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Mesenchymal Stem Cells (MSCs) as a Novel Therapeutic Option for nCOVID-19—A Review

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Abstract

The novel Coronavirus Disease (nCOVID-19) is a highly contagious viral infection which emerged as "Pneumonia of Unknown Etiology" at Hubei province of Wuhan city in China. The health authorities provided a considerable empirical evidence after this outbreak and it was notified that the causative virus, named Novel Coronavirus (subsequently SARS-CoV-2) is the culprit for progressively exerting grim effects not only on individual patients but also on the International public health, with high mutational tendencies. WHO declared nCOVID-19 as a Pandemic on 11th March 2020. The spike glycoprotein of SARS-CoV-2 plays a pivotal role in the entry of virus into the cell and it further interacts with ACE-II receptors which are widely distributed on the human cell surface especially on alveolar type II cells (AT-2) and endothelium. The mortality in nCOVID-19 patients is usually preceded by acute respiratory distress syndrome (ARDS) because of the cytokine storm. Advanced molecular biology and regenerative sciences renders a breakthrough in the treatment of severely ill nCOVID-19 patients with Mesenchymal Stem Cells (MSCs). Autologous or allogenic MSCs attenuate cytokine storm, improvise lung compliance, regulate inflammatory response, maintain functional alveoli microenvironment, promote endogenous regeneration and repair with no or minimal side effects. MSCs are naturally resistant to this novel Coronavirus. Even though it is corroborated with evidences from current clinical trials and

pilot study, we emphasize the need for conducting more clinical trials with ethical consideration to prove the efficacy and safety of MSCs in combating nCOVID-19 infection and its complications.

Keywords

Coronavirus, nCOVID-19, Mesenchymal Stem Cells, WHO, Pandemic, Level of Evidence - Level I

1. Introduction

Hui *et al.*, 2020 reported in late December 2019 that China had announced 27 residents had fallen ill with an unknown viral infection in Wuhan of Hubei Province, China and most of the infected individuals were found to be the stall holders at the city's Huanan Sea Food Market. After a week, 44 more individuals presented with similar symptoms. Later, the investigators isolated the virus from the patient samples and they genetically sequenced the viral samples and reported to WHO; in the interim the virus was given the name Novel Coronavirus discovered in 2019 (2019-nCoV/nCOVID-19) and subsequently named as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) [1]. Further, many investigations were conducted to identify the cause and it was reported that people from Hubei province might have shown the symptoms in early November, 2019. This novel coronavirus strain (SARS-CoV-2) has neither been identified nor detected in human hosts until this nCOVID-19 outbreak in China. The nCOVID-19 virus showed 79% genotypic similarity to the SARS coronavirus and was closely related to β-coronavirus from the origin of bats which suggests that bats are the most likely animal reservoir hosts for the transmission of this emerging viral pathogen, however, this transmission of infection to humans believed to have occurred due to an intermediate host transmission like pangolin [2] [3].

A substantial increase in the infection rates happened because of human-to-human transmission of this nCOVID-19 virus and by the end of February, 2020 the infection rates and new cases were found to be significantly reduced in China but on the other hand, subsequent transmission and infection rates were found to be increasing outside China including Italy, Iran, South Korea, Spain, USA, France and UK. The World Health Organization declared nCOVID-19 as a Pandemic on 11th March, 2020 and more than 174,000 confirmed positive cases and approximately 7500 deaths occurred globally [1].

Many species of animal and human hosts have been affected by coronaviruses and the family of coronaviruses remains relatively obscure probably because of no severe human diseases attributing to this virus. However, in the year 2003, it became clear that these coronaviruses were responsible for severe acute respiratory syndrome (SARS) epidemic. Since then, two new human respiratory coro-

naviruses namely Middle East respiratory syndrome related coronavirus (MERS-CoV) and nCOVID-19 have been described. In the year 1968, scientists named this virus as "coronavirus" because of its crown like morphological appearance and it belongs to the family coronaviridae with two subfamilies namely, the coronaviruses and the toroviruses. Later, it was found that these viruses were also responsible in causing enteric diseases in cattle and possibly in humans.

