Cell Transplantation
Volume 29: 1–9

DOI: 10.1177/0963689720952089
journals.sagepub.com/home/cll
SAGE

Immunomodulation and Regeneration Properties of Dental Pulp Stem Cells: A Potential Therapy to Treat Coronavirus Disease 2019

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic, originating from Wuhan, China, is known to cause severe acute respiratory symptoms. The occurrence of a cytokine storm in the lungs is a critical step in the disease pathogenesis, as it causes pathological lesions, pulmonary edema, and acute respiratory distress syndrome, potentially resulting in death. Currently, there is no effective treatment that targets the cytokine storm and helps regenerate the damaged tissue. Mesenchymal stem cells (MSCs) are known to act as anti-inflammatory/immunomodulatory candidates and activate endogenous regeneration. As a result, MSC therapy is a potential treatment approach for COVID-19. Intravenous injection of clinical-grade MSCs into COVID-19 patients can induce an immunomodulatory response along with improved lung function. Dental pulp stem cells (DPSCs) are considered a potential source of MSCs for immunomodulation, tissue regeneration, and clinical application. Although some current clinical trials have treated COVID-19 patients with DPSCs, this therapy has not been approved. Here, we review the potential use of DPSCs and their significance in the development of a therapy for COVID-19.

Keywords

dental pulp stem cells, COVID-19, coronavirus, cytokines storm, immunomodulation, regeneration

Introduction

A new infection emerged in Wuhan, China toward the end of 2019. This infection induced pneumonia and had clinical manifestations that were shortly recognized to be caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. This novel disease was named coronavirus disease 2019 (COVID-19) by the World Health Organization[2]. Over 13,500,000 cases and 580,000 deaths (https://www.worldometers.info/coronavirus/) have been reported worldwide, as of July 17, 2020. COVID-19 can be divided into three stages corresponding to different clinical manifestations, based on the pathological findings of infected respiratory tract data[3]. Under asymptomatic conditions, the virus binds to epithelial cells in the nasal cavity via the main receptor expressed in the respiratory epithelium, angiotensin-converting enzyme II (ACE2)[4,5]. At this stage, the infection rate is diagnosed using the polymerase chain reaction from nasal swab samples. By conducting the airway response stage, the virus spreads downward the respiratory tract, along the conducting airways, eliciting a vigorous innate immune response and exhibiting clinical symptoms. During stage 3, the virus infects the alveolar cells, which continue to undergo apoptosis, and the patient subsequently develops pulmonary infiltration, hypoxia, and very severe disease.

As reported, the immune-mediated inflammation markers, interleukin (IL)-2, -6, -7, monocyte chemoattractant protein-1, and tumor necrosis factor alpha (TNF-α), play a significant role in the pathogenesis of COVID-19[6,7]. Recruitment of these inflammatory cytokines into the lung tissues causes edema, lung dysfunction, and acute respiratory

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Submitted: June 9, 2020. Revised: July 17, 2020. Accepted: August 3, 2020.

