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Research Article

# Therapeutic potential of Umbilical Cord Derived Mesenchymal Stem Cells in Bleomycin-Induced Idiopathic Pulmonary Fibrosis in BALB/c Mice

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## Abstract

**Background:** Idiopathic Pulmonary Fibrosis is characterized by excessive matrix deposition that disrupts the normal architecture of the lung parenchyma and causes airway remodeling. It is a progressive and fatal lung disorder with high mortality rate. The current treatments provide only minimal benefits and have significant side effects, highlighting the need for novel treatment approaches to Pulmonary Fibrosis.

**Objective:** The aim of this study was to investigate the therapeutic potential of umbilical cord derived MSC (uMSC) bleomycin induced fibrosis.

**Methods:** A mouse model of bleomycin induced pulmonary fibrosis was used in the study. Wharton jelly derived mesenchymal stem cells were injected intravenously and inflammation, fibrosis and regeneration was examined using a series of assays such as assessment of total cell count, inflammation, hydroxyproline, cell proliferation and clonogenic potential and histology.

**Results:** We found in an increase in the total cell count ($p < 0.001$) and collagen content and a decrease in clonogenic potential ($p < 0.01$) in the lung after bleomycin treatment as compared to the control group. Interestingly, intravenous administration of umbilical cord derived MSC showed reversal of these effects by decrease in collagen content, a decrease in total inflammatory cell count ($p < 0.01$) and increase in clonogenic potential ($p < 0.05$) in the lung. Upon umbilical cord derived MSC administration, reactive oxygen species and reactive nitrogen species generation in the lung decreased as compared to only bleomycin treated group. Histological study of bleomycin treated lung revealed extracellular matrix decomposition, abnormal collagen degradation and distorted lung morphology compared to control groups; stem cell treatment assisted in restoration of lung morphology.

**Conclusion:** The present research suggests that administration of umbilical cord derived mesenchymal stem cells led to reduction in inflammation and collagen content, increased proliferative ability of the cells and restored lung morphology. Thus these cells may be used for future reference to formulate effective therapeutic protocols in managing bleomycin induced IPF.

***Keywords:*** *Idiopathic Pulmonary Fibrosis; Bleomycin; Wharton Jelly Derived Mesenchymal Stem Cells*

## Abbreviations

IPF: Idiopathic Pulmonary Fibrosis; BALF: Bronchoalveolar Lavage Fluid; MSC: Mesenchymal Stem Cell; TGF: Transforming Growth Factor; PBS: Phosphate Buffer Saline; NO: Nitric Oxide; NBT: Nitro Blue Tetrazolium

## Introduction

Lung diseases remain a significant cause of morbidity and mortality worldwide till date [1]. Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive form of interstitial lung disorder of unknown etiology characterized by alveolar epithelial injury, initiation of inflammatory cascades, exaggerated pro-fibrotic cytokine expression, increased extracellular matrix deposition, development of fibrotic

lesions and abnormal deposition of mesenchymal cells [2-7]. The risk of pulmonary fibrosis development is associated with bacterial infection, inhalation of organic and inorganic dusts, smoking, genetic factors, diabetes mellitus, and trauma [8-12]. In cases of unknown etiology, pulmonary fibrosis is referred to as idiopathic pulmonary fibrosis. Due to lack of effective therapy IPF is considered as the most life-threatening idiopathic disease with a median survival of about 3 years from initial diagnosis [10]. Treatments currently available for pulmonary fibrosis provide only minimal benefits and have significant side effects, highlighting the need for novel treatment approaches to IPF.

Animal models play an important role in the disease investigations. The standard agent for induction of experimental pulmonary fibrosis in animals is bleomycin [13]. Intra-tracheal instillation of bleomycin has easy dosage and administration. Bleomycin has been previously used for induction of experimental pulmonary fibrosis in animals [14]. Although the exact mechanisms by which bleomycin causes pulmonary fibrosis remains unclear, it has been reported that, reactive oxygen species generated by bleomycin, such as superoxide anions, hydrogen peroxides, and hydroxyl radicals play an important mediator of bleomycin-induced lung fibrosis [15-19]. A rapidly growing number of investigations of stem cells and cell therapies in lung biology and diseases as well as *ex vivo* lung bioengineering have offered novel potential therapeutic approaches against lung associated diseases.

