Dear Editors and Reviewers:

Thank you very much for your comments concerning our manuscript entitled "The status, challenges and future prospects of stem cell therapy in pelvic floor disorders" (ID: 53849), those comments are all valuable and very helpful for revising and improving our papers, as well as the important guiding significance to our researches. We have revised the manuscript, according to the comments of reviewer, and responded, point by point to, the comments as listed below, and the amendments are highlighted in red in the revised manuscript. The revised manuscript has been edited and proofread by American Journal Experts, We would like to re-submitted this revised manuscript to your journal and hope that the revision is acceptable, look forward to hearing from you soon.

Responds to comments of editors:

1. Please re-provide the original figure documents. All submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes; For line drawings that were automatically generated with software, please provide the labels/values of the ordinate and abscissa in text boxes; Please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor.

Response: We have uploaded the original PPT file to the column of image file of the system to facilitate reprocessing graphics or text.

2. Please verify that the references are cited by Arabic numerals in square brackets and superscripted in the text, and that the numbering order is correct. There should be no space between the bracket and the preceding word or the following punctuation. When references in the text and tables are cited with author name(s), it is necessary to manually verify that the name(s) is consistent with the first author's surname in the corresponding reference list.

Response: As shown in lines 541 to 783 of the revised manuscript, we have verified that the references are cited by Arabic numerals in square brackets and the order is correct. In addition, as shown in lines 80, 81, 87, 93, 96, etc of

the revised manuscript, the references are cited by Arabic numerals in square brackets and superscripted in the text and the references in the text and tables which are cited with author name(s) is consistent with the first author's surname in the corresponding reference list.

Responds to comments of reviewers:

1. Thought out the manuscript, there is problem of space adjustment between two words, which hindrance in flow of heading. Authors need to edit the manuscript carefully.

Response: We are very sorry for our negligence of this problem, we readjusted the space between the two words carefully throughout the revised manuscript to make the sentence more fluent, such as in line 153 the two words of "requirementsfor" were corrected as "requirements for", in line with 156 the two words of "followinghematopoietic" were corrected as "following hematopoietic", in line with 181 the two words of "skeletalmuscle" were corrected as "skeletal muscle", in line with 366 the two words of "engraftedBMSCs" were corrected as "engrafted BMSCs", in line with 366 the two words of "ofautologous" were corrected as "of autologous", in line with 420 the words of "earlystage ofdifferentiation" were corrected as "early stage of differentiation", in line with 498 the two words of "collagendeposition" were corrected as "collagen deposition" etc.

2. Authors should explain the pathophysiology of the disorder: cellular, molecular, structural and functional defects. What all different cell types affected in the diseased individual, showing tissue section slides of the normal and diseased person. Does the disease causes inflammation, what kind of immune cells are involved and cytokine profile in the tissue milieu need to be clearly mentioned. Finally, how the subject is tested for the recovery is necessary to understand by the readers. This will justify whether stem cells, at all, any role to alleviate the defect.

Response: We agree with the reviewer very much and thank you again for your valuable comments.

firstly, in lines 120 to 134 of the revised manuscript, " Pelvic floor dysfunction (PFD) is a disease that includes

POP, SUI and genital fistula, and it is mainly manifested as uterine prolapse, anterior vaginal wall (posterior wall) prolapse, urinary incontinence, urinary retention, urinary fistula, fecal fistula, uterine prolapse and sexual dysfunction. The changes in the anatomy and position of the pelvic floor organs (uterus, bladder, urethra, rectum and anus) caused by quantitative and qualitative defects of collagen, elastin and fibrin proteins of weak pelvic floor tissues (such as ligament, fascia defects and levator ani muscle, urethral and anal sphincter function compromise) due to parity, vaginal delivery, menopause and aging have been identified as important etiologic factors in the development of PFD. Additionally, nerve (sacral plexus and pudendal nerve) defects and declines in various cell types of the pelvic floor tissues, including muscle cells, fibroblasts, and smooth muscle cells, among others, also contribute to PFD[12, 13]. The pathophysiology of PFD is directly related to the functional anatomy of the pelvic floor. Therefore, any force or process that interrupts the integrity of the connective or neuromuscular tissue supports and weakness in any of constituents without supplement may lead to an increase in PFD." were added to further explain the pathophysiology of the disorder.