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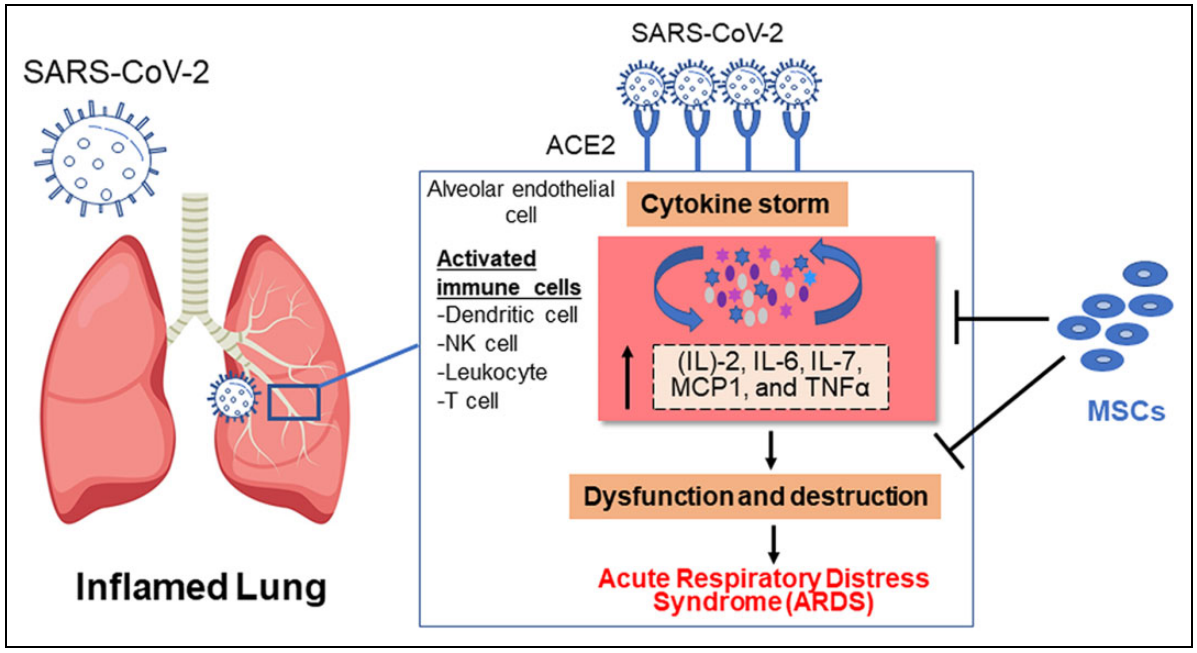

Figure 1. Release of a cytokine storm in response to SARS-CoV-2 and a potential mechanism of MSCs in the treatment of COVID-19 patients. Data are obtained from Refs[6,7,17,18]. COVID-19: coronavirus disease 2019; MSC: mesenchymal stem cell; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

distress syndrome (ARDS) (Fig. 1), which may lead to death[6]. The development of COVID-19 is accompanied by a decline in lymphocytes and a significant increase in neutrophil numbers[8]. The number of B cells, T cells, and natural killer (NK) cells decreases in patients with severe infection[8]. To date, hygiene measures and alleviation strategies have been directed at minimizing the transmission of the infection. Symptomatic and supportive treatments, oxygen supplementation, and mechanical ventilation are currently used to combat the disease. Mesenchymal stem cells (MSCs) are promising in cell-based therapies for many diseases[9] including infectious diseases such as avian influenza H9N2, infections by hepatitis viruses B and C, and human immunodeficiency virus (HIV) infection[10,11]. MSCs are thought to aid COVID-19 treatment through two strategies: (1) immunomodulation of immune cells, decreasing the inflammation[12], and (2) regeneration of the damaged lung tissues[13] (Fig. 1). A preliminary study described that the transplantation of MSCs was reliable and efficient in treating patients with COVID-19 with severe conditions[14]. MSCs can be isolated from different sources[9,15]. Dental pulp stem cells (DPSCs) are known for their unique immunomodulatory and regenerative effects[16]. In this article, we highlight DPSCs as a potential therapeutic option for patients with COVID-19.

MSCs Versus Non-cellular Treatments for COVID-19

Scientists are attempting to develop new potential therapeutic approaches to combat COVID-19. Some of these treatments involve the use of vaccines, monoclonal antibodies, peptides, small-molecule drugs, interferon-based therapies, protease inhibitors, and adjunctive medications[19]. However, some medications such as hydroxychloroquine and azithromycin are not recommended for use in the clinic, owing to reported toxicity and cardiovascular complications[20]. Remdesivir, an antiviral drug, was approved by the Food and Drug Administration for the treatment of COVID-19. However, a recent study showed that it only reduces the time of clinical improvement and that there was no significant difference when compared with a placebo group[19,21]. Lopinavir/Ritonavir or other protease inhibitors, used for HIV treatment, were also not suggested because of harmful pharmacodynamics and insignificant clinical trial outcomes[19]. Though interferons are beneficial in the treatment of SARS and Middle East Respiratory Syndrome, they lack efficacy against COVID-19[22]. Monoclonal antibodies were suggested as a therapeutic tool; however, they only provide protection against the early stage of the disease[23]. Adjunctive medications such as antimicrobial agents, corticosteroids, and ACE inhibitors are reported as a potential treatment; however, more clinical studies are required to clarify their mechanisms in COVID-19[24]. Hence, no pharmacological treatments efficiently stop disease progression.