2. Etiology of nCOVID-19

There are 2 speculations regarding the origin of SARS-CoV-2 - 1) natural selection of animal host before zoonotic transfer and 2) natural selection in humans following zoonotic transfer [4]. The zoonotic source is not confirmed however genomic analysis suggested bats as the main reservoir. The isolated β-CoV shows 88% similarity to the sequence of two bat-derived SARS-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 [5]. People with history of visit to Wuhan's seafood market contracted this infection but in due course, it was revealed that in some individuals no record of seafood market visit had also contracted infection suggesting its capability of human to human transmission capability [6]. This can occur via direct contact with an infected person, exposed to coughing, sneezing, respiratory droplets or an aerosol which enter human lungs via inhalation through nose or mouth and via indirect contact *i.e.* as fomite transmission. Recent case series has put forward possibility of vertical transmission of SARS-CoV-2 from infected mother to her new born; however it is not conclusive [7] [8] [9].

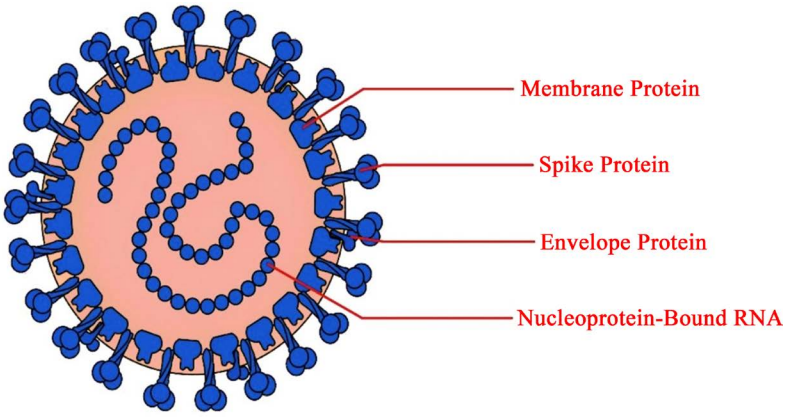
3. Coronavirus Classification/Taxonomy

Based on the serological cross-reactivity characteristics, these coronaviruses are divided into three genera, *i.e.*, group I to III [10] [11]. This was also confirmed by genome sequencing analysis [12]. The Group-I coronavirus includes animal pathogens such as transmissible gastroenteritis virus (TGEV), Porcine epidemic diarrhoea virus (PEDV), feline infectious peritonitis virus (FIPV) as well as human coronaviruses HCoV-229E, HKU1; Group-II coronavirus includes pathogens of veterinary relevance such as Bovine coronavirus (BCoV), equine coronaviruses, porcine hemagglutinating encephalomyelitis virus as well as human coronavirus OC43, NL63 and Group-III includes only avian coronavirus such as IBV, pheasant coronavirus and turkey coronaviruses [13]. But, the Group-II viruses which infects mice and rats were also found to be as coronavirus prototype that were found responsible in causing infections like enteric diseases, hepatitis, encephalitis, respiratory diseases and chronic demyelination [14]. Many controversies exist about the SARS-CoV that whether it belongs to a new group of coronaviruses or a distant member of group [15]. The sequences of coronaviruses detected in Columbia livia (feral pigeon), Anser anser (Greylag goose) and Anas

platyrhynchos (mallard) and their phylogenetic analysis also confirms that these viruses belong to group-III [16]. There were two prototypes of human coronaviruses found to cause common cold in humans namely OC43 and 229E previous to the emergence of SARS-CoV [17] and determined to have long speculations about human coronaviruses with serious health issues such as multiple sclerosis [18], hepatitis [19] and enteric diseases in new borns [20].