Secondly, in lines 453 to 471 of the revised manuscript, " In conclusion, under induction by a series of cytokines and growth factors, including VEGF，PDGFβ, CTGF, TGF-β1, FGF-2 and bFGF, MSCs were originally thought to improve tissue repair by transdifferentiation[27, 73, 74] into the required cell types, such as striated muscle cells, smooth muscle cells, fibroblasts, and peripheral nerve cells, among others. Additionally, improvements have been observed in collagen, elastin, and tenascin-C protein secretion and cell migration, proliferation and neovascularization[45, 47, 48], thus improving connective or neuromuscular tissues or any weak constituents regeneration and conferring tissue elasticity and strength in PFD. However, recent increasing evidence suggests that MSCs promote tissue repair largely due to intercellular communication mediate by exosomes through the delivery of copious quantities of bioactive molecules around the site of injury to promote angiogenesis by enhancing the migration and proliferation of tissue-specific progenitor cells such as microvascular endothelial cells

through upregulation of the expression of angiogenic factors such as VEGF-A and bFGF. Additionally, exosomes can inhibit apoptosis, fibrosis and scarring by promoting fibroblast and muscle cell proliferation and collagen and elastin protein synthesis and metabolism through upregulating expression levels of TIMP-1, TIMP-3 and type I collagen and downregulating those of MMP-1 and MMP-2[27, 52, 53], Exosomes can also inhibit inflammation by recruiting macrophages and leukocytes. Present studies are focused on exosome function in the early stage, further research is necessary." were added to respond the affected of stem cells in the diseased. Besides, lines 194 to 200, "Ding et al.[20] applied HUMSCs and smooth muscle cells differentiated from HUMSCs to produce tissue-engineered fascia equivalents in vitro, and transvaginally implanted the meshes into Sprague Dawley rats. The results showed that the PP mesh with both cells showed a trend toward better anti-inflammatory vascularization and a high collagen I/III ratio compared with the PP mesh with single stem cells, indicating the maturation of scar tissue during the wound healing stage" and lines 216 to 220, "These findings indicate that neo-tissue regeneration in POP repair may be strengthened by seeding scaffolds with EMSCs and overcome the foreign body response (FBS) of mesh used alone. Paul et al.[26] reported that bioprinting of EMSCs onto 3D melt electrospun mesh and AV-ALG hydrogel could potentially inhibit FBS and recruit a large number of anti-inflammatory M2 macrophages." and lines 241 to 245, "Kuismanen et al.[31] first demonstrated that transurethral injection of endogenic ADMSCs combined with collagen into five patients could be used to treat SUI, and the 1-year follow-up findings suggested that the treatment was safe, well-tolerated and effective based on a negative cough test and improvement of the 24-hour pad test in two of five patients as shown in Table 2." and lines 255 to 259, "After their transurethral injection into the urethral rhabdosphincter of women with SUI, symptoms obviously improved and reached a 75% success rate without bleeding or infection at the injection site and no instances of voiding dysfunction, urinary retention, or urinary tract infection reported during the 2-year follow-up" and lines 267 to 270, "The clinical trial data suggest that stem cell therapy is effective and safe with minor

complications such as pain, local reactions, mild self-limited urinary retention and urinary tract infections, which are common observed with conventional therapies." and lines 293 to 298, "According to the results, the tissue contents of elastin, collagen III and the LPP value increased significantly after pSMCs transplantation for 5 weeks, and long-term survival of these cells was observed in the host urethra for at least 10 weeks in SCID mice. Although the innervation and blood supply of the rat bladder and the urethra were likely damaged during cell transplantation after urethrolysis, no other side effects were reported[42]" and lines 425 to 427, "Researchers have implanted gelatin-coated polyamide knitted mesh seeded with EMSCs in a rat wound model of POP repair surgery, which demonstrated a significant decrease in M2 and M1 macrophages and leukocytes surrounding the mesh filaments at 90 days." and Table 2 were highlighted the corresponding content, respectively, to respond the affect of different cell types in the diseased, and the tissue section slides of the normal and diseased person were attached to supplementary materials.

Thirdly, the disease did causes inflammation and in the revised manuscript, lines 472 to 481, "MSCs also inhibit the inflammatory response and modulate both the innate and adaptive immune system, while the exact immunomodulatory mechanism of MSCs in PFD repair is still unclear. Previous studies have reported that MSCs can drive a phenotypic change from pro-inflammatory M1macrophages to anti-inflammatory M2 macrophages through the interaction of prostaglandinE2 (PGE2) with macrophage prostanoid receptors[75, 76]. It is also well known that BDMSCs can induce immunosuppression by decreasing pro-inflammatory cytokines such as TNF-a, IL-6 IL-1α, and IL-1β and increasing anti-inflammatory cytokines such as IL-10, as well as suppressing T lymphocytes, dendritic cells and natural killer (NK) cells to downregulate innate inflammatory and acquired immune responses[77, 78]." were added to introduce the kind of immune cells and cytokine profile are involved in the tissue milieu during the stem-cell based therapy for PFD.