Mesenchymal stem cells (MSCs) are multipotent stem cells which are found in human amniotic fluid, placenta, umbilical cord blood (UCB), adipose tissue and Wharton's jelly [20,21]. They are capable of differentiating into a number of different cell types such as chondrocytes (cartilage cells), osteoblasts (bone cells), adipocytes (fat cells) and myocytes (muscle cells) [22]. They have been reported to demonstrate anti-proliferative, immunomodulatory, and anti-inflammatory effects [23]. MSCs have tissue protective properties that can control the local environment of the injured tissue, inhibiting inflammation and contributing to tissue repair (Alvarez., *et al*. 2015). As a result of their advantageous property, MSCs holds promise as a novel therapeutic agent against multiple diseases [24]. Some studies suggest that MSCs may be capable of differentiation not only to cells of mesenchymal origin, but also to cells of ecto- and endo-dermal (including epithelial) origin [25,26].

MSCs cultured in airway growth media differentially express lung-lineage specific epithelial markers, including surfactant protein-C, club cell (Clara cell), secretory protein, and thyroid transcription factor-1 [27,28]. Both *in vivo* and *in vitro* studies have shown that murine or human bone marrow MSCs from and human umbilical cord blood cells may differentiate into cells with markers of lung epithelium [27,29]. The umbilical cord is derived from the extra embryonic mesoderm and develops from the proximal epiblast during the formation of the embryonic primitive streak [30]. The umbilical cord contains two arteries and a vein which is surrounded by a matrix which is rich in hyaluronic acid known as Wharton's jelly (WJ). The Wharton jelly derived stem cells have the advantage of ready availability and do not require invasive bone marrow biopsies.

Based on these studies, it was hypothesized that MSCs derived from Wharton's jelly of the human umbilical cord would repair lung injury and prevent fibrosis. In the present study, we examined the therapeutic potential of umbilical cord derived MSC (uMSC) in a bleomycin-induced model of lung injury which shares many features in common with the phenotype of IPF in human.

## Materials and Methods

This animal research study was performed at Ballygunge Science College, University of Calcutta, Kolkata, West Bengal, India from December 2015 to August 2016 by Ms. S. Kar as a part of her doctoral degree program.

## Animals

BALB/c mice were obtained from National Institute of Nutrition, Hyderabad, India. All mice were housed in the animal house of the Department of Zoology, University of Calcutta, India, under pathogen-free conditions and were routinely given food and water. All experiments were performed according to the rules and regulations of the Institutional and Departmental Animal Ethics Committee, University of Calcutta, Kolkata. Weight of mice and other parameters were measured on a regular basis according to rodent health monitoring programme.

**Experimental groups and treatment**

Twelve mice were randomly divided into 3 experimental groups with four animals in each group. Mice were anaesthetized using Propofol (Neon Laboratories Limited, Mumbai, India). The mice in the bleomycin treatment group were subjected to a single intra-tracheal (40 µl) and intranasal (20 µl) instillation of 0.075 U/ml Naprobleo (bleomycin; Miracalus Phrama Pvt Ltd) at day 0. The mice in the control group received an intra-tracheal injection of normal saline. Umbilical cord derived mesenchymal stem cells were administered intravenously via tail vein injection (40 µl; 1x $10^6$ cells/ml) at day 1 and day 3, respectively. The mice were euthanized on day 21 of the experiment and their lung, bronchoalveolar lavage, and peripheral blood were collected. Figure 1 demonstrates the experimental plan and treatment groups used for this study.

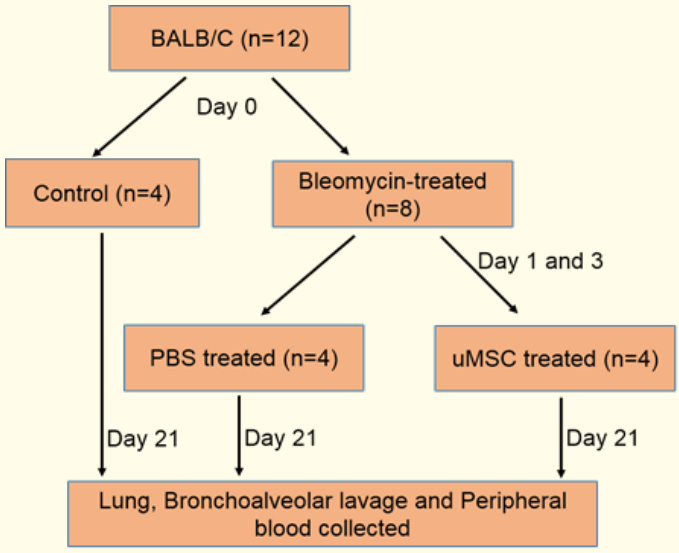


***Figure 1:*** *Experimental groups in the study.*

*BABLB/c mice were divided into three experimental groups. The control group was left untreated. The bleomycin treated mice were administered with either PBS or uMSC on Day 1 and 3. All the control and bleomycin treated mice were sacrificed on Day 21 and tissues were collected for analysis.*

