IMMUNOREGULATION

2024. Vol 7

Review Paper:
A Therapeutic Hypothesis for Intrauterine Adhesions: Combining Mesenchymal Stem Cells With Pirfenidone

Kimiya Rashidan[1], Malaksima Ayadilord[1], Seyed Mahmoud Hashemi[1*]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Use your device to scan and read the article online

Citation Rashidan k, Ayadilord M, Hashemi SM. A Therapeutic Hypothesis for Intrauterine Adhesions: Combining Mesenchymal Stem Cells With Pirfenidone. Immunoregulation. 2024; 7:E9. http://dx.doi.org/10.32598/Immunoregulation.7.9

http://dx.doi.org/10.32598/Immunoregulation.7.9

Article info:
Received: 10 Jan 2024
Accepted: 23 Mar 2024
Available Online: 20 Apr 2024

Keywords:
Intrauterine adhesions, Mesenchymal stem cells, Pirfenidone, Combination therapy

ABSTRACT

Intrauterine adhesions (IUAs), characterized by fibrous tissue bands within the endometrial cavity, present significant challenges to reproductive health. This condition leads to abnormal menstruation, infertility, and recurrent pregnancy losses. Current treatment modalities primarily focus on endometrial regeneration through hormone therapy and surgical removal of adhesions. However, the success rates of these approaches are unsatisfactory. Stem cell therapies, particularly those involving mesenchymal stem cells (MSCs), have emerged as promising tissue repair and regeneration strategies, inhibiting inflammation and fibrosis. Nonetheless, while some limitations have been observed in the application of MSCs, their therapeutic effectiveness can be significantly enhanced by combining them with other medications. Furthermore, pirfenidone, an FDA-approved idiopathic pulmonary fibrosis (IPF) drug, has demonstrated anti-fibrotic and anti-inflammatory properties. Consequently, the combined administration of MSCs and pirfenidone can potentially improve therapeutic outcomes by simultaneously targeting the key pathological characteristics of IUAs, namely fibrosis and inflammation. Furthermore, this approach could target other factors involved in IUA pathogenesis. MSCs reduce tissue damage and promote vascularization, while pirfenidone could inhibit fibrotic signaling pathways and improve autophagy defects. This combination offers a comprehensive strategy for treating IUAs by effectively preventing or mitigating these pathogenic mechanisms. Also, this combination could reduce the pirfenidone dosage and prevent potential adverse effects. This article proposes a hypothesis that encourages further investigation of the synergistic effects of MSCs and pirfenidone as potential treatments for IUAs.

*** Corresponding Author:**
Seyed Mahmoud Hashemi, Assistant professor.
Address: *Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
Phone: *+98 (21) 22439970*
E-mail: *smmhashemi@sbmu.ac.ir*

Introduction

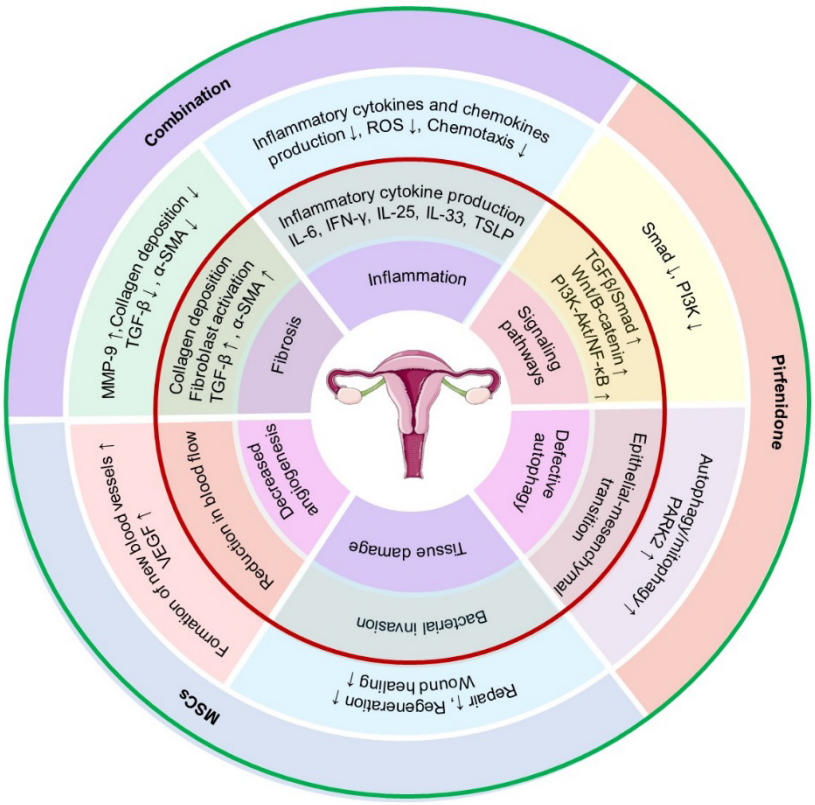
Intrauterine adhesions (IUAs), also known as Asherman's syndrome or intrauterine synechiae, are fibrous tissue bands that develop within the endometrial cavity, frequently due to uterine surgery. IUAs have been identified as potential factors contributing to abnormal menstruation, infertility, and/or recurrent pregnancy loss in women [1]. Infection or trauma are the most significant and frequent causes of IUAs, particularly after pregnancy [2]. Serious injury to the basal layer may result in the loss of most endometrial cells, and the endometrium may fail to recover [3]. IUAs treatment aims to rebuild a normal uterine cavity and regain uterine function. Current treatment lines induce endometrial regeneration from remaining progenitor cells and endometrial stem cells by combining hormone therapy with hysteroscopic surgery for adhesion removal [4]. Despite developments in therapy modalities, the successful pregnancy rate remains low, and severe IUAs provide a significant therapeutic challenge with poor prognosis. The most recent advancement in IUA treatment is the identification of appropriate biological tissues and functional bioactive scaffolds for intrauterine placement to promote or induce endometrial repair or provide an ideal environment for endometrial cell self-repair to improve patients' reproductive function. However, various hurdles remain in the face of IUA regeneration and optimum therapeutic biomaterials to produce effective treatments for IUAs [5]. In recent years, stem cell therapies have emerged as a novel approach for repairing and regenerating damaged tissues through their ability to self-renewal, differentiate, and immunoregulation. Among stem cells, mesenchymal stem cells (MSCs) have garnered significant interest due to their ease of isolation and diverse sources. MSCs can be collected from various sources, including the bone marrow, umbilical cord, adipose tissue, and peripheral blood [6]. MSCs have been extensively employed in preclinical animal models and have demonstrated successful outcomes in various clinical applications for treating IUAs [7]. However, the application of MSC therapy encounters challenges, including biased differentiation and cellular senescence, which may hinder its effectiveness. Researchers have explored the potential of pharmacological medications as enhancers to address these limitations and enhance the therapeutic potency of MSCs. Additionally, it is essential to continue investigating other drugs and compounds to optimize MSC therapy and overcome existing limitations, thereby maximizing its therapeutic efficacy [8].

