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# Mesenchymal Stem Cell Therapy for rheumatic diseases

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**Abstract:** Mesenchymal stem cell therapy (MSCT) is an innovative treatment for rheumatic diseases. Underlying mechanism of how MSCT works in rheumatic diseases are still uncertain and with various hypotheses. Animal studies in MSCT show conflicting results mainly attributed by the differences in administration methods of MSCT, types of MSC use and randomization procedures. Human studies of MSCT are so far small scale but with satisfactory results in patients with systemic lupus erythematosus (SLE). Human studies of MSCT, however, showed less rewarding results in patients with rheumatoid arthritis (RA) and systemic sclerosis (SSc). Larger scale studies are needed to confirm the efficiency of MSCT as well as the safety profile in human use.

**Keywords:** Mesenchymal Stem cells, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis

## 1 Introduction

Mesenchymal stem cell (MSC) is a multipotential non-haematopoietic progenitor cell that is able to differentiate into various cell lineages. MSC can differentiate into osteoblasts, chondrocytes, myocytes, adipose tissues, endothelial cells, neuron-like cells, cardiomyocytes and hepatocytes. MSCs lack expression of co-stimulatory molecules, are capable of escaping from alloantigen recognition and, hence, have low immunogenicity. This is why MSCs are candidate cells to prevent rejection in transplant therapy and can be a potential treatment option for rheumatic diseases.

There are various sources of MSCs. They can be obtained from both infant and adult tissues. Infant tissues mainly include umbilical cord (UC) tissues, cord blood cells and developing tooth buds of the mandibular third molar. MSCs can be derived from adult tissues as well, most commonly from the bone marrow, others from the umbilical cord, placenta, adipose tissue, muscles, peripheral blood, synovial fluid and articular cartilages. Infant tissues MSCs contain more multipotent primitive MSCs compared with adult tissues. They are able to differentiate into cells of all three germ layers, whilst the adult MSCs have restricted differentiation potential.

Detection of MSCs is mainly based on Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy minimal criteria (1). MSCs should have a long thin body with a large nucleus; have a fibroblast-like morphology; express on their surface CD73, CD90 and CD105; lack expression of CD11b, CD14, CD19, CD34, CD 45 or HLA-DR molecules; and possess differentiation capability. However, no single isolation method is regarded as the standard practice so the criteria allow only a retrospective classification of cultured cells as containing MSCs.

Functions of MSCs in vivo in normal individuals are not fully understood. There are multiple hypotheses for underlying functions of MSCs. MSCs can differentiate into osteoblasts, which are essential components of the bone marrow (BM) environment. Thus, MSCs can have regulatory role of haematopoietic development in bone marrow.

### 1.1 Mechanisms of MSCT in autoimmune diseases

There are various mechanisms of MSCs in regulation of autoimmune disease activity. First, MSCs can work as immunomodulators that can balance Th1/Th2 cells numbers and expressions. Second, MSCs promotes the production of Tregs, which suppress the activity of autoreactive T cells. Third, MSCs can differentiate into

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different cell types, for example, endothelial cells that may help reconstruct the structure of nephrons and improve renal function. At present, no data can confirm or refute these hypotheses.

## 2 MSCT in SLE

### 2.1 Mechanisms

There are multiple mechanisms of MSCT in the treatment of systemic lupus erythematosus (SLE). MSCs can increase the levels of IL-10, which is important for the Treg cell activation and function in the peripheral blood. UC-derived MSCs depress IL-4 levels, which can suppress antibody production. Patients with SLE usually suffer from osteoblastic niche deficiency in the bone marrow, in both patients and mouse models, compared with normal healthy subjects. MSCs can differentiate into osteoblasts that may regulate inflammatory reaction.

### 2.2 Timeline of MSCT development in SLE

Sun *et al.* (2) and Nie *et al.* (3) suggested that there were functional abnormalities in bone-marrow-derived MSCs in patients with SLE, compared with mice and age and sex-matched healthy individuals, respectively. As patients with SLE have defective bone-marrow-derived MSCs, allogenic rather than autologous MSC transplantation may be an effective treatment for SLE.

In order to show that autologous MSCT is useful in SLE, various animal studies had been performed, but they showed conflicting results. Schena *et al.* (4) suggested that mice with SLE, after intravenous infusion of bone-marrow-derived MSC, had no effect on autoantibody production, proteinuria or mortality, despite improvement in the histological features of the kidney. Youd *et al.* (5) concluded that, the use of allogenic bone-marrow-derived MSCs in mice with SLE worsened the disease activity, increased the level of autoantibody and proteinuria. In contrast, in Gu *et al.* (6) and Zhou *et al.* (7), after the use of UC-derived MSC and bone-marrow-derived MSC, respectively, SLE mouse models had clinical improvements, together with reduction in anti-double-stranded DNA (anti-ds DNA), proteinuria and creatinine levels.