**Isolation and culture of umbilical cord derived MSC**

Human umbilical cord was collected from the hospital with consent of the parents. Human uMSC were isolated from the Wharton's jelly of the umbilical cord. Briefly, after collecting cord blood, the cord was quickly washed in ethanol then washed with PBS and transferred onto a petri dish containing 7 ml growth medium comprising of Dulbecco's Modified Eagle Media (HiMedia Laboratories Pvt Ltd, Nasik, India) supplemented with 10% Fetal Bovine Serum (HiMedia Laboratories Pvt Ltd, Nasik, India) and 1% Penicillin/Streptomycin (HiMedia Laboratories Pvt Ltd, Nasik, India, 10,000 U/ml penicillin and 10,000 µg/ml streptomycin, 25 mg Amphotericin). The inner content of the umbilical cord was squeezed out into the media using forceps and scalpel. The remaining cord was chopped into small sections using surgical blades, and the sections were opened up for better interaction with the growth media. Blood vessels were removed and the remaining Wharton's jelly was cut into small pieces (1 - 2 $cm^2$). Sections were placed in a 100 cm tissue culture plate and 20 ml media was added. The inner content containing media was also added to the plate and the entire plate was kept at $37^0$C incubation (with 5% $CO_2$) for 5 days. The setup was kept undisturbed for 3 days and then observed under microscope for any adherent cells. After 5 days in culture, adherent cells were observed proliferating from individual explanted tissue of the Wharton's jelly. At this time, the Wharton's jelly was removed from culture and the adherent cells were cultured. After reaching confluence, the cells were passaged using trypsin-EDTA. The cells were imaged at 10X magnification using Nikon Eclipse Ti-S inverted microscope.

### Isolation of cells from Bronchoalveolar lavage fluid (BALF) and lung tissue

The mouse lungs were lavaged three times with 1 ml of PBS. The retained BALF was centrifuged at 400 x g for 5 min at 4°C. The supernatant was collected and stored at -70°C for further analysis. The cell pellets were resuspended in culture media and used for assays. The lung tissue was minced using a scalpel to isolate the cells for assays.

### Total Cell Count

The total cell count of BALF and lung sample was estimated using trypan blue (HiMedia Laboratories Pty Ltd, Nasik, India) exclusion method.

### Colony Forming Unit assay

For quantification of committed progenitors of all lineages, Colony-Forming Units in culture was performed as described previously (Kar et al, 2016). All colony types were counted using Floid Cell Imaging Station (Life Technologies, India) and the numbers were pooled to obtain the total number of colony forming units.

### Cell Proliferation Assay (MTS Assay)

The MTS assay was performed using Promega CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay Kit (Promega, India) to determine the number of viable cells in culture as per manufacturer's protocol (Kar et.al, 2016). The absorbance was immediately measured at 490 nm using Thermo Scientific *Multiscan* EX.

### Collagen concentration

The collagen content in mice lung homogenates was measured using the hydroxyproline assay as described previously (Kar et al.,2016). The optical density was measured at 550 nm using Thermo Scientific *Multiscan* EX.

### Determination of superoxide generation

A semi-quantitative microscopic nitroblue tetrazolium (NBT) assay was used to determine the production of superoxide anion [$O_2$(-)] in various phagocytic cells as previously described (Kar et.al, 2016). The absorbance was then measured at 620 nm using Thermo Scientific *Multiscan* EX.

### Determination of Nitric Oxide Content (NO Assay)

To examine the production of nitric oxide from macrophages as a result of inflammation, NO assay was performed as described elsewhere (Kar et.al, 2016). The absorbance was measured spectrophotometrically by measuring the absorbance at 540 nm using Thermo Scientific *Multiscan* EX.

### Histology

The mice were euthanized by cervical dislocation and tissues collected. One part of the lungs was carefully excised and fixed in 10% (w/v) PBS-buffered (HiMedia Laboratories Pty Ltd, Nasik, India) formaldehyde solution and paraffin embedded. Five micrometer thick tissue sections were cut using a microtome (RM-2135, Leica Microsystems, Bensheim, Germany). To evaluate the histopathological changes, the sections were stained with haematoxylin and eosin. To identify the density of the accumulated collagen fibers, the sections were stained using Masson's trichrome staining.

