**Name of Journal:** *World Journal of Stem Cells*

**Manuscript NO:** 53683

**Manuscript Type:** MINIREVIEWS

**Adipose-derived stem cell therapy shows promising results for secondary lymphedema**

Hu LR *et al*. ADSC therapy for secondary lymphedema

Li-Ru Hu, Jian Pan

**Li-Ru Hu,** State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

**Li Ru-Hu, Jian Pan,** Department of Oral and Maxillofacial Surgery, West China Hospital of Stomatology, Sichuan University, Chengdu 610041, Sichuan Province, China

**Author contributions:** Hu LR wrote the paper and collected the data; Pan J and Hu LR approved the final version to be published.

**Supported by** The Project of Cadre Institution of Sichuan Province, No. 2019-901; and The Project of Human Resources and Social Security Department Academic and Technical Leader Training Fund in Sichuan, No. 2017-A.

**Corresponding author: Jian Pan, PhD,** **Professor, Chief,** Oral and Maxillofacial Surgery, West China Hospital of Stomatology, Sichuan University, No. 14, Section 3, Renmin South Street, Chengdu 610041, Sichuan Province, China. jianpancn@163.com

**Received:** January 3, 2020

**Revised:** March 29, 2020

**Accepted:** June 2, 2020

**Published online:**

**Abstract**

Lymphedema is mainly identified by progressive soft tissue swelling in impaired lymphatic system. Secondary lymphedema attributed to cancer therapy, parasite infection, and trauma remains a serious global disease. Patients with lymphedema suffer swelling, pain, and fatigue, with the dysfunction of the deformed extremities reducing the quality of life and increasing the risk of infection and lymphangiosarcoma. Adipose-derived stem cells (ADSCs) possess prominent regenerative potential to differentiate into multilineage cells, and produce various lymphangiogenic factors, making ADSC therapy a promising approach for lymphedema. The development of lymphedema consists of local inflammation, the fibrosis of lymphatic vessels, and the deposition of adipose fat. Existing animal models do not mimic the chronic inflammation environment, therefore suitable models are required in further studies. Some signal pathways and molecular mechanisms in physiological and pathological lymphagiogenesis remain unclear. In previous animal and human trials, ADSC therapy reduced edema in varying degrees. A larger number of trials with larger samples and longer follow-up periods are required to verify the efficiency and feasibility of ADSC therapy. ADSCs are of easy availability and immune exemption, making them a candidate for lymphedema treatment. Whether ADSCs enhance malignant characteristics or trigger the malignant change deserves further exploration and study before ADSC therapy can be made widely available.

**Key words:** Secondary lymphedema; Adipose-derived stem cells; Lymphangiogenesis; Stem cells; Cell therapy; Lymphatic regeneration

Hu LR, Pan J. Adipose-derived stem cell therapy shows promising results for secondary lymphedema. *World J Stem Cells* 2020; In press

**Core tip:** Secondary lymphedema attributed to cancer therapy, parasite infection, and trauma remains a serious global disease. Adipose-derived stem cells (ADSCs) possess prominent regenerative potential to differentiate into multilineage cells, and produce various lymphangiogenic factors, making ADSC therapy a promising approach for lymphedema. However, suitable animal models are required for further studies and a larger number of clinical trials are necessary to verify the efficiency and feasibility of ADSC therapy. This review exhibits the pathophysiology of lymphedema and elaborates how ADSCs can improve lymphatic function.

**INTRODUCTION**

Secondary lymphedema remains a serious global disease with primary lymphedema due to genetic defects accounting for only a subset of those afflicted[1,2]. The common causes of secondary lymphedema are attributed to cancer therapy, parasite infection, and trauma[3]. Most sufferers are those with cancer who require radiotherapy or lymph node dissection[4]. For example, the development of breast cancer–related lymphedema results from axillary lymph node dissection, chemotherapy, radiotherapy, and postoperative complications[5]. Patients with lymphedema suffer swelling, pain, and fatigue, with the dysfunction of the deformed extremities reducing the quality of life and increasing the risk of infection and lymphangiosarcoma[4].

Conservative treatments include pharmacotherapy and physiotherapy (compression and lymphatic drainage massage), whilst surgical treatments include lymphaticovenular anastomosis, lymph node transfer, fluid drainage, and liposuction[6-8]. However, no effective treatments for lymphedema are available and impairment during surgical therapy can reversibly aggravate lymphedema.