Pirfenidone is a synthetic non-peptide compound with a high level of oral bioavailability. It exhibits many therapeutic effects, including anti-fibrotic, anti-inflammatory, antioxidant, and apoptosis-regulating properties [9–13]. Pirfenidone is one of the two medications approved by the FDA for treating idiopathic pulmonary fibrosis (IPF) [14]. Apart from IPF, pirfenidone has an anti-fibrotic effect on various tissues, including the kidney, liver, heart, gastrointestinal system, eye, and skin, because fibrosis is the outcome of multiple common stages in many illnesses and pirfenidone targets such pathways [15–20]. However, it is worth noting that in some cases, the administration of high doses of pirfenidone has been accompanied by adverse effects [21]. Consequently, the current monotherapy approach utilizing pirfenidone alone has demonstrated limited effectiveness. Therefore, it is crucial to explore and develop improved treatment strategies that can address these limitations and enhance therapeutic outcomes [22].

The combination of pirfenidone and MSCs has the potential to synergistically enhance their anti-fibrotic and anti-inflammatory effects, thereby making a valuable contribution to the treatment of IUAs. Additionally, this combination therapy may reduce the required pirfenidone dosage. By effectively targeting fibrosis and inflammation through their combined action, pirfenidone and MSCs offer a promising approach to improve therapeutic outcomes in IUAs.

The Hypothesis

Our hypothesis suggests combining pirfenidone and MSCs could offer therapeutic advantages for managing IUAs. This combination is expected to synergistically leverage the anti-fibrotic and anti-inflammatory properties of pirfenidone, complementing the similar effects of MSCs and effectively targeting fibrosis and inflammation caused by IUAs. Additionally, pirfenidone alone can inhibit fibrotic signaling pathways and promote autophagy, addressing the autophagy defect observed in IUAs. In contrast, MSCs possess regenerative capabilities, promoting tissue repair and regeneration and enhancing angiogenesis to counteract tissue damage and mitigate the reduced vascularization seen in IUAs. As such, the combined administration of pirfenidone and MSCs addresses multiple components of IUA pathogenesis, offering a comprehensive and promising therapeutic approach. Figure 1 shows our hypothesis.



IMMUNOREGULATION

Figure 1. Pathogenesis of IUAs and effects of treatment with pirfenidone and MSCS, schematic representation of our proposed hypothesis

Note: The red circle illustrates several crucial features contributing to the pathogenesis of IUAs, providing detailed insights. Meanwhile, the green circle highlights how MSCs, pirfenidone or a combination of both can target each of these pathogenic mechanisms. IUAs, intrauterine adhesions; MSCs, mesenchymal stem cells.

Basis of the Hypothesis

Characteristics of IUAs

Inflammation

IUA formation appears to be strongly linked to increased endometrial inflammation, as described by Moquin-Beaudry et al., who found significantly higher preoperative inflammation in IUA patients than in non-IUAs participants [23]. Bacterial invasion of the endometrium leads to local inflammatory reactions, increasing pro-inflammatory cytokines, such as interleukin-6 (IL-6) and interferon-gamma (IFN-γ). In addition, damaged epithelial cells release cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin. These cytokines can directly or indirectly stimulate the T helper two immune response, thereby promoting the development of fibrosis [24]. Additionally, by promoting the expression of inflammatory cytokines, the transcription factor NF-κB plays a critical role in inflammatory disorders [25], and its up-regulation has been observed in patients with endometrial adhesions [26]. Transforming growth factor beta (TGF-β), tumor necrosis factor-alpha (TNF-α), IL-1, and IL-18 are among the pathogenic cytokines that interact closely with NF-κB and are involved in intrauterine adhesion [25].

Fibrosis

Fibrosis is characterized by scar tissue formation between the uterine layers, which binds the uterine walls together. The primary pathogenic characteristic of IUAs is endometrial fibrosis. Excessive fibrous structures, avascular and insensitive to hormonal stimulation, largely replace the endometrial stroma in IUAs [27]. TGF-β has been widely recognized as a key mediator of the fibrotic response due to its ability to stimulate fibroblasts in the synthesis and contraction of extracellular matrix (ECM) [28]. Moreover, inflammatory conditions within the endometrium subsequently stimulate an excessive process known as epithelial-mesenchymal transition (EMT),

activating a significant population of myofibroblasts. Myofibroblasts are characterized by elevated metabolic activity and substantial expression of α-smooth muscle actin (α-SMA). This expression of α-SMA facilitates a notable augmentation in the synthesis of fibrotic collagen, including types I, III, V and VI [29]. Therefore, administrating agents exhibiting anti-inflammatory and/or anti-fibrotic characteristics holds promise for potentially yielding therapeutic effects on IUAs. The signaling pathways primarily implicated in endometrial fibrosis are the Wnt/β-catenin, PI3K-Akt/NF-κB, and TGF-β1/Smad signaling pathways [30]. Hence, inhibiting these pathways may be beneficial in IUA progression.

Defect in autophagy

Autophagy, a programmed cellular degradation process, is a crucial response mechanism to environmental stress and significantly influences the cyclic changes occurring within the endometrium [31]. Defective autophagy contributes to EMT [32], and its involvement in the pathogenesis of endometrial fibrosis in IUAs has been demonstrated [33–35].

Decreased vascularization

Angiogenesis and uterine blood flow play crucial roles in facilitating the growth of the endometriumcrucial [36, 37]. Intrauterine scarring plays a critical role in this regard, as it results in a decrease in vascularization, leading to a reduction in the blood supply to the surface of the uterine cavity [36–38].

Functions of pirfenidone

Anti-inflammation and anti-fibrotic

Pirfenidone has been studied in several in vitro systems and animal models of fibrosis to determine its anti-fibrotic and anti-inflammatory qualities [39]. This drug can reduce fibroblast proliferation, fibrosis-related protein and cytokine production, and ECM accumulation and biosynthesis in response to cytokine growth factors, such as TGF-β [40]. Pirfenidone exhibited notable therapeutic potential in managing postoperative intraabdominal adhesions, addressing adhesion formation and inflammation. Furthermore, pirfenidone administration decreased metallopeptidase inhibitor 1, TNF-α, and TGF-β1 proteins concentrations while increasing the matrix metalloproteinase (MMP-9) protein [41]. Moreover, this drug has demonstrated its ability to disrupt collagen synthesis and fibrillogenesis by attenuating the synthesis of specific profibrotic and growth factors, thus reducing ECM deposition [42–44]. Recent studies revealed that pirfenidone significantly reduces EMT and the expression of α-SMA, type I, and type III collagen [45]. Research has indicated that pirfenidone can diminish chemotaxis and the synthesis of pro-inflammatory ROS and cytokines, such as TNFα, IL-1β, and IL-6, along with chemokines, such as MCP-1, IL-8, and MIP-1α. Moreover, pirfenidone enhances the production of anti-inflammatory interleukin-1 receptor antagonists [46]. These functions of pirfenidone may address the fibrosis and inflammation observed in IUAs.