### Statistical analysis

All the comparisons were performed using GraphPad Prism 6 statistical software. The effect of uMSC administration in the mice with pulmonary fibrosis was analysed using Student's t-test, at $\alpha = 5\%$. A p value of $< 0.05$ was considered to indicate a significant difference.

## Results

In this study, we used a bleomycin-induced lung injury model to examine the anti-inflammatory, anti-fibrotic and regenerative potential of umbilical cord derived mesenchymal stem cells in idiopathic pulmonary fibrosis.

### Culture of umbilical cord derived MSC

Wharton's jelly derived mesenchymal stem cells were cultured in tissue culture dishes. After 5 days of culture, the cells were seen to proliferate from individual explanted tissue of the Wharton's Jelly. Initial cultures contained a population of adherent fibroblast-like cells with long and short processes (Figure 2A). By day 15 they reached approximately 90% confluency as shown in figure 2B.

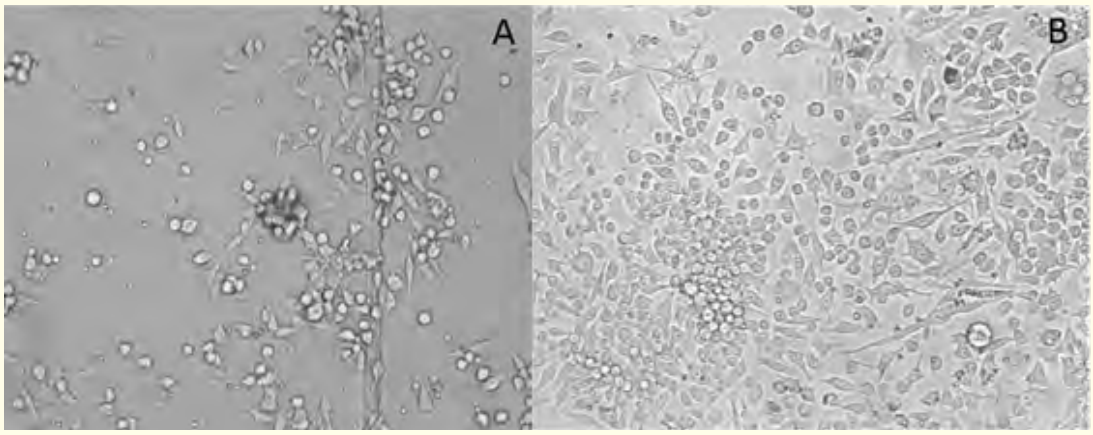


***Figure 2:*** *Culture of umbilical cord derived mesenchymal stem cells.*

*Wharton's jelly was dissected from the umbilical cord and cultured in a tissue culture dish. The uMSC proliferated and demonstrated fibroblast-like morphology.*

### Total cell count

On examination of the total cell count in BALF, we found a significant increase of 3.9 fold ($p = 0.0036$) in the bleomycin treated group as compared to the control group (Figure 3A). Further, upon treatment with uMSC the total cell count decreased 2 fold as compared to the bleomycin treated group ($p = 0.0164$).

The total cell count was estimated in the lung samples collected from all the treatment groups using a hemocytometer. We found a significant increase of 2.5 fold in the total cell count in bleomycin treated group as compared to the control group ($p = 0.0004$; Figure 2B). This suggests that bleomycin was highly effective in causing fibrosis in mice. On treatment with uMSC, the cell count significantly decreased (1.8 fold; $p = 0.0012$) in comparison with the bleomycin treated group.

These results suggest that uMSC treatment perhaps reduced the inflammatory cell count in bleomycin treated mice.

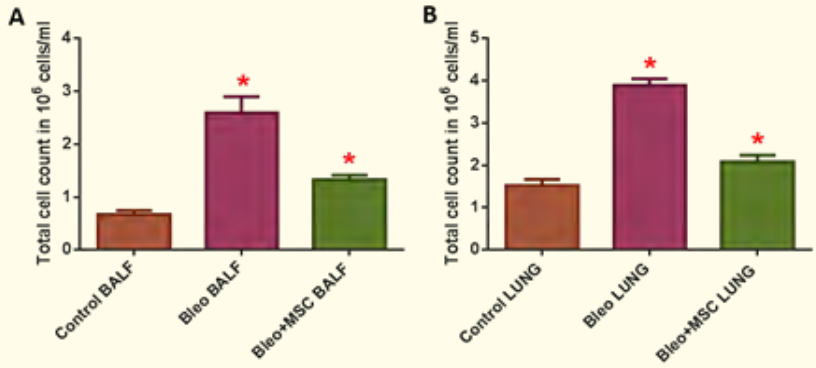


***Figure 3:*** *Comparison of Total cell count upon uMSC administration.*

*Total cell count was examined using a hemocytometer. The figure shows the results obtained from comparison of total cell count between the treated and the control samples A) Bronchoalveolar lavage (BALF) and B) Lung sample. The total number of polymorphonuclear cells was estimated using a hemocytometer. Bleomycin treated (Bleo) showed a significant increase in the total cell count which was significantly reduced upon treatment with uMSC (Bleo+MSC).*