Finally, in the revised manuscript, lines 437 to 452, "Overall, stem cell-based therapy generally trans- or

periurethral injections of different stem cell preparations (including stem cells alone, stem cells combined with cytokines or seeded on mesh, or the secretion of exosomes) into the bladder neck, external sphincter or submucosa[66-68] for PFD treatment. To assess the success of treatment, the Incontinence Score (a 24-h voiding diary, 24-h pad test and patient questionnaire)[69], Incontinence Quality of Life (I-QOL) test[70], Urodynamic tests (including pressure flow studies, urethral pressure profiles, voiding cystometry and urethral profilometry)[71], kinesiological electromyography (EMG) measurements and the TUUS (high-frequency transducers) imaging technique among others, were used before and after treatment to assess the pattern of individual muscle activity, any therapeutic effect on urethral closure pressures and the morphology and function of the urethra and rhabdosphincter[72], MSCs have shown impressive efficacy on most of PFD patients, with alleviation of LPP, improvement of quality of life, the pad test and negative cough test, and an increase in resting tone and voluntary contractile force, and improvement of the rhabdosphincter, urethral submucosa regeneration without serious side effects such as scars, hyperplasia, tumors or inflammation, as shown in Table 2." were added to introduce different methods for testing subject recovery and to justify whether the stem cells play any role to alleviate the defect.

3. In line 229, authors should define how MDSCs are characterized in the referred literature.

Response: As reviewer suggested that, as shown in lines 249 to 255 of the revised manuscript, " Muscle-derived stem cells are regarded as candidates for SUI therapy due to their properties of easy collection from striated muscle biopsies and capabilities of long-term self-renewal, proliferation, multipotent differentiation (including differentiation into endothelial, neuronal and myogenic lineages) and expression of the surface markers desmin, MyoD and Sca1 and a series of mesenchymal surface markers[33-35]. Klaudia et al. evaluated the morphology, confirmed the cellular fusogenic potential and assessed the cell surface maker expression of desmin to characterize MDCs." were added to introduce the characterized of MDSCs which referred in the literature.

4. Line 265, does use of human cells in animal may like to cause any tissue rejection?

Response: We had added this part in lines 288 to 298 of the revised manuscript to answer the reviewer's question. "Another similar study demonstrated that human ESC/iPSC-derived pSMCs can facilitate the restoration of the sphincter structure in a SUI rat model when injected periurethrally into urethral injury female immunodeficient Rowett nude (RNU) rats or intramuscularly into 8-10-week-old CB17 severe combined immunodeficiency (SCID) female mice to investigate the effects of pSMCs on injured urethra and long-term survival in vivo. According to the results, the tissue contents of elastin, collagen III and the LPP value increased significantly after pSMCs transplantation for 5 weeks, and long-term survival of these cells was observed in the host urethra for at least 10 weeks in SCID mice. Although the innervation and blood supply of the rat bladder and the urethra were likely damaged during cell transplantation after urethrolysis, no other side effects were reported", thus, weather these human derived pSMCs used in animal may cause any tissue rejection were unknown, further research are needed to investigate these problems.

5. What is the best route of application of the cells and alternate route, if any?

Response: Through reading a large number of literatures, we concluded the route of application of the cells as shown in lines 437 to 440 of the revised manuscript, "Overall, stem cell-based therapy generally trans- or periurethral injections of different stem cell preparations (including stem cells alone, stem cells combined with cytokines or seeded on mesh, or the secretion of exosomes) into the bladder neck, external sphincter or submucosa[66-68] for PFD treatment." to introduce the different route of application of the cells, and in lines 501 to 507," cell-free treatments that achieve the same therapeutic effect through the paracrine action of stem cells and that deliver biomolecules including mRNA and miRNA into recipient cells can avoid FBR of stem cells via transplant directly into the damaged tissue. Recently, an increasing number of studies have been dedicated to investigating the effect of exosomes secreted by stem cells on the treatment of POP, which indeed alleviated POP and reduced the incidence of FBR compared with stem cells transplanted directly into the body[50]." were

highlighted to illustrate the use of exosomes may be the best route of application of the cells, while the studies focusing on the function of exosomes are in the early stage, and thus, comprehensive studies of the absorption, distribution, metabolism, and excretion of exosomes are urgently needed. Besides, the alternate route is not clear at present, and further research are needed in the future.

In all, we found the reviewer's comments are quite helpful, and we revised our manuscript point by point, thank you and the review again for your help!

With kindest regards,

Yours sincerely,

Juan Cheng

Corresponding author:

Name: Prof. Yali Miao, PhD

E-mail: miaoyali2006@163.com