4. Virion Structure and Function

Coronaviruses (nCOVID-19/SARS-CoV-2) are enveloped viruses with round or pleomorphic structure of approximately 80 to 120 nm in diameter containing positive single-stranded RNA genome of 30 kb size [21] [22] (as shown in **Figure 1**). The RNA genome is complexed with basic nucleocapsid protein (N) to form a helical viral protein and these are spike proteins (S) which are the Type-I glycoprotein that forms the peplomers on the virion surface giving it a crown-like structure (as shown in **Figure 1**). The membrane protein (M) which spans three times the viral membrane has a short N-terminal ectodomain and a cytoplasmic tail. The small membrane protein (E) is found to be highly hydrophobic in nature [23] and this spans twice the N and C terminals on the interior part of the virion [24]. There are still many other minor proteins present in the viral structure yet undetected and the genomes of all the coronaviruses were found to have similar structural characteristics [25]. For all coronaviruses, the structural proteins are encoded in order of S-E-M-N within the one third of the genome and each group of coronaviruses encodes a group of unique small proteins while these are non-essential proteins and have been found to serve as accessory proteins to interact or interfere with the host innate immune responses which has not been demonstrated for any of these proteins [26] [27]. Untranslated regions of coronaviruses (UTRs) on both 5' and 3' ends of the genome were believed to interact with the host and control the RNA replication process and the viral transcription has been reviewed in recent studies [28].



Courtesy: First Electron microscopic image of COVID-19 virus (NIV, Pune, India)

Figure 1. Structural representation of SARS-CoV-2.

5. Replication of nCOVID-19

Coronaviruses (nCOVID-19/SARS-CoV-2) attach to specific cellular receptors like ACE-2 with its spike protein (S) and this triggers a conformational change in the viral spike protein which mediates a fusion reaction between the virus and the cell membrane resulting in injecting the viral genetic material into the host cell. Upon entry, the 5' end of the RNA genome, ORFs 1a and 1b are translated into polyproteins (pp 1a and pp 1b) and here, the polyproteins pp 1ab are translated through a frameshift mechanism occurring at a high frequency of 25% to 30% [20] [28]. ORF 1a encodes one or two papain like protease enzymes and a picornavirus 3C-like protease which functions in processing the polyproteins into mature replicase proteins [29] and encoded in X domain of ORF 1a and processed by an RNA-dependent RNA polymerase (RdRP) along with helicase enzyme [30] as well as with other enzymatic activities [31]. An additional putative enzymatic activity of cyclic phosphodiesterase encodes the downstream process in ORF 2a and these multiple enzymatic activities play a vital role in metabolism of coronavirus RNA interaction with the host cell process [32]. The interaction between the viral spike protein (S) and the ACE-2 receptor on the host cell surface significantly initiates the infection process and the cryo-EM structure analysis has revealed the binding affinity efficacy of nCOVID-19 (SARS-CoV-2) S protein to ACE-2 receptor of about 10 to 20 times higher than that of SARS-CoV S protein [33] [34] and has higher transmissibility and contagiosity of nCOVID-19 as compared to SARS-CoV [35].

On the basis of genome sequence alignment and homology, SARS-CoV and SARS-CoV-2 (nCOVID-19) are found to have shared a highly conserved receptor-binding domain (RBD) and there is 76% similarity of the domain of S protein sequence [22] [36] and 83% of similarity in their active sites [4]. Due to their much higher binding affinity to the cellular receptors the human to human transmission of the virus is also visibly higher when compared to that of SARS-CoV coronavirus outbreak and additional studies are required in this pandemic situation for better investigation of this possibility of higher transmission among individuals [37]. Based on these evidences, the natural evolution of nCOVID-19 or the SARS-CoV-2 was supported based on its overall molecular mechanisms and also scientists have found that SARS-CoV-2 backbone differed substantially from those known coronaviruses. Additionally, their viral resemblances were found in bats and pangolins. Hence, the scientists concluded that the nCOVID-19 coronavirus is the product of natural evolution ending any deliberate genetic engineering [38].