**Clonogenic potential assay**

Clonogenic potential of BALF was estimated using colony forming assay. We found that the clonogenic potential of bleomycin treated sample significantly decreased by 1.6 fold as compared to control (Figure 4B; p = 0.005). On uMSC treatment, the clonogenic potential showed a significant increase of 1.3 fold as compared to bleomycin treated samples (Figure 4A; p = 0.017).

Similarly, the clonogenic potential in the lung was decreased by 1.7 fold as compared to the control group after treatment with bleomycin (p = 0.0034), whereas the total number of colonies significantly increased by 1.4 fold (p = 0.018) upon administration of uMSC as compared to the bleomycin treated group (Figure 4B).

These results suggest that bleomycin effectively decreased the clonogenic potential of the lung and uMSC administration repaired the damage.

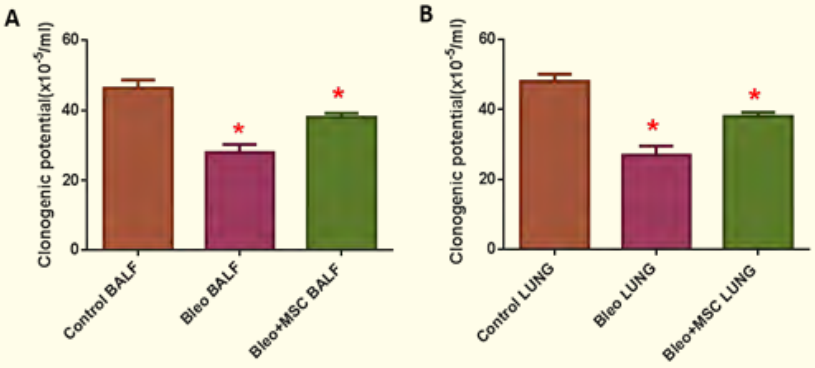


***Figure 4:*** *Comparison of clonogenic potential of cells upon uMSC administration.*

*Clonogenic potential was measured by colony-forming unit assay. Five million cells were plated for each group and colony formation was measured at 7 and 14 days. The figure shows clonogenic potential of cells obtained from A) BALF and B) Lung of treated and control samples. Clonogenic potential reduced on bleomycin treatment (Bleo) and was restored upon administration of uMSC (Bleo+MSC).*

**Cell proliferation assay**

The MTS assay is an indicator of the proliferative potential of cells. We found that, the cell number in the bronchoalveolar lavage fluid decreased by more than 7 fold on bleomycin administration as compared to the control group (Figure 5a; p = 0.019). On comparison of the uMSC treated group with bleomycin treated group we observed a 10 fold increase in cell numbers (p = 0.0001). These results suggest that uMSC administration was effective in restoring cell proliferation.

In the lung sample we observed that the cell numbers significantly decreased on bleomycin treatment, as compared to the control (Figure 5d; p= 0.006). Upon uMSC treatment we observed a similar proliferative potential as in the bleomycin treated lungs. Perhaps, administration of uMSC led to only a small alteration in proliferative potential in the lung.

**Estimation of Superoxide generation**

Nitroblue tetrazolium assay was performed to assess superoxide generated due to oxidative stress in BALF and lung from all treatment groups. In BALF the superoxide generation increased significantly (7 fold; p = 0.0017) after bleomycin administration (Figure 5b). Administration of uMSC reduced the superoxide generation, however not significantly.

In lung sample, we observed a significant increase in superoxide generation after bleomycin treatment (1.7 fold) as compared to the untreated control group (Figure 7e). The superoxide content decreased by 1.4 fold after administering uMSC as compared to only bleomycin treated group. The differences were not statistically significant, however these results suggest that administration of uMSC facilitated reduction of oxidative stress in the tissue.

**Estimation of nitric oxide concentration**

Nitric oxide (NO) is produced by macrophages as a defense mechanism against oxidative stress. Further, we estimated NO production to examine the effect of oxidative stress on BALF and lung cells. We observed that NO levels did not alter much in BALF. The concentration of nitric oxide in BALF increased by 1.1 fold on bleomycin treatment as compared to the untreated control group (Figure 5c). However, on comparison with bleomycin treated samples, we found that, nitric oxide concentration decreased by 1.1 fold in uMSC treated group.