Signaling pathways inhibition

Pirfenidone treatment also down-regulated the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), p38, and c-Jun N-terminal kinase (JNK) induced by TGF-β1. These results indicate that pirfenidone can attenuate EMT and fibrosis in vivo and in vitro by interfering with the MAPK pathway [45]. In an experiment using human intestinal fibroblasts stimulated with TGF-β1, pirfenidone demonstrated a strong capability to inhibit cell proliferation and induce apoptosis. This effect was achieved by inhibiting Smad and PI3K/AKT pathways [47]. This function of pirfenidone might address the involvement of fibrotic signaling pathways observed in IUAs.

Autophagy activation

It was revealed that the administration of pirfenidone activated autophagy/mitophagy through upregulating PARK2 expression. This activation partially suppresses the differentiation of myofibroblasts in the presence of TGF-β [48]. This function of pirfenidone may address the defensive autophagy observed in IUAs. Nonetheless, pirfenidone possesses certain limitations in its clinical application, including adverse effects, and requires ongoing refinement to fully harness its antifibrotic effects [21,49–51].

Functions of MSCs

Anti-inflammation

MSCs significantly impact all stages of wound healing, including the inflammatory, proliferative, and remodeling phases. In the inflammatory phase, MSCs actively regulate the actions of inflammatory cells and prevent the harmful consequences of inflammatory cytokines like TNF and IFN-γ [52]. New research suggests that MSCs have immunomodulatory and homeostatic functions, and their use may provide a means of controlling

inflammation and speeding the healing of injured tissue in inflammatory illnesses [53]. The immune-modulating effect of MSCs involves the inhibition of immune cell proliferation and the secretion of various cytokines (such as IL-1, IL-6, IL-8, IL-12, TNF-α, and IFN-γ) as well as chemokines (including CCL2 (Chemokine ligand 2) and CCL5) [54, 55]. This MSC function might address inflammation in IUAs.

Anti-fibrotic

The potential antifibrotic properties of MSCs have been explored in the context of fibrotic conditions, including IUAs. For instance, human umbilical cord-derived MSCs (hUC-MSCs) have demonstrated their ability to diminish fibrotic factors and collagen accumulation in pulmonary fibrosis [56] and to downregulate the expression of TGFβ1 and genes associated with fibrosis in IUAs [57]. This MSC function might address the fibrosis in IUAs.

Endometrium regeneration

The regenerative properties of stem cells, characterized by their capacity for self-renewal and multi-lineage differentiation, hold significant promise in addressing tissue damage within the uterine cavity. In recent years, there has been a growing research focus on investigating the therapeutic potential of stem cells for treating IUAs [58]. MSCs are stem cells that play an essential role in regeneration and repair due to their capacity to differentiate into multiple lineages. They can differentiate into connective tissues, skeletal muscle, and vascular cells [1]. MSCs can display various therapeutic functions during tissue healing, contributing to the repair and regeneration of damaged tissue [59]. Menstrual blood-derived MSCs [60], bone marrow-derived stem cells [61], adipose-derived stem cells, and UC-MSCs can promote the regeneration and repair of the damaged endometrium by differentiating into endometrial epithelial cells and stromal cells [62, 63]. This function of MSCs might contribute to endometrium regeneration in IUA treatment.

Angiogenesis

Align with regenerative properties, MSCs contribute to angiogenesis, which involves the formation of new blood vessels through a complex mechanism involving the action of several growth factors, including hepatocyte growth factor, vascular endothelial growth factor, and fibroblast growth factor [59]. This function of MSCs might address the reduced blood flow observed in IUAs.

RGS2 upregulation is a potential mechanism for synergistic effects of pirfenidone and MSCs

Recent studies have revealed significant downregulation of the RGS2 expression in animal models of fibrosis, indicating its crucial role in regulating fibrosis-related pathogenesis. Pirfenidone treatment reduced bleomycin-induced pulmonary fibrosis in mice with intact RGS2. At the same time, its efficacy was not observed in RGS2 knockout mice [64], suggesting that a novel mechanism underlying the fibrosis-ameliorating effects of pirfenidone involves the up-regulation of RGS2 [65, 66]. The therapeutic effects of MSCs and pirfenidone and their synergistic mechanism have been investigated in only one study and the context of pulmonary fibrosis. Therefore, further investigations are required to explore this phenomenon in other fibrotic conditions, such as IUAs. Wu et al. demonstrated that the combination of pirfenidone and hUC-MSCs demonstrated superior therapeutic effects compared to individual treatments. This combination increased RGS2 protein levels and significantly reduced fibrosis markers. The group treated with both hUC-MSCs and pirfenidone showed higher expression of RGS2 than those treated with pirfenidone alone, suggesting a potential role of hUC-MSCs in this effect. Knocking down RGS2 did not affect fibrosis markers induced by TGF-β1 with either pirfenidone treatment or the combination of pirfenidone and hUC-MSCs, emphasizing the importance of RGS2 in mediating the antifibrotic effects of these treatments. The researchers suggested that the combination of hUC-MSCs with pirfenidone synergistically enhanced RGS2 expression, leading to a more potent antifibrotic effect, and speculated that hUC-MSCs may upregulate RGS2 expression in myofibroblasts through the paracrine secretion of certain substances. However, further exploration is needed to better understand the mechanisms underlying this combination treatment [22].