6. Pathogenesis of nCOVID-19 with Human Host

nCOVID-19 patients show clinical manifestations such as fever, non-productive cough, dyspnoea, fatigue, myalgia, normal or decreased leukocyte counts with radiographic evidence of pneumonia. Recent evidences shows anosmia, hyposmia and dysgeusia to be considered in the list of symptoms associated with

nCOVID-19 infection [39]. As per several reports, the envelope spike glycoprotein play pivotal role in the entry. It recognizes and binds to its cellular receptor angiotensin I converting enzyme 2 receptor (ACE2R) for SARS-CoV and SARS-CoV-2, CD209L (a C-type lectin, also called L-SIGN) for SARS-CoV, DPP4 for MERS-CoV [40]. Moreover the entry mechanism is also dependent on cellular protease like human airway trypsin like protease (HAT), cathepsins and transmembrane protease serine 2 (TMPRSS2) which facilitates splitting of spike protein and further penetration changes are established [41] [42]. Molecular studies explicatively stated that ACE2 receptors are widely distributed on the human cell surface, especially the alveolar type II cells (AT2) and capillary endothelium with AT2 highly expressing TMPRSS2. Viral proteins and genome RNA are subsequently assembled into virions in endoplasmic reticulum or Golgi. Lastly from endoplasmic-reticulum-Golgi intermediate compartment (ERGIC), the viral particles in the vesicles fuse with plasma membrane to release the virus [5] [6]. The Structural proteins are encoded by the four structural genes, including spike (S), envelope (E), membrane (M) and nucleocapsid (N) genes (as shown in **Figure 1**). The ORF1ab is the largest gene in SARS-CoV-2 which encodes the pp1ab protein and 15 nsps. The genomic variation between SARS-CoV and SARS-CoV-2 such as the absence of 8a protein and fluctuation in the number of amino acids in 8b and 3c protein in SARSCoV-2 [6].

The mortality in nCOVID-19 patients is associated with onset of acute respiratory distress syndrome (ARDS) due to the cytokine storm mechanism resulting in uncontrolled systemic inflammatory response from the release of pro-inflammatory cytokines (IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-8, IL-33, TNF-α, TGF-β) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL-10 etc) [5] [40]. It culminates into edema, dysfunction of the air exchange, acute respiratory distress syndrome, acute cardiac injury and the secondary infection which may lead to death [42]. The key to treatment aims at avoiding or attenuating cytokine storm, and to restrict the entry of mononuclear cells via CD4 and CD8T cells (as shown in **Figure 2**). Case studies and analysis throw light on application of mesenchymal stem cells in such a situation. MSCs, with their powerful immunomodulatory ability, may benefit by preventing or attenuating the cytokine storm [40].

7. Emerging Stem Cell Based Therapy for nCOVID-19

Recent advances in molecular biology and regenerative medicine has promised induction of the activity of micro-molecules such as stem cells, bioactive materials and growth factors promoting improved quality, structure, function and bio-mechanical strength of tissue regeneration and healing process. The cellular therapy induces cellular proliferation, chondrogenesis, angiogenesis and rejuvenation of degenerated tissue to attain micromolecular homeostasis.

The art of cellular therapy has revolutionized the field of regenerative medicine towards natural healing cascade. Among cellular therapy, mesenchymal stem cells (MSCs) has homing ability, immunoregulatory nature, anti-inflammatory effects, and multilineage differentiation potential as reported by Ullah *et al.* *[43]* and Ramesh R *et al.* [44] (as shown in **Figure 3**).

