REVIEW

Therapeutic Potential of Mesenchymal Stem Cells for Postmastectomy Lymphedema: A Literature Review

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Upper limb lymphedema is one of the most common complications after breast cancer surgery and radiotherapy. Despite various physical therapy and surgical options available, the impaired lymph fluid drainage may be progressive due to lymphatic vascular insufficiency making treatment more difficulty. Stem cell therapy provides a promising alternative in the treatment of various chronic diseases. The wide applicability of cell therapy has been reviewed throughout literature. This review provides an overview of recent progress in the therapeutic effect of adult stem cells for primary and secondary lymphedema after breast surgery in preclinical studies and clinical cases. We start with a brief introduction about the pathophysiological mechanisms of postmastectomy lymphedema. Regarding existing treatments, we systematically summarize the benefits and limitations of recent progress. Because of their multidirectional differentiation potential and growth factor secretion, stem cell therapy shows promising results in the management of light to severe lymphedema. Increasing evidences have demonstrated a noticeable reduction in postmastectomy lymphedema and increased lymph-angiogenesis after specific stem cell therapy. Current data suggests that stem cell therapy in lymphedema treatment provides reversal of pathological reorganization associated with lymphedema progression. Finally, we propose potential strategies for overcoming the challenges in the development of multipotent progenitor cells for the treatment and prevention of lymphedema in clinical practice.

Breast cancer is the most common malignant tumor in the female population, and the incidence rate is noticeably increasing. The cure rate of breast cancer is gradually improving, but complications, such as upper limb lymphedema, remain a challenging problem. Postmastectomy lymphedema is currently the most common iatrogenic lymphedema in clinical practice. According to reports in the literature, in patients with axillary lymph node dissection, the incidence of upper limb lymphedema can reach up to 65%.[1] Postmastectomy lymphedema is a secondary lymphedema, which occurs due to surgical damage to the upper extremity lymphatic system. It is different from primary lymphedema, which is a rare inherited condition caused by lymphatic development pathology.

PATHOPHYSIOLOGICAL MECHANISMS OF POSTMASTECTOMY LYMPHEDEMA DEVELOPMENT

Physiological function of the lymphatics is to return proteinaceous fluid, including proteins, lipids, and water, from the interstitium to the intravascular space. Main pathological changes of postmastectomy lymphedema are the impaired lymph fluid drainage and lymphatic vascular insufficiency.[2] Disrupted outflow of lymphatic fluid in the upper extremity causes a cascade of pathological reorganization in tissue structure. The specific structure of the lymphatic vessels allows

for the transfer of large proteins and lipids into the lymph, as well as large amounts of interstitial fluid. The lymphatic system intakes the surplus interstitial component incapable of transfer into the venous system.[3] For normal functioning, the pressure in the lymphatic vessels should remain ≤ 0 mm H_2O. Axillary blockage and disruption of lymphatic outflow due to surgical intervention causes a significant increase in intralymphatic pressure.[4,5] The increased intralymphatic pressure reduces passive protein transport into the lymphatic system, leading to stable pathophysiological changes resulting in edema.

Increased intralymphatic pressure causes accumulation of protein-rich fluid in the extracellular matrix. Along with disruption of fluid outflow due to surgical excision of lymphatic tissue, this causes a co-aggravating pathological system to exist.[6] An increase in protein concentration causes a significant increase in intercellular fluid retention, which cannot be drained due to increase intralymphatic pressure and proximal lymphatic blockage. Secondary lymphedema can be classified into different stages based on clinical symptoms,[7] pathological changes,[8,9] and x-ray images,[10] see **Table 1**.

CURRENT TREATMENT APPROACHES

Early prevention and treatment of secondary lymphedema is highly important. Adipocytes in the patients

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Chen and Sinelnikov are both regarded as the first authors.