On the other hand, human application of MSCT in patients with SLE, compared with animal studies, showed more rewarding results. In an initial small-scale human study in Carrion *et al.* (8), autologous bone-marrow-derived MSCs were administered intravenously

in two patients with SLE. It showed no clinical improvement in the disease activity, and renal flare was even diagnosed in one patient 4 months after MSCT. However, as the follow-up period was 14 months only, conclusion of inefficacy could not be drawn. Subsequent studies showed more promising results in MSCT. Sun *et al.* (9) demonstrated the success of MSCT in four patients with refractory SLE. Two of them had completely taken off cyclophosphamide after 6 months of MSCT use. All patients were able to tail down the daily steroid dose to less than 10 mg of prednisolone. Improvements in secondary outcomes such as significant reduction on the systemic lupus erythematosus disease activity index (SLEDAI) and proteinuria were observed in all patients at 12 months after MSCT. Liang *et al.* (10) and Sun *et al.* (11) illustrated the improvement in the disease activity in patients with SLE. In Liang *et al.*, 16 patients suffered from SLE with life-threatening organ involvement and who were refractory to treatment were enrolled. After MSCT, there was significant reduction in anti-ds DNA and anti-nuclear antibody (ANA) titers in 1 month and 3 months post-transplant. There was also significant improvement of renal function and urine protein excretion. Patients even had improvements in non-lupus-nephritis-related manifestations, for example, constitutional symptoms of fatigue, weight loss and low-grade fever, cutaneous lesions, arthralgia and refractory pancytopenia.

Subsequent human studies of MSCT in patients with refractory SLE guide our methods of application of MSCT in order to have an optimal response. In Wang *et al.* (12), 58 patients with refractory SLE were randomized into two groups, with 30 patients receiving single MSCT and 28 patients receiving double MSCT. In patients receiving double MSCT, the second MSCT was received a week after the first infusion of MSCs. Surprisingly, the rate of complete remission was higher in the single transplantation group compared with multiple transplantation group ( $p = 0.006$ ). On the other hand, there was no significant difference in the rate of disease relapse between single transplantation group and double transplantation group ( $p = 0.784$ ). This study highlighted that single MSCT would be the mode of future application in patients with SLE. In Wang *et al.* (13) and Wang *et al.* (14), larger number of subjects in multiple centres were recruited for intravenous bone marrow or umbilical cord MSCT. Both studies showed positive results with better survival rates and clinical responses. One important point to note in Wang *et al.* was that patients were randomized into two groups, one with and one without cyclophosphamide pre-treatment. There was no significant difference in complete clinical remission, SLEDAI score, proteinuria and serum albumin

level between the two groups. Hence, cyclophosphamide pre-treatment may not be necessary before MSCT.

Contrary to MSCT in patients with SLE, haematopoietic stem cell (HSC) transplantation was only once studied in patients with SLE. It not only caused significant clinical improvement but also with significant mortality and morbidity, for example, mucositis, transplantation-related infection and lung injury.

## 3 MSCT in RA

### 3.1 Mechanisms

In patients with rheumatoid arthritis (RA), there is influx of fibroblast-like synoviocytes (FLS) into the joints. FLS are considered to be the 'diseased' MSCs. They are involved in pannus formation, synovial inflammation and tissue damage. FLS are able to produce inflammatory cytokines that cause further joint destruction and suppress MSC repair function within the joint. Hence, they are considered to be pathogenic cells that contribute to chronicity and progression of RA.

MSCs are able to produce anti-inflammatory cytokines to reduce deleterious Th1/Th17 response and inhibit B cells function and differentiation. They can also reduce the expression of human leukocyte antigen DR (HLA-DR) and CD80 and CD 86 co-stimulatory molecules on antigen-presenting cells, which can help reduce synovial inflammation.

Apart from anti-inflammatory effect, MSCs can differentiate into different cell types, for example, osteoblasts for bone regeneration. Also, MSCs can produce osteoprotegerin, which can block receptor activator of nuclear factor-kappa B ligand (RANKL)-receptor activator of factor-kappa(RANK) interaction and, hence, inhibit osteoclastogenesis.

Current conventional disease modifying anti-rheumatic drugs (DMARDs) and biological DMARDs are able to suppress joint inflammation. In contrast to DMARDs, effective MSCT in patients with RA may help cartilage and joint regeneration in addition to inflammation suppression effect, which sounds more attractive.