Studies on the pathogenesis of COVID-19 suggest that a dysregulated immune response occurs, resulting in extreme inflammation and deadly ARDS[25] (Fig. 1). Thus, immunomodulation can be an efficient therapy. The safety and efficacy of MSCs have been presented in preclinical models of

ARDS[26], making them promising candidates for COVID-19. Moreover, the use of MSCs to treat patients with COVID-19 was safe and had no harmful effects[14]. In brief, MSCs represent a potential agent for treating COVID-19 because they can modulate the immune response and uphold the host immune system.

MSCs and Infectious Diseases

MSCs are multipotent stromal cells that originate from stromal tissues. They are fibroblast-like cells; adhere to plastic substrates; express different clusters of differentiation (cell surface markers) CD90, CD105, CD29, CD44, CD54, CD166, Stro-1, and MHC-I; and express the absence of hematopoietic markers CD45, CD31, CD11b, CD19, CD34, CD14, and HLA-DR[27,28]. Owing to their attractive cell biology and roles in the field of tissue engineering, MSCs have been extensively explored over the past 30 years[9]. MSCs have a pioneering characteristic to differentiate intrinsically and release several beneficial growth factors and cytokines. MSCs possess effective anti-inflammatory and immunomodulatory properties that can treat inflammatory and immune-mediated conditions[29]. They have been reported to be safe and efficient for clinical application and their mechanism of action is well established[30,31]. MSC preconditioning with proinflammatory cytokines licenses the immunosuppressive activity by inducing the expression of anti-inflammatory factors[32–34], reducing inflammation.

Owing to the above-mentioned potential, MSCs are promising agents for the treatment of different tissue disorders[9] and for the control of infectious diseases[11]. MSCs have antimicrobial properties that reduce acute lung injury (ALI) induced by bacterial infections[35]. MSCs improve the phagocytic function of host macrophages, leading to more effective eradication of bacteria[35]. Other reports also validate MSC therapy for malaria, a parasitic disease that causes severe destruction of red blood cells and anemia[36]. Gupta et al. demonstrated that transplantation of allogeneic hematopoietic stem cells that did not express CCR5, the receptor to enter target immunological cells, could effectively cure HIV[37]. Earlier studies further showed that hematopoietic stem cells could be engineered to target HIV-infected cells[38]. Additionally, bone marrow–derived MSCs (BMMSCs) recover liver function in hepatitis B by regulating the Treg/Th17 cell balance[39]. In the lung, MSCs and their derived extracellular vesicles were shown to reduce the levels of chemokines and proinflammatory cytokines, decreasing the migration of inflammatory cells into the lungs. This can be a promising approach to cure ALI, which is induced by influenza viruses[10,40]. Improved oxygenation and decreased pulmonary edema were shown following the transplantation of human MSCs in ARDS in a sheep model[41]. MSCs have also been suggested for the treatment of the H7N9 viral infection that has similar clinical effects on the lung as COVID-19[42]. As noted, there are various sources from which MSCs can be isolated. However, bone marrow[43] and adipose tissue[44] are the most common adult tissue sources for MSCs. As a result, the infusion of adipose tissue–derived MSCs into patients with COVID-19 could improve the clinical outcomes and immunomodulatory functions in severe cases[14]. Human umbilical cord Wharton's jelly-derived MSCs were used to treat patients with severe COVID-19[45]. Dental tissue–derived MSCs have also shown promise in tissue regeneration because of their potent capacities to differentiate into multiple lineages, immunomodulate, and secrete trophic factors. This collectively supports the use of MSCs as a potential treatment for COVID-19.