Evaluation of the hypothesis

Our hypothesis necessitates experimental testing using in vitro and/or animal models, such as rats or mice, which can exhibit pathologies similar to human IUAs. However, regarding ethical considerations surrounding animal experiments, we strongly recommend using alternative approaches, such as 3D models like spheroids and organ-on-a-chip systems. An in vitro model of IUAs can be established by inducing cellular damage by exposing endometrial cells to hydrogen peroxide. Subsequently, to investigate the effects of our treatment on the damaged cells, in the treatment groups, MSCs were added to the upper chamber of the insert chambers. In contrast,

damaged cells were coated on the lower compartment, followed by the addition of the optimum concentration of pirfenidone. Subsequently, the endometrial cells were examined for the expression of fibrosis and adhesion transcription factors and proteins. This approach enabled the investigation of the impacts of MSCs, pirfenidone, and their combination on damaged cells. For the animal model, IUAs should be induced in female animals utilizing thermal, chemical, or mechanical methods [67]. Following model induction, it is essential to evaluate and score changes in tissue morphology, the number of endometrial glands, the degree of endometrial fibrotic area (as determined by H&E-Masson's trichrome staining), and the expression levels of TGF-β in the endometrium to validate the establishment of the IUAs model [68]. Once the model is confirmed, pirfenidone and MSCs administration should be initiated. Pirfenidone can be administered orally (via gavage), intravenously, or intraperitoneally at optimal concentrations. Similarly, a sufficient number of MSCs (5×10^5 per mouse [69]) can be transplanted locally or systemically. The treatment protocol will span several days (for example, 500 mg/kg of pirfenidone for eight days [70]). Following the treatment period, a subset of female mice will be mated with male mice to assess pregnancy outcomes, and the remaining mice will be euthanized for pathological and molecular examinations. These examinations will involve analyzing uterine tissue samples for fibrosis and adhesion markers, proteins, collagen deposition, number of glands, and endometrial thickness. Additionally, evaluating pregnancy outcomes will provide valuable insights. It is crucial to note that animal and in vitro models have limitations in completely replicating the pathogenesis of IUAs in humans. Therefore, if the results are promising, this treatment could be the subject of clinical trials (Figures 2 and 3).

Discussion and consequences

We have presented evidence that the anti-fibrotic and anti-inflammatory properties of MSCs enhance pirfenidone power in fibrosis and inflammation inhibition and might lead to a more efficient therapy for IUAs. Endometrial fibrosis constitutes the core of IUAs, and research indicates that various types of MSCs can mitigate the risk of endometrial fibrosis and enhance the chances of pregnancy [71]. In addition, other roles of MSCs in enhancing tissue repair and angiogenesis, together with pirfenidone ability to inhibit fibrotic signaling pathways and address decreased autophagy, make this combination a promising treatment that can target different aspects of IUAs pathogenesis. Another point to consider is the attempt to mitigate the potential side effects associated with high doses of pirfenidone, such as occasional drowsiness, skin rash, or gastric discomfort [72]. A strategy to reduce the effective dosage involves combining low doses of pirfenidone with MSCs. Wu et al. investigated the therapeutic effect of hUC-MSCs in combination with low-dose pirfenidone on pulmonary fibrosis in mice and explored the possible mechanisms involved [22]. This combination has been demonstrated to significantly augment the antifibrotic effects of pirfenidone by a factor of three in the context of pulmonary fibrosis [22]. A similar outcome could be achieved in the case of IUAs.

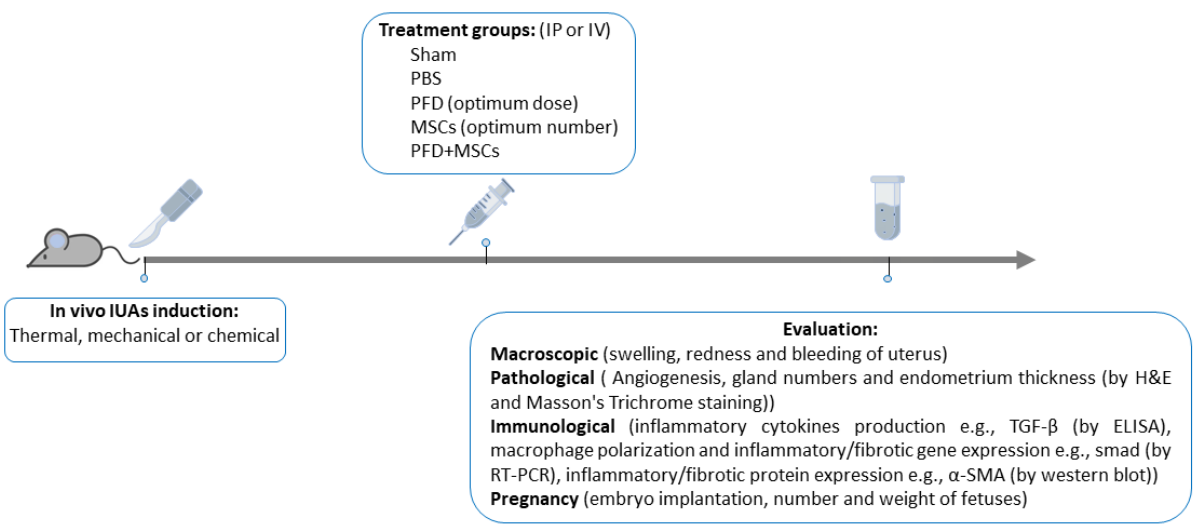


Figure 2. In vivo hypothesis testing

IMMUNOREGULATION

Abbreviations: IUAs: Intrauterine adhesions; MSCs: Mesenchymal stem cells; PFD: Pirfenidone.

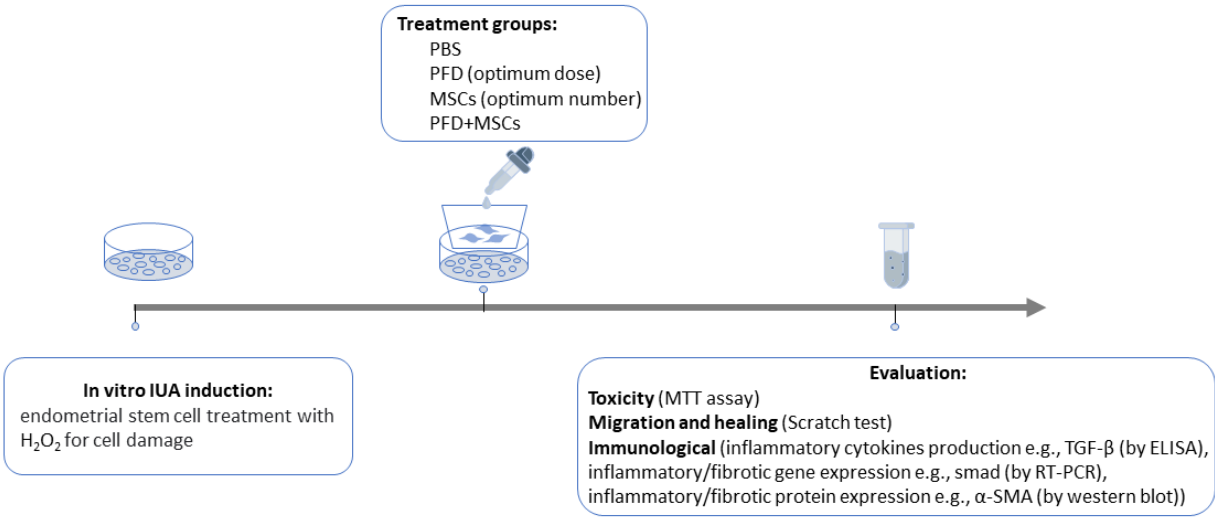


Figure 3. In vitro hypothesis testing

IMMUNOREGULATION

Abbreviations: IUAs: Intrauterine adhesions; MSCs: Mesenchymal stem cells; PFD: Pirfenidone.