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Table 1 Different staging systems for clinic postmastectomy lymphedema

Staging system	Detailed information
Clinical stage system[7]	Stage 0: No clinical or morphological changes. Stage 1: 1A-No edema, with presence of lymphatic dysfunction. 1B-Mild edema, reversible with declivous position and night rest. Stage 2: Persistent edema that regresses only partially with declivous position and night rest. Stage 3: Persistent edema that continually becomes more severe (recurrent acute erysipeloid lymphangitis). Stage 4: Fibrotic lymphedema (with initial lymphostatic warts) and column-shaped limb. Stage 5: Severe clinical lymphedema (manifestation of elephantiasis) and heavy complications.
International Society of Lymphology[8]	Stage 0: Latent or subclinical lymphedema where swelling is not evident despite impaired lymphatic transport (ICG). It may exist months or years before overt edema occurs. Stage 1: Early accumulation of fluid relatively high in protein content that subsides with limb elevation. Pitting may occur. Stage 2: Pitting may or may not occur as tissue fibrosis develops. Limb elevation alone rarely reduces tissue swelling. Stage 3: Lymphostatic elephantiasis where pitting is absent. Trophic skin changes, such as acanthosis, fat deposits, and warty overgrowths, often develop.
Pathological progression stage[9]	Stage 0: Free transport of lymph, some compensatory changes, such as smooth muscle cell proliferation can be seen. Stage 1 Ectasis type: The microvascular networks gradually regress as progressive compensatory changes occur. In addition, the lymphatic vessel lumen is found to be dilated in the ectasis type, which is associated with an increase in endolymphatic pressure. Stage 2 Contraction type: Increase in smooth muscle cell proliferation and fibrosis resulting in thickening of the lymphatic vessel wall is observed. Stage 3 Sclerosis type: Decompensatory changes. As the lymphedema progresses, the lymphatics become irreversibly fibrotic, loose elasticity and the ability to move peristaltically to transport lymphatic fluid. Multiple blockages can be seen.
Lymphoscintigraphy stage[a10]	M. D. Anderson lymphedema classification based on indocyanine green (ICG)* lymphangiographic findings. Stage 1: Many patent lymphatic vessels, with minimal, patchy dermal backflow. Stage 2: Moderate number of patent lymphatic vessels, with segmental dermal backflow. Stage 3: Few patent lymphatic vessels, with extensive dermal backflow involving the entire arm. Stage 4: No patent lymphatic vessels seen, with severe dermal backflow involving the entire arm and extending to the dorsum of the hand.

[a]ICG images were used for staging the lymphedema into the linear or dermal back-flow pattern. The severity of lymphedema increased with ICG dermal back-flow from splash, stardust, to diffuse pattern.

with lymphedema display hypertrophic changes and have more collagen fiber deposits.[11–13] Because the circulation of lymphocytes and macrophages is blocked, adipose-derived stromal stem cells (ASCs) and M2 macrophages *in situ* declined in number within the lymphedematous adipose tissue. Cell-mediated immunity is weakened, whereas edematous skin is easily damaged, and the protein-rich medium is highly susceptible to secondary infection, causing even more dangerous complications.[14,15]

Current trends in lymphedema treatment include surgical and nonsurgical treatment methods. Nonsurgical treatment mainly includes physical therapy, pharmacological therapy, and other noninvasive methods. Patients receive such treatment for a long period with high costs, and the efficacy for patients with moderate and severe upper extremity lymphedema is limited. Although, traditional compression sleeve therapy (CST) is also effective for lymphedema reduction, it provides highly volatile results and requires constant monitoring, and does not assess the pathophysiological mechanisms in the underlying pathology.[16–18] The use of anti-inflammatory agents, such as pharmacological substances, cell infiltration, and physiotherapy, have shown positive impact on overall treatment outcome.[19–21] Surgical treatment includes vascularized lymph node transfer (VLNT) and lymphatic venous anastomosis (LVA).[22–24]

The principles behind VLNT and LVA are important in understanding the applicability of new treatment methods in postmastectomy lymphedema.[25,26] Increased interstitial fluid pressure in the surrounding tissue causes compensational opening of LVA, providing movement of lymph node into the circulatory system.[27] High intravascular pressure, exceeding interstitial pressure, causes blockage of lympho-venous communication in the lymph node.[28] This mechanism often occurs in patients with postmastectomy lymphedema due to venous hypertension from scar tissue in the axillary region. Therefore, an existing compensatory measure for fluid drainage is blocked and disease progression continues.