Analysis of lung samples showed that nitric oxide concentration significantly increased by 1.6 fold (p = 0.0061) after administration of bleomycin as compared to the control group (Figure 5f). Nitric oxide concentration decreased by 1.4 fold after treatment with uMSC as compared to the bleomycin group; although this difference was not statistically significant.

We observed minimal alteration upon uMSC treatment in inflammation in the BALF and in proliferation in the lung; reduction in inflammation and superoxide generation in the lung is not statistically significant. However, the trends in the tissue show beneficial effect of MSC administration in this model.

**Collagen concentration estimation**

In fibrosis, collagen concentration is greatly increased along with extracellular matrix complex. In this study we examined the collagen concentration using hydroxyproline assay and the results are shown in figure 6. We found that there was a 1.4 fold increase in collagen concentration after bleomycin treatment as compare to control group We observed that, after treatment with uMSC the collagen concentration was reduced by 2 fold as compared to the bleomycin treated individuals. These results again suggest that, bleomycin was effective in inducing fibrosis in mice and administration of uMSC demonstrated anti- fibrotic action, however not statistically significant.

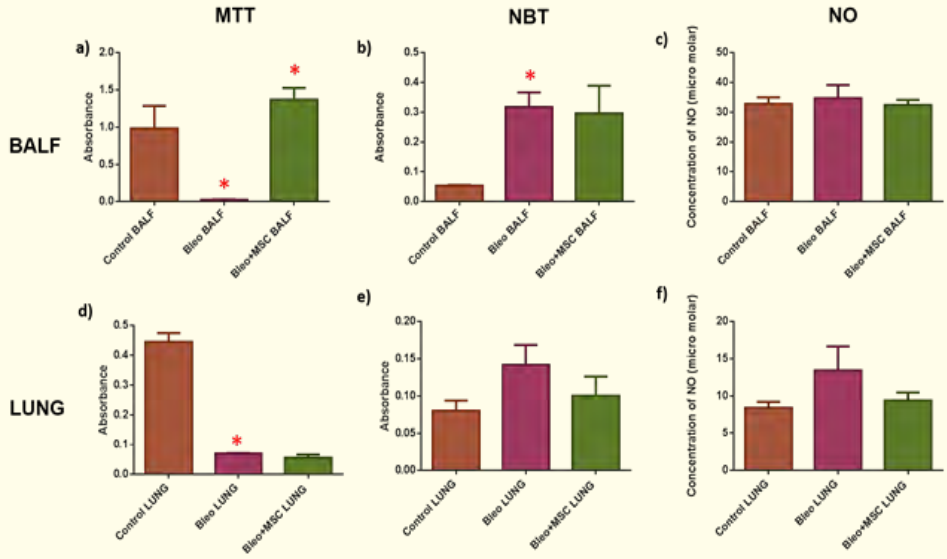


***Figure 5:*** *Comparison of cell-based assays upon uMSC treatment.*

*The number of viable cells present in bronchoalveolar lavage fluid was measured by MTS assay and the absorbance of formazan was measured at 490 nm. The proliferative potential was compared in A) BALF and B) Lung tissue. The number of cells reduced on bleomycin (Bleo) administration and increased again on treatment with uMSC (Bleo+MSC) in the a) BALF while minimal change was observed in the b) lung. Production of superoxide anion was measured by NBT assay. The figure shows comparison of superoxide levels in b) BALF and e) Lung tissue of the treated and untreated samples. We found an increase in superoxide anion production after bleomycin administration which reduced after treatment with uMSC. The concentration of nitric oxide was measured using NO assay. NO content was estimated in c) BALF and f) Lung tissue obtained from treated and control samples. Nitric oxide concentration was increased after bleomycin administration (Bleo), which decreased after addition of uMSC (Bleo + MSC).*

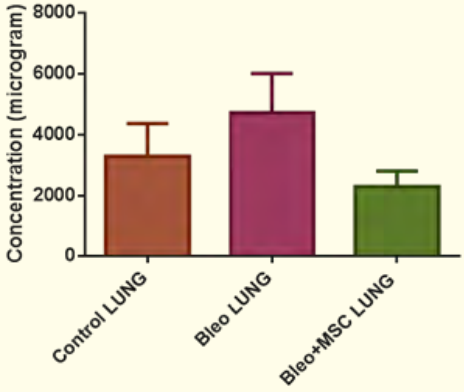


***Figure 6:*** *Collagen content analysis of in lung sample.*

*Collagen concentration was estimated by hydroxyproline assay. Collagen concentration was greatly reduced after treatment with bleomycin (Bleo), while administration of uMSC showed a decrease in collagen concentration (Bleo+MSC). These changes were not statistically significant.*