In addition to their recognized antifibrotic properties, MSCs have been found to exhibit certain profibrotic effects, such as the secretion of TGF-β [73]. MSCs can undergo EMT in a fibrotic environment, converting them into myofibroblasts [74, 75]. These findings contribute to the controversial effects of MSCs in fibrosis treatment. However, the multi-cytokine inhibitor pirfenidone counteracts these effects by inhibiting the production of cytokines, including TGF-β1, basic fibroblast growth factor, and connective tissue growth factor, in myofibroblasts, thus suppressing their growth and collagen synthesis [76, 77]. Also, Wu et al. discovered that treating hUC-MSCs with pirfenidone significantly decreased the expression of fibronectin and α-SMA messenger ribonucleic acid (mRNA). These results indicate that pirfenidone inhibits the transformation of hUC-MSCs into mesenchymal cells [22]. The markers associated with myofibroblasts in hUC-MSCs decreased after treatment with pirfenidone, suggesting that pirfenidone treatment reduces the conversion of hUC-MSCs into myofibroblasts, thereby allowing hUC-MSCs to exert their full antifibrotic effects [22]. This may explain why the combination of MSCs and pirfenidone could yield superior results compared to pirfenidone alone in fibrotic conditions, such as IUAs. Finally, feasibility, transport, storage issues, and the possible tumorigenicity and teratogenicity of MSC-based treatments, might limit their clinical use [78]. This issue could be addressed using MSC-derived exosomes instead of MSCs [79].

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive specific grants from public, commercial, or not-for-profit funding agencies.

Authors' contributions

Investigation: Kimiya Rashidan and Malaksima Ayadilord; Conceptualization: Seyed Mahmoud Hashemi; Writing the original draft, review, editing and Validation: All authors.

Conflicts of interest

The authors declared no conflicts of interest.

References

[1] Asherman JG. Amenorrhoea traumatica (atretica). The Journal of Obstetrics and gynaecology of the British Empire. 1948; 55(1):23-30. [DOI:10.1111/j.1471-0528.1948.tb07045.x] [PMID]

[2] Lin YH, Jang TN, Hwang JL, Huang LW, Seow KM, Hsieh BC, et al. Bacterial colonization with balloon uterine stent placement in the uterus for 30 days: A randomized controlled clinical trial. Fertility and Sterility. 2015; 103(2):513-8. e2. [DOI:10.1016/j.fertnstert.2014.10.032] [PMID]

[3] Zhou Q, Wu X, Hu J, Yuan R. Abnormal expression of fibrosis markers, estrogen receptor α and stromal derived factor-1/chemokine (C-X-C motif) receptor-4 axis in intrauterine adhesions. International Journal of Molecular Medicine. 2018; 42(1):81-90. [DOI:10.3892/ijmm.2018.3586]

[4] Myers EM, Hurst BS. Comprehensive management of severe Asherman syndrome and amenorrhea. Fertility and Sterility. 2012; 97(1):160-4. [DOI:10.1016/j.fertnstert.2011.10.036] [PMID]

[5] Ma J, Zhan H, Li W, Zhang L, Yun F, Wu R, et al. Recent trends in therapeutic strategies for repairing endometrial tissue in intrauterine adhesion. Biomaterials Research. 2021; 25(1):40. [DOI:10.1186/s40824-021-00242-6] [PMID] [PMCID]

[6] Elahi KC, Klein G, Avci-Adali M, Sievert KD, MacNeil S, Aicher WK. Human mesenchymal stromal cells from different sources diverge in their expression of cell surface proteins and display distinct differentiation patterns. Stem Cells International. 2016; 2016:5646384. [DOI:10.1155/2016/5646384] [PMID] [PMCID]

[7] Li C, Hu Y. Extracellular vesicles derived from mesenchymal stem cells as cell-free therapy for Intrauterine Adhesion. International Journal of Stem Cells. 2023; 16(3):260-8. [DOI:10.15283/ijsc21177] [PMID] [PMCID]

[8] Al-Azab M, Idiiatullina E, Safi M, Hezam K. Enhancers of mesenchymal stem cell stemness and therapeutic potency. Biomedicine & Pharmacotherapy. 2023; 162:114356. [DOI:10.1016/j.biopha.2023.114356] [PMID]

[9] Qiu ZZ, He JM, Zhang HX, Yu ZH, Zhang ZW, Zhou H. Renoprotective effects of pirfenidone on chronic renal allograft dysfunction by reducing renal interstitial fibrosis in a rat model. Life Sciences. 2019; 233:116666. [DOI:10.1016/j.lfs.2019.116666] [PMID]

[10] Lv Q, Wang J, Xu C, Huang X, Ruan Z, Dai Y. Pirfenidone alleviates pulmonary fibrosis in vitro and in vivo through regulating Wnt/GSK-3β/β-catenin and TGF-β1/Smad2/3 signaling pathways. Molecular Medicine. 2020; 26(1):49. [DOI:10.1186/s10020-020-00173-3] [PMID] [PMCID]

[11] Misra HP, Rabideau C. Pirfenidone inhibits NADPH-dependent microsomal lipid peroxidation and scavenges hydroxyl radicals. Molecular and Cellular Biochemistry. 2000; 204(1-2):119-26. [DOI:10.1023/A:1007023532508] [PMID]

[12] Shihab FS, Bennett WM, Yi H, Andoh TF. Effect of pirfenidone on apoptosis-regulatory genes in chronic cyclosporine nephrotoxicity. Transplantation. 2005; 79(4):419-26. [DOI:10.1097/01.TP.0000151721.99418.48] [PMID]

[13] Zou WJ, Huang Z, Jiang TP, Shen YP, Zhao AS, Zhou S, et al. Pirfenidone inhibits proliferation and promotes apoptosis of hepatocellular carcinoma cells by inhibiting the Wnt/β-catenin signaling pathway. Medical Science Monitor. 2017; 23:6107-13. [DOI:10.12659/MSM.907891] [PMID] [PMCID]