The pathophysiological mechanisms of lymphedema progression after a mastectomy show a lack of treatment aimed at specific physiological mechanisms of disease progression. Reconstruction of the lymphatic network to restore normal lymphatic circulation is the key to treating lymphedema. Surgical methods, including VLNT and LVA can effectively reduce local lymphedema but cannot completely cure lymphedema in the entire range of the affected limb. Surgical treatment can temporarily alleviate the patient's symptoms, but with the lymphatic reflux obstructed, the edema will still relapse.[29]

Through understanding the current approaches to lymphedema treatment, it is noticeable that multifactorial treatment of associated pathologies is required for management of

disease progression. All the pathophysiological components of lymphatic disease must be addressed for best treatment results. We believe that stem cell-based therapy can provide sufficient management of occurring physiological abnormalities in postmastectomy lymphedema.

THERAPEUTIC EFFECT OF STEM CELL IN LYMPHEDEMA

Stem cells are undifferentiated cells with self-renewal and multidirectional differentiation potential. The self-renewal of stem cells allows them to proliferate *in vitro* under suitable culture conditions. The multidirectional differentiation potential indicates that stem cells can be induced to differentiate into various types of somatic cells. These two characteristics enable stem cells to be used for cell replacement, tissue repair, organ regeneration, gene therapy, and other aspects, providing a new treatment method for many major diseases that are difficult to treat clinically.

Stem cells are classified into embryonic stem cells and adult stem cells according to different stages of development. Embryonic stem cell refers to the cell group inside the blastocyst when the fertilized egg divides and develops into a blastocyst. It has a higher level of development and can be differentiated into all somatic cells of the human body; it is an omnipotent stem cell. Adult stem cells exist in adult tissues, including hematopoietic stem cells (HSCs), bone marrow mesenchymal stem cells, urine stem cells, etc., and they are pluripotent or single-potent stem cells with low developmental grade.[30]

Advantages and properties of stem cells
From the perspective of lymphedema pathology, the lymphatic circulation network is destroyed, and the lymphatic canal wall structure is changed. Stem cells have a wide range of therapeutic effects in anti-inflammatory, antifibrosis, anti-oxidative stress, and promoting lymph vessel regeneration. We can take advantage of these properties by using stem cell transplantation to promote the regeneration of lymphatic vessels, rebuild lymphatic circulation, and successfully treat lymphedema. Preclinical (**Table 2**) and clinical (**Table 3**) outcomes have shown significant importance of stem cell activity in disease regression.

Stem cells derived from adipose tissue have a multipotent growth potential and have a specific set of characteristic effects, which can help manage lymphedema progression, and possibly reverse the pathological process. Stem cells possess anti-inflammatory effects to secrete biological factors to inhibit T cells in the lymphedema tissue.[31–33] Similarly, the antifibrotic effects of stem cells can potentially mediate lymphatic vessel structure recovery via preventing fibroblast overgrowth[34] by releasing from stem cells, reversing the core dysfunction of lymph vessels, and pathophysiological progression induced by accumulated lipoprotein compounds.[35–37]

The anti-oxidative stress capacity of stem cells provides another target in the treatment of lymphedema. Human mesenchymal stem cells (hMSCs) offer the profile of cytokine secretion of dendritic cells, T cells (naive and effector subtypes), natural killer cells, and other local immune response cells. The modified secretion profile exabits lower tumor-necrosis factor α, interferon γ levels, and increased interleukin-10, interleukin-4, and prostaglandin E_2. In addition, the anti-oxidative effect of hMSC increases the levels of regulatory T cell proliferation[38] and decreases chronic inflammation.[39,40] Thus, immunomodulation of stem cells positively inhabits the pathological chain of events in lymphedema by reduction of inflammation, fibrosis, and oxidative stress, as well as stimulation of vascular growth for lymphatic regeneration.