MSCs could act upon two ways in the nCOVID19 treatment, namely immunomodulatory effects and differentiation ability. In nCOVID19 cases, acute respiratory distress is the result of SARS-CoV-2 infecting alveolar type II cells (AT2) and the capillary endothelium as mentioned by Hamming *et al.* [45]. The

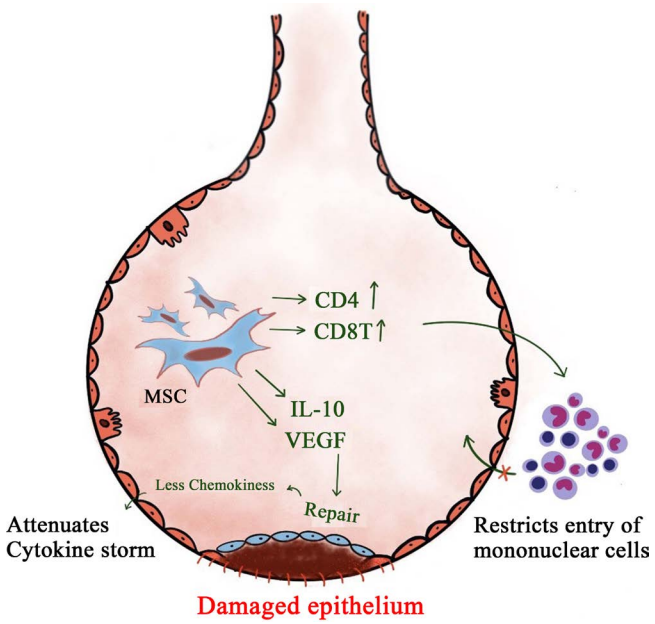


Figure 2. Mechanism of action of MSCs in nCOVID-19 treatment.

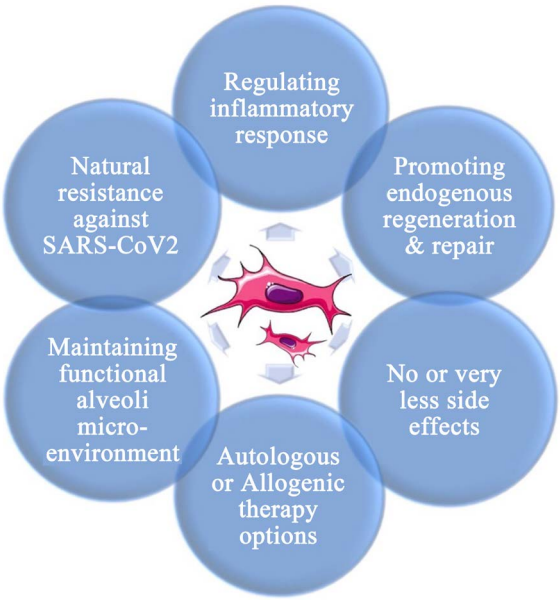


Figure 3. Role of MSCs in nCOVID-19 treatment.

pulmonary cells generally express ACE2 (Angiotensin-converting enzyme 2) receptor and TMPRSS2 (transmembrane serine protease 2) on their membrane.

SARS-CoV-2 uses this ACE2 for the entry and the TMPRSS2 serine protease aids in S protein priming. These two membrane proteins (ACE2 and TMPRSS2) mediate viral entry into cells and also the spread of virus within the host [46]. According to F. Qi *et al.*, the expression of ACE2 and TMPRSS2 are not only limited to pulmonary cells [47], they are widely expressed in the Cardiac, renal and hepatic tissues. Hence, patients with severe infection possibly along with dyspnoea also suffer from multiple organ failure, shock, heart failure, arrhythmias, and renal failure.

MSCs are bestowed with the property of not exhibiting ACE2. In addition, Z. Leng *et al.* have shown the absence of TMPRSS2 in MSC through single-cell transcriptome analysis [38]. By naturally lacking the key entry points, MSCs are immune to SARS-CoV-2 infection.