[14] Neighbors M, Cabanski CR, Ramalingam TR, Sheng XR, Tew GW, Gu C, et al. Prognostic and predictive biomarkers for patients with idiopathic pulmonary fibrosis treated with pirfenidone: Post-hoc assessment of the CAPACITY and ASCEND trials. The Lancet. Respiratory Medicine,. 2018; 6(8):615-26. [DOI:10.1016/S2213-2600(18)30185-1] [PMID]

[15] Cho ME, Kopp JB. Pirfenidone: An anti-fibrotic therapy for progressive kidney disease. Expert Opinion On Investigational Drugs. 2010; 19(2):275-83. [DOI:10.1517/13543780903501539] [PMID] [PMCID]

[16] Di Sario A, Bendia E, Macarri G, Candelaresi C, Taffetani S, Marzioni M, et al. The anti-fibrotic effect of pirfenidone in rat liver fibrosis is mediated by downregulation of procollagen alpha1(I), TIMP-1 and MMP-2. Digestive and Liver Disease. 2004; 36(11):744-51. [DOI:10.1016/j.dld.2004.05.012] [PMID]

[17] Aimo A, Cerbai E, Bartolucci G, Adamo L, Barison A, Lo Surdo G, et al. Pirfenidone is a cardioprotective drug: Mechanisms of action and preclinical evidence. Pharmacological Research. 2020; 155:104694. [DOI:10.1016/j.phrs.2020.104694] [PMID]

[18] Cui Y, Zhang M, Leng C, Blokzijl T, Jansen BH, Dijkstra G, et al. Pirfenidone inhibits cell proliferation and collagen I production of primary human intestinal fibroblasts. Cells. 2020; 9(3):775. [DOI:10.3390/cells9030775] [PMID] [PMCID]

[19] Stahnke T, Kowtharapu BS, Stachs O, Schmitz KP, Wurm J, Wree A, et al. Suppression of TGF-β pathway by pirfenidone decreases extracellular matrix deposition in ocular fibroblasts in vitro. Plos One. 2017; 12(2):e0172592. [DOI:10.1371/journal.pone.0172592] [PMID] [PMCID]

[20] Shi K, Wang F, Xia J, Zuo B, Wang Z, Cao X. Pirfenidone inhibits epidural scar fibroblast proliferation and differentiation by regulating TGF-β1-induced Smad-dependent and -independent pathways. American Journal of Translational Research. 2019; 11(3):1593-1604. [PMID]

[21] King Jr TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. The New England Journal of Medicine. 2014; 370(22):2083-92. [DOI:10.1056/NEJMoa1402582] [PMID]

[22] Wu X, Gou H, Zhou O, Qiu H, Liu H, Fu Z, et al. Human umbilical cord mesenchymal stem cells combined with pirfenidone upregulates the expression of RGS2 in the pulmonary fibrosis in mice. Respiratory Research. 2022; 23(1):270. [DOI:10.1186/s12931-022-02192-6] [PMID] [PMCID]

[23] Moquin-Beaudry G, Colas C, Li Y, Bazin R, Guimond JV, Haddad E, et al. The tumor-immune response is not compromised by mesenchymal stromal cells in humanized mice. Journal of Immunology. 2019; 203(10):2735-45. [DOI:10.4049/jimmunol.1900807] [PMID]

[24] Placek K, Schultze JL, Aschenbrenner AC. Epigenetic reprogramming of immune cells in injury, repair, and resolution. The Journal of Clinical Investigation. 2019; 129(8):2994-3005. [DOI:10.1172/JCI124619] [PMID] [PMCID]

[25] Yimam M, Lee YC, Moore B, Jiao P, Hong M, Nam JB, et al. Analgesic and anti-inflammatory effects of UP1304, a botanical composite containing standardized extracts of Curcuma longa and Morus alba. Journal of Integrative Medicine. 2016; 14(1):60-8. [DOI:10.1016/S2095-4964(16)60231-5] [PMID]

[26] Wang X, Ma N, Sun Q, Huang C, Liu Y, Luo X. Elevated NF-κB signaling in Asherman syndrome patients and animal models. Oncotarget. 2017; 8(9):15399-406. [DOI:10.18632/oncotarget.14853] [PMID] [PMCID]

[27] Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome--one century later. Fertility and Sterility. 2008; 89(4):759-79. [DOI:10.1016/j.fertnstert.2008.02.096] [PMID]

[28] Park JO, Lee BH, Kang YM, Kim TH, Yoon JY, Kim H, et al. Inflammatory cytokines induce fibrosis and ossification of human ligamentum flavum cells. Journal of Spinal Disorders & Techniques. 2013; 26(1):E6-12. [DOI:10.1097/BSD.0b013e3182698501] [PMID]

[29] Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, et al. Recent developments in myofibroblast biology: Paradigms for connective tissue remodeling. Pulmonary Pharmacology & Therapeutics. 2012; 180(4):1340-55. [DOI:10.1016/j.ajpath.2012.02.004] [PMID] [PMCID]

[30] Liu HD, Wang SW. Role of noncoding RNA in the pathophysiology and treatment of intrauterine adhesion. Frontiers in Genetics. 2022; 13:948628. [DOI:10.3389/fgene.2022.948628] [PMID] [PMCID]

[31] Choi J, Jo M, Lee E, Oh YK, Choi D. The role of autophagy in human endometrium. Biology of Reproduction. 2012; 86(3):70. [DOI:10.1095/biolreprod.111.096206]

[32] Zhou Z, Wang H, Zhang X, Song M, Yao S, Jiang P, et al. Defective autophagy contributes to endometrial epithelial-mesenchymal transition in intrauterine adhesions. Autophagy. 2022; 18(10):2427-42. [DOI:10.1080/15548627.2022.2038994] [PMID] [PMCID]

[33] Zhao G, Li R, Cao Y, Song M, Jiang P, Wu Q, et al. ΔNp63α-induced DUSP4/GSK3β/SNAI1 pathway in epithelial cells drives endometrial fibrosis. Cell Death & Disease. 2020; 11(6):449. [DOI:10.1038/s41419-020-2666-y] [PMID] [PMCID]

[34] Song M, Cao C, Zhou Z, Yao S, Jiang P, Wang H, et al. HMGA2-induced epithelial-mesenchymal transition is reversed by let-7d in intrauterine adhesions. Molecular Human Reproduction. 2021; 27(2):gaaa074. [DOI:10.1093/molehr/gaaa074] [PMID] [PMCID]

[35] Song M, Zhao G, Sun H, Yao S, Zhou Z, Jiang P, et al. circPTPN12/miR-21-5 p/ΔNp63α pathway contributes to human endometrial fibrosis. Elife. 2021; 10:e65735. [DOI:10.7554/eLife.65735] [PMID] [PMCID]