Stem cell therapy for primary lymphedema
According to the pathological principle of lymphedema, both primary and secondary lymphedema are caused by compensatory changes in the wall structure of the lymphedema.[17] Stem cell therapy provides alternative in the treatment of lymphedema (**Figure 1**).

GATA2-related disorders include the recently described congenital lymphedema (Emberger syndrome). GATA2 deficiency presents with a spectrum of phenotype manifestations, including increased sensitivity to viral and bacterial infections, cytopenias, myelodysplasia, myeloid leukemias, pulmonary alveolar proteinosis, and lymphedema.[41,42] Studies have shown that allogeneic stem cell transplantation in these cases remains the treatment of choice.[43] Rastogi *et al.* reported a case of Emberger syndrome with a GATA2 mutation in a 9-year-old girl who presented with lymphedema.[44] She received hematopoietic stem cell transplantation (HSCT) and had neutrophil engraftment on day 15 and full donor chimerism by day 30. She was disease free on day 475 after treatment. Ramzan *et al.* showed positive HSCT treatment results in a patient with GATA2 deficiency and lymphedema (Emberger syndrome).[45] Most patients with GATA2 anomaly died due to the development of acute myelinolysis or active infections. Saida with co-authors used reduced-intensity stem cell transplantation for GATA2 deficiency treatment to achieve positive outcome.[46] Lübking *et al.* described successful HSCT in the treatment of symptomatic B and NK lymphocytes deficiency (DCML).[47]

The rare "yellow nail syndrome" (YNS) can also cause lymphedema. Gregoire *et al.* treated two cases of YNS with HSCs. Onset or worsening of YNS symptoms followed graft-vs.-host disease (GVHD) manifestations. YNS after HSCT might be a microvascular manifestation of endothelial GVHD and corticosteroids prove to be an effective treatment.[48] Several cases and studies have shown that HSCT is feasible and safe in the treatment of primary lymphedema syndrome caused by gene deficiency.

Applying the knowledge behind successful treatment of primary lymphedema with stem cell technology can provide substantial insight into the application of these methods in postmastectomy lymphedema treatment.

Stem cell therapy for lymphedema
Understanding the detailed mechanisms governing lymphatic vessel formation and function in pathophysiologic conditions is essential to prevent and treat secondary lymphedema.[49] According to the advantages of the induced differentiation of stem cells, we can induce stem cells under the action of

Table 2 Preclinical outcomes of stem cell therapy for lymphedema

Cell type	Methods	Inducing factor	Outcomes	References
ASC	PRP and ASC stimulation of lymphangiogenesis in a murine tail lymphedema model	–	PRP and ASC showed to promote lymphangiogenesis and prevent lymphedema	52
	Treatment of mouse lymphedema model using ASC induced by VEGF-C factor	VEGF-3	ASC containing VEGF-C factor can efficiently generate LEC and promote the formation of a new lymphatic network	53,54
	Guide ASC to differentiate into LEC by Prox1 in a mouse for treatment secondary lymphedema	Prox1	Proix1 successfully induces ASC differentiation into stable LEC	56
hiPSC	CV and AF cells were reprogrammed to hiPSC treatment of lymphedema	Olycistronic lentiviral vector (hSTEMCCA-loxP) encoding	The successful reprogramming of both CV and AF cells into hiPSC	61

AF, amniotic fluid; ASC, adipose derived stem cells; CV, chorionic villus; hiPSC, human induced pluripotent stem cells; LEC, lymphatic endothelial cells; Prox1, Prospero homeobox protein 1; PRP, platelet-rich plasma; VEGF-C, vascular endothelial growth factor C.