8. Clinical Trials on MSCs and nCOVID-19

A multicentric trial by Beijing 302 hospital (Trial no: NCT04252118) is expected to recruit 20 patients to evaluate the safety and functional outcome of umbilical cord mesenchymal stem cell therapy (UC-MSCs) for patients with nCOVID-19 pneumonia. A group of 10 patients will receive 3 intravenous infusion of UC-MSCs along with the conventional treatment and remaining 10 patients will receive only conventional treatment. The clinical, immunological, pulmonary functional and radiological outcomes will be evaluated during 180 days follow up period. The phase I trial researchers expected the potential beneficial effect of MSC based treatment could be principally due to the immunomodulation and regenerative potential of pulmonary parenchymal cells [48].

A clinical trial was started by Wuhan Union Hospital (Trial no: NCT04273646) to investigate effectiveness and safety of UC-MSCs in treating nCOVID-19 severe pneumonia patients. They recruited 48 patients, with 24 patients receiving 4 IV transfusion of 5.0×10^6 cells/kg of UC-MSCs along with conventional treatment. Another 24 patients receiving conventional treatment (in the form of supportive treatment) will serve as a control group. Based on their study protocol the patients will be followed up from 90 days to 96 weeks. These researchers proclaimed that UC-MSCs migrate to damaged tissues and exert strong anti-inflammatory & immunomodulatory functions to promote the repair, regeneration and rejuvenation and to resist necro-apoptosis and fibrosis of damaged pulmonary parenchymal tissues [49].

A clinical trial was started by the Puren Hospital (Trial no: NCT04293692) affiliated to Wuhan University of Science and Technology to explore the safety and efficacy of UC-MSCs therapy for coronavirus pneumonia patients. The researchers recruited 48 patients with 24 patients (study group) receiving 0.5×10^6 UC-MSCs /kg body weight IV treatment 4 times every alternate day along with conventional treatment and the other 24 patients (control group) receiving the

conventional treatment plus 4 times of placebo intravenously. In this ongoing trial, the efficacy of UC-MSCs will be evaluated within 24 hours and at 1, 2, 4, 8 weeks after treatment in terms of haematological and radiological improvement along with 28-days mortality of the patient [50]. The end results of this trial are yet to be published.

9. Discussion

Mesenchymal stem cells (MSCs) have self-renewal and multi-differential abilities. They are readily accessible and expandable in-vitro with exceptional genomic stability and few ethical issues, marking its importance in cellular therapy, regenerative medicine and tissue rejuvenation and repairment [51]. Tsai MS *et al.* reported immunomodulatory effects and differentiation abilities of mesenchymal stem cells [52]. The immunomodulatory effects of MSCs are triggered by the activation of toll like receptor in MSCs [53] [54]. Few pilot studies by Leng Z *et al.* [38] and Liang B *et al.* [55], using MSCs have produced promising outcome for patients reported positive for SARS-CoV-2 nucleic acid. By utilizing its reparative and immunomodulatory characteristics rightly, it can be a reliable therapeutic strategy, notably in the nCOVID-19 pandemic.

MSCs exert antimicrobial effect indirectly through coordination of the pro- and anti-inflammatory elements of the immune system or by increasing the phagocytotic activity in the microenvironment; and directly by the secretion of antimicrobial peptides & proteins (AMPs), and by the expression of molecules such as indoleamine 2,3-dioxygenase (IDO) and interleukin (IL)-17 [56]. AMPs-mediated cell killing occur by disrupting membrane integrity, by inhibiting protein, DNA or RNA synthesis, and by interacting with certain intracellular targets. Thus, MSC helps in curbing the microbial agent [57] [58].