[36] Smith SK. Angiogenesis, vascular endothelial growth factor and the endometrium. Human Reproduction Update. 1998; 4(5):509-19. [DOI:10.1093/humupd/4.5.509] [PMID]

[37] Smith SK. Angiogenesis and implantation. Human Reproduction. 2000; 15(S 6):59-66. [PMID]

[38] Zhao X, Zhang A, Gao B, Burjoo A, Huang H, Xu D. Cold scissors ploughing technique in hysteroscopic adhesiolysis: A comparative study. Annals of Translational Medicine. 2020; 8(4):50. [DOI:10.21037/atm.2019.11.136] [PMID] [PMCID]

[39] George PM, Wells AU. Pirfenidone for the treatment of idiopathic pulmonary fibrosis. Expert Review of Clinical Pharmacology. 2017; 10(5):483-91. [DOI:10.1080/17512433.2017.1295846] [PMID]

[40] Lancaster LH, de Andrade JA, Zibrak JD, Padilla ML, Albera C, Nathan SD, et al. Pirfenidone safety and adverse event management in idiopathic pulmonary fibrosis. European Respiratory Review. 2017; 26(146):170057. [DOI:10.1183/16000617.0057-2017] [PMID] [PMCID]

[41] Bayhan Z, Zeren S, Kocak FE, Kocak C, Akcılar R, Kargı E, et al. Antiadhesive and anti-inflammatory effects of pirfenidone in postoperative intra-abdominal adhesion in an experimental rat model. The Journal of Surgical Research. 2016; 201(2):348-55. [DOI:10.1016/j.jss.2015.11.033] [PMID]

[42] Ruwanpura SM, Thomas BJ, Bardin PG. Pirfenidone: Molecular mechanisms and potential clinical applications in lung disease. American Journal of Respiratory Cell and Molecular Biology. 2020; 62(4):413-22. [DOI:10.1165/rcmb.2019-0328TR] [PMID]

[43] Ma Z, Zhao C, Chen Q, Yu C, Zhang H, Zhang Z, et al. Antifibrotic effects of a novel pirfenidone derivative in vitro and in vivo. Pulmonary Pharmacology & Therapeutics. 2018; 53:100-6. [DOI:10.1016/j.pupt.2018.10.006] [PMID]

[44] Qin W, Liu B, Yi M, Li L, Tang Y, Wu B, et al. Antifibrotic agent pirfenidone protects against development of radiation-induced pulmonary fibrosis in a murine model. Radiation Research. 2018; 190(4):396-403. [DOI:10.1667/RR15017.1] [PMID]

[45] Li Z, Liu X, Wang B, Nie Y, Wen J, Wang Q, et al. Pirfenidone suppresses MAPK signalling pathway to reverse epithelial-mesenchymal transition and renal fibrosis. Nephrology. 2017; 22(8):589-97. [DOI:10.1111/nep.12831] [PMID]

[46] Evani SJ, Karna SLR, Seshu J, Leung KP. Pirfenidone regulates LPS mediated activation of neutrophils. Scientific Reports. 2020; 10(1):19936. [DOI:10.1038/s41598-020-76271-3] [PMID] [PMCID]

[47] Sun Y, Zhang Y, Chi P. Pirfenidone suppresses TGF-β1-induced human intestinal fibroblasts activities by regulating proliferation and apoptosis via the inhibition of the Smad and PI3K/AKT signaling pathway. Molecular Medicine Reports. 2018; 18(4):3907-13. [DOI:10.3892/mmr.2018.9423]

[48] Kurita Y, Araya J, Minagawa S, Hara H, Ichikawa A, Saito N, et al. Pirfenidone inhibits myofibroblast differentiation and lung fibrosis development during insufficient mitophagy. Respiratory Research. 2017; 18(1):114. [DOI:10.1186/s12931-017-0600-3] [PMID] [PMCID]

[49] Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, et al. An official ATS/ERS/JRS/ALAT clinical practice guideline: Treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. American Journal of Respiratory and Critical Care Medicine. 2015; 192(2):e3-19. [DOI:10.1164/rccm.201506-1063ST] [PMID]

[50] Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): Two randomised trials. Lancet. 2011; 377(9779):1760-9. [DOI:10.1016/S0140-6736(11)60405-4] [PMID]

[51] Yue YL, Zhang MY, Liu JY, Fang LJ, Qu YQ. The role of autophagy in idiopathic pulmonary fibrosis: From mechanisms to therapies. Therapeutic Advances in Respiratory Disease. 2022; 16:17534666221140972. [DOI:10.1177/17534666221140972] [PMID] [PMCID]

[52] Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA. Concise review: Role of mesenchymal stem cells in wound repair. Stem Cells Translational Medicine. 2012; 1(2):142-9. [DOI:10.5966/sctm.2011-0018] [PMID] [PMCID]

[53] Sun Z, Wang S, Zhao RC. The roles of mesenchymal stem cells in tumor inflammatory microenvironment. Journal of Hematology & Oncology. 2014; 7:14. [DOI:10.1186/1756-8722-7-14] [PMID] [PMCID]

[54] Jiang W, Xu J. Immune modulation by mesenchymal stem cells. Cell Proliferation. 2020; 53(1):e12712. [DOI:10.1111/cpr.12712] [PMID] [PMCID]

[55] Vladimirovna IL, Sosunova E, Nikolaev A, Nenasheva T. Mesenchymal stem cells and myeloid derived suppressor cells: Common traits in immune regulation. Journal of Immunology Research. 2016; 2016:7121580. [DOI:10.1155/2016/7121580] [PMID] [PMCID]

[56] Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, et al. Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. The American Journal of Pathology. 2009; 175(1):303-13. [DOI:10.2353/ajpath.2009.080629] [PMID] [PMCID]

[57] Liu Y, Cai J, Luo X, Wen H, Luo Y. Collagen scaffold with human umbilical cord mesenchymal stem cells remarkably improves intrauterine adhesions in a rat model. Gynecologic and Obstetric Investigation. 2020; 85(3):267-76. [DOI:10.1159/000505691] [PMID]

[58] Song YT, Liu PC, Tan J, Zou CY, Li QJ, Li-Ling J, et al. Stem cell-based therapy for ameliorating intrauterine adhesion and endometrium injury. Stem Cell Research & Therapy. 2021; 12(1):556. [DOI:10.1186/s13287-021-02620-2] [PMID] [PMCID]

[59] Merimi M, El-Majzoub R, Lagneaux L, Moussa Agha D, Bouhtit F, Meuleman N, et al. The therapeutic potential of mesenchymal stromal cells for regenerative medicine: Current knowledge and future understandings. Frontiers in Cell and Developmental Biology. 2021; 9:661532. [DOI:10.3389/fcell.2021.661532] [PMID] [PMCID]