Table 3 Clinical outcomes of human adult stem cell therapy

Cell type	Disease	Methods	Outcomes	References
HSCT	Lymphedema syndrome caused by GATA2 mutation	HSCT administrations alleviates GATA2 deficiency-related symptoms	GATA2 deficiency syndrome lymphedema manifestations are successfully treated with HSCT. Promising treatments results in postmastectomy lymphedema can be predicted.	41,44,45,46,47
	YNS symptoms follow lymphedema caused by GVHD	HSCT administrations alleviates YNS related symptoms	YNS after HSCT might be a microvascular manifestation of endothelial GVHD and corticosteroids prove to be an effective treatment	48
ASC	Patients (*n* = 10) with upper extremity lymphedema after breast surgery	ASC injected directly into the axillary region, which was combined with a scar-releasing fat graft procedure	No serious adverse events were observed in the 6-month follow-up period. Encouraging results in reducing lymphedema severity were achieved.	50
MSC	Patients (*n* = 40) with lower extremity lymphedema	20 cases received MSC therapy	MSC therapy can achieve reduction in limb circumference and pain relief and improvement in walking ability in patients with chronic lymphedema	60

ASC, adipose derived stem cells; GVHD, graft-vs.-host disease; HSCT, hematopoietic stem cell transplantation; MSC, mononuclear stem cells; YNS, yellow nail syndrome.
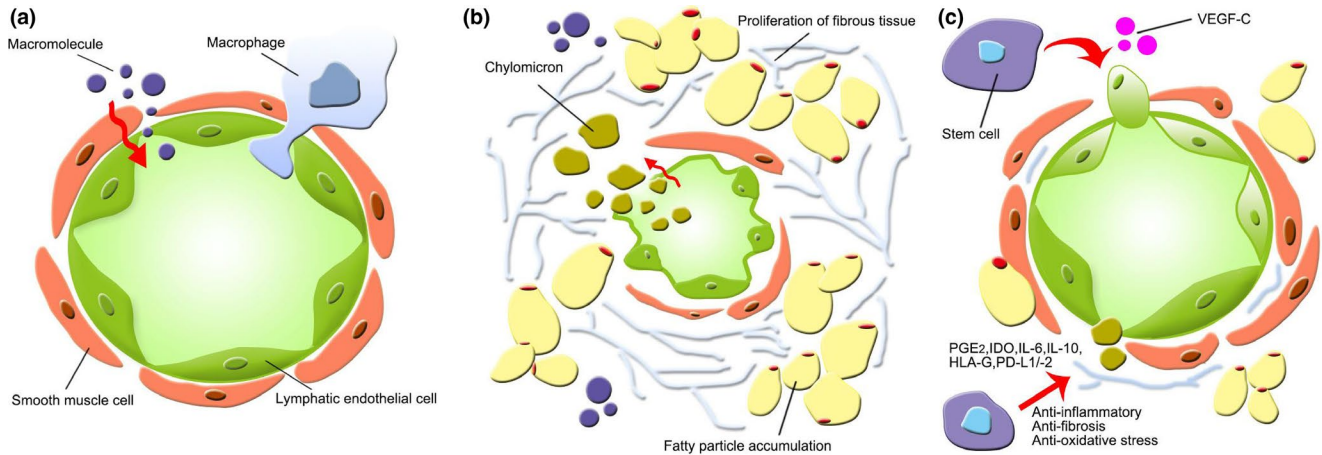
Figure 1 The pathology of lymphedema and strategy of stem cell therapy in the treatment of lymphedema. (**a**) Normal lymphatic vessel structure. (**b**) Pathological changes of tissues in lymphedema. (**c**) Therapeutic effect of stem cells.

induced factors. Lymphatic endothelial cells (LECs) can be induced and differentiated to prove neo-angiogenesis. This can lead to the formation of new lymphatic vessels, restructuration and normalization in lymphatic circulation, and the reconstruction of damaged lymphatic networks.