Immunomodulation and Regenerative Properties of DPSCs

DPSCs, isolated from the pulp tissue, are the most common form of dental tissue–derived MSCs. Other stem cells from exfoliated deciduous teeth (SHED), stem cells from apical papilla, periodontal ligament stem cells, and dental follicle precursor cells have also been reported[46]. DPSCs self-renew, are multipotent, and have unique biological properties that make them promising agents, capable of improving the endurance of affected tissues[16] (Fig. 2). DPSCs have an effective immunomodulatory function that is able to address a diverse set of autoimmune and inflammation-related diseases[47]. The immunomodulatory mechanisms associated with the release of soluble factors, such as indoleamine 2, 3-dioxygenase (IDO), prostaglandin E2 (PGE2), transforming growth factor-β (TGF-β), and human leukocyte antigen G5 (HLA-G5), and interfaces with immune cells such as B cells, T cells, dendritic cells, and macrophages are hallmarks of their function[48]. DPSCs promote high expression of trophic factors such as PGE2, IL-6, and IDO[49–51]. A mixed lymphocyte reaction assay further revealed the strong enhancement of the immunosuppression of the DPSC-conditioned medium (CM)[51]. DPSCs inhibit the function of proinflammatory M1 macrophages by inhibiting the secretion of TNF-α via the IDO-mediated pathway[52] (Fig. 2). They also polarize macrophages toward the anti-inflammatory M2 phenotype through increased secretion of IL-10, PGE2, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF)[53]. Furthermore, they have the capability to inhibit the activation of Th17 cells[54] and stimulate the growth of $CD25^+$, $CD4^+$, and $FoxP3^+$ regulatory T cells[55]. DPSCs also induce T cell apoptosis and inhibit the survival rate of NK cells and Th1[56], resulting in the reduced production of IFN-γ and IL-17 by Th1. At a level of humoral immunity, DPSCs hamper the proliferation, antibody production, and differentiation potential of B cells and inhibit the proliferation of allogeneic T and B cells by the release of TGF-β1[56]. This secretion of TGF-β can suppress the activation of human peripheral blood mononuclear cells[57].

DPSCs play an important role in tissue regeneration[58] and have the capability to repair different tissue disorders[16]. While BMMSCs are representative MSCs due to their high