### 3.2 Timeline of MSCT development in RA

Conflicting results were shown in MSCT in animal models. Some studies such as Augello *et al.* (15) and Gonzalez *et al.* (16) suggested that MSCT was able to prevent the incidence

and reduce the severity of arthritis in mice models. Both studies discussed that MSCs were not localized in the joints only and that intra-articular injection of MSCs was less effective than intraperitoneal route. Both studies had drawn a conclusion that clinical improvement might not be confined to the localized effect of MSCs in joints. In contrast, Djouad *et al.* (17), Chen *et al.* (18) and Schurgers *et al.* (19) were negative studies of MSCT in RA mice models. In Schurgers *et al.*, both intravenous and intraperitoneal route of MSC were administered but lack of response in CIA mice models was noted. One of the potential reasons for the conflicting results is that there were discrepancies in the sources of MSCs, tissues of origin, routes of administration and treatment regimens in various studies. Consequently, direct comparison of studies in mice models was not feasible.

In human studies, unfortunately, they also showed conflicting results in patients with RA. In Liang *et al.* (20), four patients with refractory RA received intravenous infusion of allogenic bone-marrow-derived or UC-derived MSCs. Refractory RA is defined as established RA with resistance to conventional DMARDs and at least one anti-tumour necrosis factor  $\alpha$  (anti-TNF $\alpha$ ). MSCT could only result in a partial and transient clinical improvement. In Wang *et al.* (21), a larger sample size was obtained, with 136 patients in treatment of intravenous injection of MSC with DMARDs and 36 patients in treatment of DMARDs without MSCs. It showed significant improvement in MSCs group in terms of clinical response and increase in the percentage of regulatory T cells in peripheral blood. Further studies will be needed for any conclusive evidence for the application of MSCT in patients with RA.

Another potential reason for conflicting results in MSCT studies in RA is that the effective mode of administration of MSCs is still unknown. A comparative study of MSCs showed that intra-articular MSCs were superior in cartilage formation compared with other modes of administration (22). In previous studies, intra-articular administration of MSCs had a low efficacy in patients with RA (23). A new delivery method of MSCs using nanofibre poly-lactic-co-glycolic acid (PLGA) was proposed (24). PLGA sheet was applied topically over joints. The pros of using PLGA were controlled biodegradability, low immunogenicity and that they were useful as a scaffold for the regeneration of bone defect and cell regeneration. Yet, the application of PLGA is still rudimentary and needs further animal and human studies. Other future perspectives of MSCT in patients with RA include identifying the subset of patients with RA responsive to MSCT and combination of MSCT and anti-TNF $\alpha$  therapy for theoretical synergistic effect.

### 3.3 MSCT in Systemic Sclerosis (SSc)

The main mechanism of MSCT in systemic sclerosis (SSc) is the inhibition of proliferation of T lymphocytes and B lymphocytes. BM-derived MSCs from patients with SSc appeared similar to those healthy individuals in their phenotype and capacity to differentiate into adipogenic and osteogenic lineages and similar immunosuppressive properties. However, patients with SSc have a diminished capacity to differentiate into endothelial progenitor cells and to contribute to vasculogenesis. In other words, theoretically, autologous MSCT may be useful in patients with SSc.

However, the use of MSCT in SSc is still preliminary. One case study suggested UC-derived MSCT in patients with SSc who had leg ulcers refractory to steroid, cyclophosphamide and plasma exchange treatment showed improvement in modified Rodnan Skin Score and health assessment questionnaire disability index score (25). Another case series, which included five patients treated with intravenous-bone-marrow-derived MSC showed the improvement in mRSS and ulcerations but no significant improvement in lung function, lung fibrosis and cardiac function (26).

In contrast to MSCT in patients with SSc, use of hematopoietic stem cells transplantation (HSCT) has been more rewarding. An international multi-centre, open-label phase III study, named Autologous Stem Cell Transplantation International Scleroderma (ASTIS) trial, enrolled 156 patients and it suggested that HSCT group patients had a significantly better long-term survival rate than the intravenous cyclophosphamide group (27).

## 4 Conclusion

MSCT in rheumatic diseases is getting more and more attention. Source of MSC varies, mainly bone marrow and umbilical cord. The route of administration is mainly intravenous, but intraperitoneal and topical are occasionally used. MSCT seems to be more rewarding in patients with refractory SLE, has mixed results in patients with RA and is not quite effective in patients with SSc. More multicenter, randomized controlled human studies should be performed in order to make a conclusion to the efficacy of MSCT in rheumatic diseases.

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