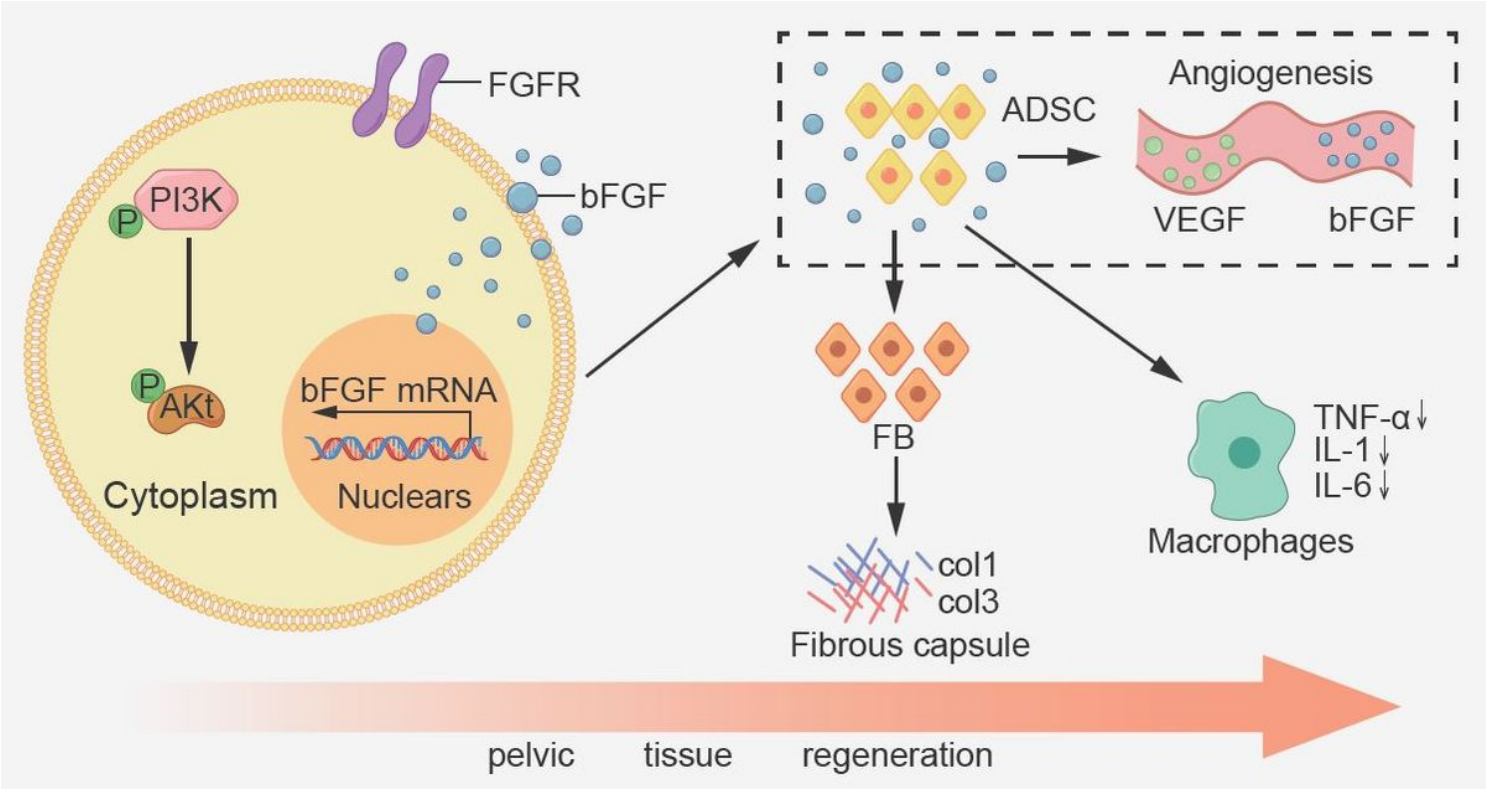

Figure 6

Schematic presentation of supposed pleiotropic effect of ADSCbFGF on pelvic floor regeneration.