### Histology and Fibrosis of lung sample

Hematoxylin and Eosin staining was performed to examine the lung histology of the treatment groups. We found that, the lung tissue section of bleomycin-treated animals showed histopathological abnormalities (Figure 7B). These include disturbance in alveolar structure, extensive thickening of the intra-alveolar septa, and dense interstitial inflammatory cells and fibroblast. These changes were not observed in the control group. In contrast, upon uMSC treatment provided protection against bleomycin induced lung tissue distortion (Figure 7C). Significant amelioration was observed in the cellular infiltrates and thin lined alveolar septa were observed in the lung tissue of umbilical cord derived mesenchymal stem cell treated groups compared to the bleomycin treated animals. Masson trichome staining was performed to examine the fibrosis in the lung. We found that bleomycin treatment led to increase in lung fibrosis, which is consistent with previous studies (Figure 7E). Upon uMSC treatment we observed fewer fibrotic lesions (Figure 7F). These results suggest that uMSC administration facilitates improvement in lung histology and fibrosis.

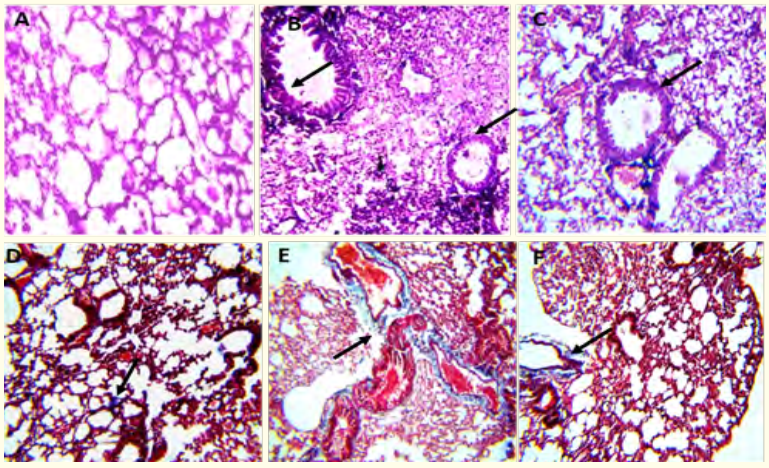


***Figure 7:*** *Histological assessment of lung tissue.*

*Histological analysis of lung tissue was performed using Hematoxylin-Eosin (A-C) and Masson's trichrome staining (D-F). Lung tissue sections (A) of control mice showed normal lung morphology; (B) of mice with bleomycin-induced pulmonary fibrosis demonstrated distorted lung morphology; (C) of uMSC treated mice showed improvement in tissue histology. Arrows indicate the changes in alveolar septa. Masson's trichome staining of lung tissue sections (D) of control animals demonstrated normal lung morphology; (E) of mice with bleomycin-induced pulmonary fibrosis showed dense collagen accumulation and (F) of uMSC treated mice showed reduced collagen deposition. Arrows indicate the collagen deposits present in the tissues. All images were taken at 40 X magnification using Dewinter FLUORE I LED.*

## Discussion

In the present study we examined the therapeutic potential of umbilical cord derived MSC in a mouse model of idiopathic pulmonary fibrosis. Consistent with previous reports, our study presents evidence that uMSC may be a promising therapeutic option in IPF.

Due to the advantageous properties of MSCs, it holds promise as a novel therapeutic agent in multiple diseases including severe graft-versus-host disease [31], sepsis [32], pancreatic islet and renal glomerular repair in diabetes [33] fulminant ischemic acute renal failure [34], acute lung injury [35], hepatic failure [36] and improving cardiac function after ischemic injury [37-39]. MSCs are a potential source of multiple cell types for use in tissue engineering [40,41] and tissue regeneration after spinal cord trauma, stroke, and connective tissue

injury [42]. In this study we analysed the effect of umbilical cord derived MSC in pulmonary fibrosis. MSCs delivered intravenously travel directly to the lungs via the right heart [43]. In our study we administered uMSC thorough an intravenous injection in the tail vein.

Umbilical cord–derived stem cells home to sites of bleomycin-induced lung injury, inhibit the production of pro inflammatory cytokines, and reduce lung injury and collagen deposition [44]. It has been demonstrated that MSCs have a beneficial effect on pulmonary fibrosis if they are administered 24 hours after bleomycin dosage early in the disease course and not when the fibrotic changes have already set-in. In the present study we administered uMSC on day 1 and day 3, before the occurrence of fibrotic changes. MSC administration optimizes cell incorporation into lung injury during early inflammation.