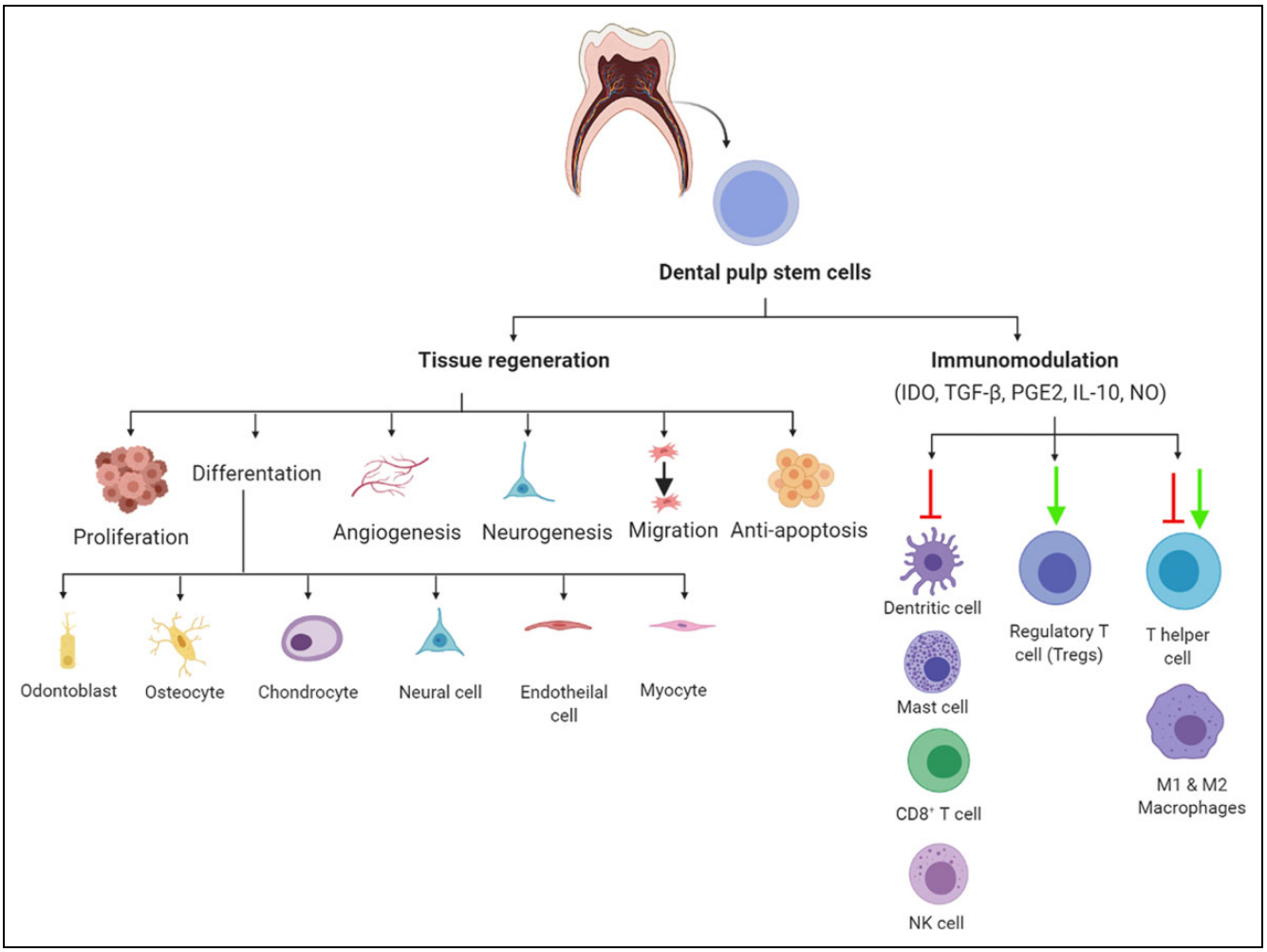

Figure 2. Schematic diagram of a potential mechanism of DPSCs in the treatment of COVID-19. Data are obtained from Refs[16,48,58]. Figure is made with biorender: https://biorender.com/. COVID-19: coronavirus disease 2019; DPSCs: dental pulp stem cells.

regenerative potential, DPSCs have higher angiogenic, neurogenic, and regenerative potential[59], exhibiting an alternative multipurpose stem cell source for cellular therapies (Fig. 2). Neovascularization in the ischemic hindlimb has been demonstrated after DPSC transplantation[60] (Fig. 3). The DPSC regeneration mechanisms via migration activity are achieved through modulating the response to granulocyte colony-stimulating factor (G-CSF), improving their regenerative potential[51]. Transplanted DPSCs produce a broad spectrum of cytokines and growth factors that modify neighboring cells. The success in restoring damaged tissues is in part related to the disclosure of paracrine factors in the host tissues. These paracrine effects promote the recruitment of progenitor cells, improve angiogenesis/neurogenesis, and modify the immune response[61]. The following paracrine factors are highly expressed in DPSCs, hepatocyte growth factor, vascular endothelial growth factor, insulin-like growth factor, fibroblast growth factor, macrophage colony-stimulating factor, stromal cell-derived factor 1, GM-CSF, G-CSF, and a small number of cytokines (IL-6, -8, -10)[61]. The release of trophic factors following DPSC transplantation induces pulp regeneration, but the DPSCs themselves are not incorporated into the new tissue[50]. DPSCs differentiate into different cell types such as cardiomyocytes, melanocytes, myocytes, neurons, and hepatocyte-like cells[16]. BMMSCs and adipose-derived stem cells (ADSCs) induce regenerated pulp tissue similar to DPSCs. The transplantation of DPSCs into pulpectomized teeth compared with BMMSCs or ADSCs resulted in the generation of a larger amount of pulp[59]. This can be explained by the increased release of trophic factors that promote angiogenesis, neurogenesis, anti-apoptosis, and chemotactic factors[49]. For these reasons, DPSCs are now considered as one of the best future sources of MSCs for use in regenerative medicine[16]. Thus, they are used to treat various tissue disorders, including dental, neurological, corneal, cardiovascular, hepatic, muscular dystrophy, pancreatic, and renal tissue diseases[16,62,63]. Moreover, the transplantation of allogeneic DPSCs (with