A single injection of ASCs improves the patient's condition based on patient-reported outcome and there are no serious adverse effects in secondary lymphedema treatment.[50,51] The study of Ackermann *et al.* on rats also showed that ASC-treated lymphedema, might represent a promising approach to improve regeneration of lymphatic vessels, and restore disrupted lymphatic circulation.[52]

Because ASCs have the property of being induced to differentiate, we can influence differentiation by experimental induction of ASCs. In the process of ASC differentiation to LECs, vascular endothelial growth factor C (VEGF-C) plays a crucial role and can be regarded as an essential factor. The ligand of VEGF-3, vascular endothelial growth factor receptor 3 (VEGFR-3) accelerates lymph-angiogenesis by binding to VEGF-C.[53,54]

Takeda *et al.* reviewed the expression of several lymph-angiogenic factors in ASCs. The authors examined by quantitative reverse transcription-polymerase chain reaction and enzyme-linked immunosorbent assay the effects of factors secreted by ASCs. These factors were shown to induce LEC proliferation, migration, and vessel formation more potently than recombinant human VEGF-C.[55] According to a study by Deng *et al.*, transplanted human LECs can help manage secondary lymphedema in an experimental animal model. The authors induced Prox1 overexpression in ASCs by using the transfection of lentiviral vectors to stimulate the differentiation of ASCs to LECs. The results showed that overexpression of Prox1 in ASCs successfully induced their differentiation into stable lymphatic endothelial cells. This study provides an interesting approach to secondary lymphedema by inducing lymphatic differentiation of human ASCs.[56] Authors Levi *et al.* obtained ASCs from an elderly patient with lymphedema and a healthy control patient. Subsequently, the ASCs were examined using single cell transcriptional analysis techniques.

Osteogenesis, adipogenesis, and angiogenesis gene expression and differentiation were assessed by quantitative real-time polymerase chain reaction and standard *in vitro* differentiation assays. The results showed varying transcription clusters of ASCs in both patients. Lymphedema-related stem cells had much higher adipogenesis gene expression and enhanced adipogenic differentiation. However, they had lower angiogenesis gene expression *in vitro*, with no significant changes in osteogenic differentiation ability. According to the results of this study, it can be concluded that ASCs in an affected limb appear to exhibit transcriptional profiles similar to those of abdominal ASCs.[57] Their adipogenic differentiation potential is greatly increased and their angiogenic capacity is impaired. This particular phenomenon suggests that the underlying pathophysiology of lymphedema drives the differentiation of adipose-derived stem cells into adipogenesis.

Most of the studies found on this topic are subject to bias and more preclinical studies and large-scale high-quality clinical trials are needed to show if this emerging therapy can satisfy expectations.[58,59] Overall, current data shows that ASCs can facilitate positive treatment results in patients with secondary lymphedema to a certain extent, more importantly, studies have shown that the potential pathological factors of lymphedema affect the differentiation of ASCs.

Other stem cells may also play a role in the treatment of lymphedema, but little research has been done. Mononuclear cells (MNCs) can potentially alleviate lymphedema through stimulation of lymph-angiogenesis.[60] Spitalieri *et al.* explored generation of human-induced pluripotent stem cells (hiPSCs) derived from autologous extra-embryonic fetal tissues. This innovative personalized regenerative technology can transform cells into embryonic stem-like cells. The authors used chorionic villus (CV) and amniotic fluid (AF) cells to demonstrate how they represent the ideal cellular resource for more efficient generation of hiPSCs. CV and AF cells were successfully reprogrammed to hiPSC by specific morphology, molecular, and immunocytochemical markers, and teratogenic

potential in *in vivo* experiments. Their data suggests that hiPSC-CV/AF can be considered an effective model to achieve significant progression in lymphedema pathogenesis research.[61]

Application of combined treatment methods for lymphedema

In situ adipogenesis from the host adipose-derived stem cells might accelerate lymphedema progression following the breast surgery. To minimize this problem, a new lymphatic pathway has to build up. A combination therapy of constructive surgery and stem cell implantation includes the surgery for rebuilding lymphatic networks and stem cells for regenerating lymphatic vessels and improving unhealthy microenvironment. After that, lymphangiography is used to monitor the lymph fluid dynamic changes to achieve the effective outcomes.