Mesenchymal Stem Cell (MSC) therapy inhibit the exaggerated response of the immune system and promotes, regenerates and rejuvenates the microenvironment. After entering the systemic circulation, MSCs invade the lung parenchyma and exerts its action by improving the pulmonary micro alveolar structure and pulmonary compliance which further prevents pulmonary fibrosis [59]. After administration of IV MSCs, due to its immunosuppressive potential, there is a downregulation of proinflammatory cytokines and chemokines and induction of regulatory dendritic cells to the repaired tissue. The increased levels of IL-10 and VEGF promoted the pulmonary parenchymal repair and rejuvenation, which lead to the clinical and functional recovery of patients with severe nCOVID-19 pneumonia [60] [61].

Leng *et al.* (2020) conducted a pilot clinical study on MSC transplantation for 7 SARS-CoV-2 positive patients, among them one critically severe, four severe, and two non-severe. 1×10^6 clinical-grade MSCs per kilogram of weight was given as an intravenous dose to all the 7 patients. Extensive follow up on various parameters including pulmonary function, peripheral lymphocytes, and C-reactive proteins along with screening for SARS-CoV-2 nucleic acid, continued until 14

days after transplantation. The treatment significantly improved the health condition of the patients. Also the study showed that SARS-CoV-2 was not infecting MSCs. In nCOVID-19 affected individuals, MSCs downregulates the inflammatory response and promote tissue repair and regeneration [38].

Liang *et al.* (2020) treated a critically ill 65-year-old patient with allogeneic human umbilical cord MSC (hUCMSC). Three intravenous infusions of hUCMSC significantly improved the patient's condition. It was evident even after the first infusion of 5×10^7 hUCMSC, with no side effects reported [55].

Mesenchymal Stem Cells (MSCs), as a therapeutic option for nCOVID-19 affected individuals, can help in improving the lung compliance, curb off pneumonia and the agent factor causing the disease per se. The choice of mesenchymal stem cells has to be validated. Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) being allogenic and with limited availability, the option of autologous Bone Marrow Mesenchymal Stem Cells (BM-MSCs) and Adipose derived Mesenchymal stem cells (AD-MSCs), which are easily accessible in larger quantities as well as autologous in nature eliminating immunological concerns, can serve the purpose of regenerating pulmonary micro alveolar epithelium and meshwork, thus decreasing morbidity & mortality and improving the functional quality of life of nCOVID-19 affected individuals.

The evidence on Mesenchymal stem cell as a therapeutic option for nCOVID-19 patients is limited; more clinical trials have to be taken up with ethical consideration to prove the efficacy and safety of MSCs in combating nCOVID-19. Also, long term follow-up is required to validate the results and to prove the long term risk-benefits of MSCs on nCOVID-19 affected individuals.

10. Conclusion

To summarise, this review focuses on the MSCs as a novel therapeutic option in managing nCOVID-19 infected patients with severe pneumonia considering its safety and efficacy. The novel cellular therapy against severe novel coronavirus infection (nCOVID-19) renders autologous or allogenic MSCs as a therapeutic option to regulate inflammatory response, maintain functional alveoli microenvironment, promote endogenous regeneration and repair, and natural resistance against it with no or minimal side-effects (as shown in **Figure 2**). To emphasize, MSCs through its anti-inflammatory and immunomodulatory potential inhibit the overactivation of immune system and play a vital role in regeneration of the affected tissues, thus enhancing recovery. To add on, this therapeutic option of treating critically ill nCOVID-19 patients with MSCs is relatively non-invasive and inexpensive. Further studies are required in larger cohort to validate this modality of treatment.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviation

ACE—Angiotensin Converting Enzyme
AD-MSCs—Adipose Derived Mesenchymal Stem Cells
ARDS—Acute Respiratory Distress Syndrome
BM-MSCs—Bone Marrow Mesenchymal Stem Cells
MERS—Middle East Respiratory Syndrome-related coronavirus
MSC—Mesenchymal Stem Cells
SARS—Severe Acute Respiratory Syndrome
TMPRSS—Transmembrane Protease Serine
UC-MSCs— Umbilical Cord Mesenchymal Stem Cells
VEGF—Vascular Endothelial Growth Factor
WHO—World Health Organisation