Mice were treated with bleomycin to induce lung injury. Bleomycin administration is known to damage the lung epithelial cells resulting in lower cell numbers (Hay., *et al*. 1991). We performed total cell count of BALF and lung tissue which demonstrates that uMSC administration reduced the total number of cells perhaps reducing infiltration of inflammatory cells in the tissue.

In our previous work we found that bleomycin administration the cells lose the ability to proliferate and form colonies on semi- solid medium [45]. Consistently, in the present study we found that colony forming assay in both BALF and lung tissue demonstrate a reduction in colony formation on bleomycin treatment. We found that the number of colonies increased significantly in the group treated with stem cells. Bleomycin results in loss of oxidative balance in the body which in turn leads to reduction in cell proliferation. However, on analysis of cell proliferation we did not observe any significant change in the uMSC treated BALF and lung as compared to the bleomycin treated samples. This may be due to small brought about by the administration of uMSC which could not be recorded.

One of the major results of an inflammatory reaction in a body is the overproduction of pro-oxidative agents such as super oxide anion, nitric oxide. In response to the increase in pro-oxidative radicals, the body attempts to maintain the oxidative balance by producing more antioxidants, which can scavenge the harmful radicals. This leads to an increase in the proliferation of cells. Bleomycin is known to activate macrophages, which in turn can release large amounts of cytotoxic mediators, including superoxide anion and nitric oxide [46] that decrease cell proliferation. This has been demonstrated in our results where treatment with bleomycin led to an increase in NO concentration and super oxide anion generation. Administration of uMSC ameliorated the effect of bleomycin in the lung. Administration of bone marrow derived cells has shown similar reduction in inflammation which further facilitates differentiation into lung cells [47,48]. Our results indicate that uMSC is capable of lowering the generation of both superoxide and nitric oxide from the lung perhaps by inhibiting specific inflammation regulatory pathways.

The immunomodulatory and anti-inflammatory effects of MSCs are well known and have been reported in multiple studies [49]. Factors secreted by MSCs in response to inflammation include, but are not limited to, interleukins, tumor necrosis factor and prostaglandins. MSC administration affects secretion of TGFβ which plays an important role in modulating immune responses to ameliorate inflammation. At the same time MSCs can also act as a target for transforming growth factor β (TGFβ), which results in an increase in collagen expression and deposition [50].

Bleomycin has been shown to generate toxic hydroxide, superoxide free radicles which involve in the formation of hydroxylated proline (Bhatnagar and Liu, 1972). It greatly induces collagen deposition with a significant increase in hydroxyproline content and development of fibrosis in a maximum of 15 to 30 days after injury, resulting in the development of fibroblastic foci [51]. We found an increase in collagen deposition in mice treated with bleomycin which reduced after uMSC injection. This is consistent with previous reports which have used adipose or bone marrow derived MSCs in a model of pulmonary fibrosis [48,52]. Administration of stem cells derived from amniotic fluid have been reported to demonstrate similar effect [53]. These findings claim for the role of uMSC as an anti-fibrotic agent in bleomycin-induced pulmonary fibrosis in BALB/c mice.

Histological study of bleomycin induced lung revealed abnormal collagen decomposition and distorted lung morphology compared with control groups. Some amelioration was observed in the lung morphology of the uMSC treated groups suggesting that blunting of

inflammation at an early stage leads to the attenuation of some downstream events which were necessary for collagen deposition and fibrosis. These results are consistent with previous reports where they have observed restoration of lung morphology and reduction in collagen deposition [54,55].

To date there is no definitive approach in the treatment of IPF because evidence for effective medical therapy is still lacking. Multiple clinical trials using stem cells treatment in IPF are listed in the Clinicaltrials.gov. A stem cell-based therapy using uMSC obtained from discarded placental tissue provides an alternate source for allogenic stem cell therapy further reducing chances of rejection and may impact on several pathways in the pathogenesis of major lung injury [56,57].

## Conclusion

In summary, we found that, transplantation of umbilical cord derived MSC reduced inflammatory and fibrotic mediators in a model of bleomycin-induced pulmonary fibrosis. Further studies examining the molecular pathways affected by these stem cells may help in improving their efficacy in treatment of idiopathic pulmonary fibrosis.

## Acknowledgments

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## Conflict of Interest

The authors have declared that no conflict of interest exists.

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