[60] Zhang S, Chang Q, Li P, Tong X, Feng Y, Hao X, et al. Concentrated small extracellular vesicles from menstrual blood-derived stromal cells improve intrauterine adhesion, a preclinical study in a rat model. Nanoscale. 2021; 13(15):7334-47. [DOI:10.1039/D0NR08942G] [PMID]

[61] Chen F, Gong Y, Jiang N, Xiao J, Wang Y, Chen L, et al. Transplantation of bFGF-transfected bone mesenchymal stem cells on collagen scaffolds promotes the regeneration of injured rat endometrium. American Journal of Translational Research. 2022; 14(9):6712-25. [PMID]

[62] Ding L, Li X, Sun H, Su J, Lin N, Péault B, et al. Transplantation of bone marrow mesenchymal stem cells on collagen scaffolds for the functional regeneration of injured rat uterus. Biomaterials. 2014; 35(18):4888-900. [DOI:10.1016/j.biomaterials.2014.02.046] [PMID]

[63] Xin L, Lin X, Pan Y, Zheng X, Shi L, Zhang Y, et al. A collagen scaffold loaded with human umbilical cord-derived mesenchymal stem cells facilitates endometrial regeneration and restores fertility. Acta Biomaterialia. 2019; 92:160-71. [DOI:10.1016/j.actbio.2019.05.012] [PMID]

[64] Xie Y, Jiang H, Zhang Q, Mehrotra S, Abel PW, Toews ML, et al. Upregulation of RGS2: A new mechanism for pirfenidone amelioration of pulmonary fibrosis. Respiratory Research. 2016; 17(1):103. [DOI:10.1186/s12931-016-0418-4] [PMID] [PMCID]

[65] Jang HS, Kim JI, Noh M, Rhee MH, Park KM. Regulator of G protein signaling 2 (RGS2) deficiency accelerates the progression of kidney fibrosis. Biochimica et Biophysica Acta. 2014; 1842(9):1733-41. [DOI:10.1016/j.bbadis.2014.06.022] [PMID]

[66] Zhang P, Su J, King ME, Maldonado AE, Park C, Mende U. Regulator of G protein signaling 2 is a functionally important negative regulator of angiotensin II-induced cardiac fibroblast responses. American Journal of Physiology. Heart and Circulatory Physiology. 2011; 301(1):H147-56. [DOI:10.1152/ajpheart.00026.2011] [PMID] [PMCID]

[67] Liang S, Huang Y, Xia Y, Liang S, Wu Q, Zhi Z. Animal models in intrauterine adhesion research. Journal of Obstetrics and Gynaecology. 2022; 42(8):3409-15. [DOI:10.1080/01443615.2022.2124854] [PMID]

[68] Feng Q, Gao B, Zhao X, Huang H, Yi S, Zou L, et al. Establishment of an animal model of intrauterine adhesions after surgical abortion and curettage in pregnant rats. Annals of Translational Medicine. 2020; 8(4):56. [DOI:10.21037/atm.2020.01.134] [PMID] [PMCID]

[69] Li T, Chan RWS, Li RHW, Ng EHY, Zhang S, Yeung WSB. Endometrial mesenchymal stromal/stem cells improve regeneration of injured endometrium in mice. Biological Research. 2024; 57(1):6. [DOI:10.1186/s40659-024-00484-3] [PMID] [PMCID]

[70] Fuentes-Orozco C, Agredano-Jiménez R, Alvarez-Villaseñor AS, Mares-País R, Barbosa-Camacho FJ, Cortés-Flores AO, et al. Effects of oral zafirlukast, sildenafil, or pirfenidone on the formation of postsurgical intra-abdominal adhesions in an experimental rat model. European Surgical Research. 2022; 63(3):145-54. [DOI:10.1159/000521036] [PMID]

[71] Wu F, Lei N, Yang S, Zhou J, Chen M, Chen C, et al. Treatment strategies for intrauterine adhesion: Focus on the exosomes and hydrogels. Frontiers in Bioengineering and Biotechnology. 2023; 11:1264006. [DOI:10.3389/fbioe.2023.1264006] [PMID] [PMCID]

[72] Raghu G, Johnson WC, Lockhart D, Mageto Y. Treatment of idiopathic pulmonary fibrosis with a new antifibrotic agent, pirfenidone: Results of a prospective, open-label Phase II study. American Journal of Respiratory and Critical Care Medicine. 1999; 159(4 Pt 1):1061-9. [DOI:10.1164/ajrccm.159.4.9805017] [PMID]

[73] Li X, Yue S, Luo Z. Mesenchymal stem cells in idiopathic pulmonary fibrosis. Oncotarget. 2017; 8(60):102600-16. [DOI:10.18632/oncotarget.18126] [PMID] [PMCID]

[74] Gazdhar A, Grad I, Tamò L, Gugger M, Feki A, Geiser T. The secretome of induced pluripotent stem cells reduces lung fibrosis in part by hepatocyte growth factor. Stem Cell Research & Therapy. 2014; 5(6):123. [DOI:10.1186/scrt513] [PMID] [PMCID]

[75] Mezey E. The therapeutic potential of bone marrow-derived stromal cells. Journal of Cellular Biochemistry. 2011; 112(10):2683-7. [DOI:10.1002/jcb.23216] [PMID] [PMCID]

[76] Oku H, Shimizu T, Kawabata T, Nagira M, Hikita I, Ueyama A, et al. Antifibrotic action of pirfenidone and prednisolone: Different effects on pulmonary cytokines and growth factors in bleomycin-induced murine pulmonary fibrosis. European Journal of Pharmacology. 2008; 590(1-3):400-8. [DOI:10.1016/j.ejphar.2008.06.046] [PMID]

[77] Myllärniemi M, Kaarteenaho R. Pharmacological treatment of idiopathic pulmonary fibrosis - preclinical and clinical studies of pirfenidone, nintedanib, and N-acetylcysteine. European Clinical Respiratory Journal. 2015; 2. [DOI:10.3402/ecrj.v2.26385] [PMID] [PMCID]

[78] Benor A, Gay S, DeCherney A. An update on stem cell therapy for Asherman syndrome. Journal of Assisted Reproduction and Genetics. 2020; 37(7):1511-29. [DOI:10.1007/s10815-020-01801-x] [PMID] [PMCID]

[79] Zhou Y, Zhang XL, Lu ST, Zhang NY, Zhang HJ, Zhang J, et al. Human adipose-derived mesenchymal stem cells-derived exosomes encapsulated in pluronic F127 hydrogel promote wound healing and regeneration. Stem Cell Research & Therapy. 2022; 13(1):407. [DOI:10.1186/s13287-022-02980-3] [PMID] [PMCID]