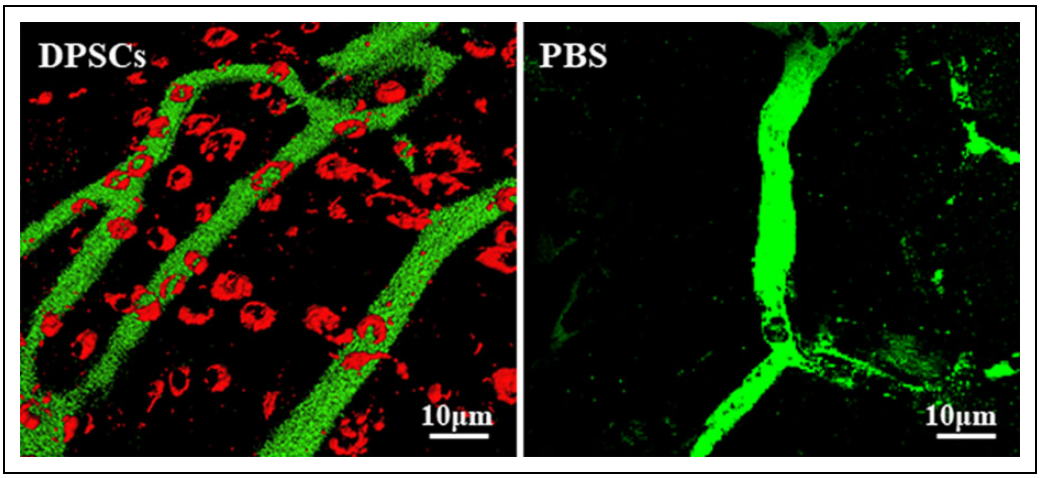

Figure 3. Neovascularization in ischemic hindlimb after transplantation of DPSCs. Three-dimensional confocal laser micrograph. Red: 1,1′-dioetadeeyl-3,3,3′.3′- tetramethylindocarboeyanine dioetadeeyl (DiI)-labeled DPSCs. Green: capillaries with labeling with fluorescein isothiocyanate dextran. Data are obtained from Iohara et al[60]. DPSCs: dental pulp stem cells.

matched and mismatched dog leukocyte antigen) into pulpectomized teeth of dogs regenerated pulp tissue in both matched and mismatched transplanted teeth. There was no toxicity or adverse event associated with the transplantation of DPSCs[64]. These results showed that DPSCs have high immunosuppressive, immunomodulatory, and tissue regeneration activities. Therefore, it is of interest in the development of COVID-19 treatment options (Fig. 2).

DPSCs and Lung Injury

Loss of alveolar structures and accumulation of inflammatory cells, followed by fibrosis, are the main characteristics of ARDS[65]. The activation of macrophages plays a role in the pathophysiology of ARDS[66], where M1 macrophages release proinflammatory cytokines and increase tissue fibrosis[67]. Therefore, managing macrophages is a valuable strategy to treat ARDS. Wakayama et al. showed that intravenous infusion of SHED and their CM were assessed in chemically induced ALI in a mouse model. The results showed that the survival rate and regeneration improved after the administration of either SHED or CM. Moreover, this treatment upregulated the anti-inflammatory effect by activating M2 macrophages[68]. In COVID-19, the reported clinical studies used the intravenous injection of MSCs as a satisfactory and less invasive method. Fortunately, MSCs injected intravenously are trapped mostly in the lungs, the most affected organ in COVID-19, and to a smaller extent in other tissues[69]. This suggests that intravenous administration of DPSCs will be localized to the lung tissues. Compared with other sources of MSCs, DPSCs exhibit higher potential for treating COVID-19 for the following reasons:

A. DPSCs can be easily isolated in a less invasive manner from discarded teeth and comply with ethical considerations[70].
B. DPSCs are abundantly available, are easy to harvest, and have effective therapeutic abilities[49,71,72].
C. *In vitro*, they show a high proliferative ability[73], provide sufficient cell numbers in a short period. Besides, demonstrated a multi-differentiation potential[46].
D. DPSCs and SHED have immunomodulatory functions similar to those of BMMSCs. Consequently, they are considered good candidates for cell-based approaches for immune- and inflammation-related diseases[48].

Even though the above-mentioned facts highlight the potential of DPSCs in combating COVID-19, all MSC sources have a restricted lifespan *in vitro* and lose their original characteristics with serial passaging. To overcome these challenges, immortalized DPSCs have been developed[74]. The piggyBac system, composed of mutant baculovirus strains derived from the cabbage looper moth *Trichoplusia ni*, was used to immortalize DPSCs[75]. Orimoto et al. transduced a mutant cyclin-dependent kinase 4 ($CDK4^{R24C}$), cyclin D1, and telomerase reverse transcriptase into DPSCs to establish an immortalized DPSC cell line with a high proliferative rate[74]. In addition, immortalized DPSCs using human telomerase reverse transcriptase were reported[76].

Clinical Trials of DPSCs in COVID-19 Patients

DPSCs are thought to fight against COVID-19 by preventing the cytokine storm through immunomodulation. Moreover, DPSCs can regenerate and reconstruct damaged tissues via reparative properties and effects of trophic factors (Fig. 2).

Table 1. The Clinical Trials of DPSCs on COVID-19 Patients.

Name of the study	Number of the study	Status	Reference
Safety and efficacy study of human allogeneic dental pulp mesenchymal stem cells to treat severe COVID-19 patients[77].	NCT04336254	Recruiting	https://clinicaltrials.gov/
Novel Coronavirus induced severe pneumonia treated by dental pulp mesenchymal stem cells.	NCT04302519	Not yet recruiting	https://clinicaltrials.gov/

COVID-19: coronavirus disease 2019; DPSCs: dental pulp stem cells.

Currently, there are many clinical trials using MSCs from different sources to treat COVID-19 (https://clinicaltrials.gov/). Two of the early recorded trials used DPSCs (Table 1). A protocol for a clinical trial by Ye et al. to assess the safety and efficiency of DPSCs from allogeneic donors in severe cases of COVID-19 was recently published[77]. The study reported on the safety and efficacy of DPSCs for the treatment of COVID-19. The time consumed for clinical improvement and the improvement of laboratory tests such as blood tests, liver and kidney functions, inflammatory markers, and immunological tests influenced the outcomes of the study[77]. However, the specific underlying mechanisms and outcomes of these trials are still not clear. Going forward, the appropriate cell dosage and concentration should be optimized to increase the efficacy and safety of DPSC-based COVID-19 therapies.

Conclusion

COVID-19 is a global pandemic and requires the simultaneous development of an effective therapy and vaccine. One of the most prominent findings in COVID-19 has been the presence of a cytokine storm in the lungs in severely impacted patients. MSCs are ACE2-negative at the gene level (improbable of getting infected) and have been extensively used in other immune-related diseases. MSCs, specifically DPSCs, can be great candidates for the treatment of this novel disease, owing to their ability to inhibit the release of cytokines through their immunomodulation and regenerative capacity.

Acknowledgments

We would like to thank Editage (www.editage.com) for English language editing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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