Combined treatment methods of VLNT with ASC transplantation show promising results. From the pathological point of view in upper limb lymphedema, the underlying cause is the destruction of the lymphatic network and blockage of lymphatic vessels after extensive lymph node dissection. Hayashida *et al.* experimentally induced lymphedema of the lower extremities in mice. The mice were divided into four groups: group 1 was treated with VLNT using an abdominal flap; group 2 was treated with ASC administration; group 3 received VLNT combined with ASC therapy; and group 4 received no treatment. Photodynamic imaging, using indocyanine green fluorescence, provided lymphatic flow assessment. A water-displacement plethysmometer provided volume assessment of the affected limb. Tissue quantification of lymphatic vessels, and functional analysis of lymphatic vessels and nodes was performed. The results showed better overall outcome in group 3 mice, treated with combined ASC and VLNT method ($P < 0.05$). Combined ASC and VLNT approaches could effectively decrease lymphedema volume and restore lymphatic function by lymphangiogenesis and the lympho-venous circulation route restoration in secondary lymphedema.[62]

Lymphoscintigraphy-assisted stem cell therapy is a promising method of targeted treatment. Peña Quián *et al.*[63] applied lymphoscintigraphy technology for stem cell implantation by multiple superficial and deep injections in the trajectory of the lymphatic vessels in the groin area for better understanding and evaluation of the anatomic function of the lymphatic system. Hadrian *et al.*[64] established various animal models to obtain insights into the pathophysiological mechanisms underlying lymphedema progression to evaluate the best treatment options. These results showed that lymphography modalities are essential for detecting the extent of lymphatic dysfunction and determining the appropriate treatment method.

Although adult stem cells are theoretically considered to differentiate into the targeted cells after being transplanted, no clear evidences demonstrate that stem cells in the body give rise into LEC. Labeling technology using green fluorescent protein, a fluorescent tracking reagent originally isolated from Aequorea Victoria, might track the fate of implanted stem cells and determine if stem cells reach the location of required differentiation. This could provide the feedback to improve the cell therapy methods in determining the optimal cell type, passages, concentrations, timing, and frequency of implantation, thereby increasing the treatment efficiency.[65]

Chen *et al.*[2] analyzed the existing data on human and animal application of stem cell treatment in current practice. The authors state that current knowledge and experimental data provide significant insight into the efficacy of stem cell therapy procedures, even with existing differences in animal modeling of lymphedema.[2] The early clinical trials provide encouraging treatment outcomes, which offers an alternative to solve tough clinical problems that has long remained unsolved. Further large-scale, randomized, clinical trials are required to develop evidence-based clinical recommendations on stem cell therapy in the management of postmastectomy lymphedema.

CONCLUSIONS

As treatment of upper limb lymphedema after breast cancer surgery is still in the exploratory stage, no unified treatment protocol is available. Pathophysiologically, the destruction of the corresponding lymphatic network during lymph node dissection is the main cause of lymphedema, resulting in lymphatic obstruction. As a result, the original pressure balance in the upper limb tissue is broken, leading the dilated and deformed lymphatic vessels, smooth muscle cell growth lymphatic concentrating dysfunction of the lymphatic vessels. With the development of regenerative medicine and tissue engineering, stem cells provide an effective way to rebuild a normal lymphatic network.[66] Stem cells possess regenerative potential to promote lymph tissue repair by cell differentiation and paracrine effects in anti-inflammatory, antifibrosis, anti-oxidative stress function, and stimulate lymphatic vessel regeneration. More extensive experimental and clinical studies are necessary to fully understand the effect of stem cell therapy on lymphedema treatment. The well-developed animal models of lymphedema are required to develop and to better understand the pathological progression and evaluate various treatments.[67] Understanding the cellular and molecular mechanisms and lymphatic fluid dynamic alternations will help optimize the stem cell therapy in the treatment of lymphedema after breast surgery.

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