In the last decade, cell-based therapies have emerged as a research hotspot due to their capacity to promote tissue regeneration. Mesenchymal stem cells (MSCs) are multipotent adult progenitor cells with favorably low immunogenicity and unique regenerative potential[9-11], making them a therapeutic option for lymphatic regeneration. MSCs include three major stem cell types, namely, bone marrow-derived MSCs (BM-MSCs), umbilical cord-MSCs (UC-MSCs), and adipose-derived stem cells (ADSCs). ADSCs have attracted increased attention due to their ease of accessibility, avoidable ethical concerns, and adequate sources. ADSCs grow stably[12] and produce various lymphangiogenic factors such as vascular endothelial growth factor C (VEGF-C), making ADSC therapy a promising approach for diseases of the lymphatic system[11].

In this review, we discuss the basic information regarding the pathophysiology of lymphedema including local inflammation, fibrosis, and the deposition of adipose tissue (AT). We discuss the development of lymphedema and outline how ADSCs can improve lymphatic function. Previous studies associated with ADSC-based therapy for the treatment of lymphedema are discussed, including both animal and human studies, with a specific focus on the outcomes of ADSC therapy in the clinic. The focus of this review is to explore the efficiency and feasibility of ADSC-based therapy. In addition, the future perspectives of ADSCs in the field of lymphatic regeneration are discussed.

**EMBRYONIC LYMPHANGIOGENESIS**

***Lymphatic endothelial cells specification***

Lymphatic specification can be observed from embryonic day 9.5 (E9.5) in a subset of cells in the walls of the cardinal veins. VEGF-C and its receptor vascular endothelial growth factor receptor 3 (VEGFR3) comprise the most essential signaling pathways during initial lymphatic development, in addition to lymphatic endothelial cell (LEC) proliferation, migration, and maintenance during early embryonic growth[13-15].

Prospero homeobox protein 1 (Prox1) determines the fate of differentiation[16,17], and is a master lymphatic transcription factor during cell proliferation and the maintenance of lymphatic integrity. In *Prox1* knockout mice, LEC budding is observed during the early stages of development, suggesting that the early expression of lymphatic vascular hyaluronan receptor - 1 (Lyve-1) together with that of Prox1 represents the first indication of lymphangiogenesis[18]. Coup-TFII directs the polarized expression of Prox1 in endothelial cells within the cardinal vein[19]. Notch signaling regulates normal lymphatic vessel patterning, the deletion of which leads to excessive Prox1+ LEC differentiation and lymphatic overgrowth[20]. LEC precursors migrate out of the vein under the control of the VEGF-C/VEGFR-3/Prox1 axis[21].

***Lymphatic sprouting growth***

Mature lymphatic structures are composed of capillaries, pre-collectors, and collecting vessels, and rely on the formation of lymph sacs and lymphatic plexuses. At approximately E10.5, Prox1 + LECs begin to migrate. At E11.5, VEGF-C/VEGFR-3 signaling stimulates lymph sac morphogenesis, and its hyperactivation leads to the overgrowth of lymph sacs[22].

***Lymphatic maturation***

Lymph sacs and lymphatic plexuses undergo further remodeling to form a functional lymphatic vessel network from E15.5 to early post-birth stages[23]. The formation of lymphatic valves, the recruitment of mural granulosa cells, and the deposition of the basement membrane are signs of maturity for collecting vessels. The transition of the LEC junctions from zippers to buttons characterizes the process of lymphatic capillary maturation[24].

**PATHOLOGICAL CHANGES DURING LYMPHEDEMA**

The pathophysiology of lymphedema remains poorly understood due to the lack of suitable animal models[25,26]. Rodent tails and hindlimb models fail to accurately recapitulate latent onset in the human body, which ranges from 3 mo to 3 years[27,28]. However, a positive feedback loop is widely accepted during lymphedema development, involving local inflammation, the fibrosis of lymphatic vessels, and the deposition of adipose fat[29,30]. Lymphedema is therefore chronic, potentially progressive, and irreversible.

***Local chronic inflammation and pathological lymphangiogenesis***

In animal models of lymphedema, the infiltration of lymphocytes and macrophages is observed. Lymphatic function can be improved through immunosuppressive drug targeting at T cells, including tacrolimus[31] and atorvastatin[27]. In contrast to acute inflammation, active T cells play an important role in the progressive development of lymphedema including neolymphatic vessel formation and fibrosis[4,32].CD4+ cells play a key role in impaired lymphangiogenesis and lymphatic dysfunction, whilst the depletion of CD8+ cells has minimal effects[33]. *CD4* knockout mice with acquired lymphedema exhibit lower levels of swelling and improved lymphatic function. The number of CD4+ cells is also positively associated with the severity of edema[4]. However, CD4+ T cells have different roles when cooperating with macrophages. Ogata *et al*[27] found that the addition of CD4+ T cells had no effect on tube formation. However, when CD4+ T cells were co-cultured with macrophages, new lymphatic tubes were observed. Macrophages are essential during lymphangiogenesis. Recent studies show that RAMP1 signaling accelerates lymphedema by inhibiting the recruitment of macrophages[34].

T cell-derived cytokines including interleukin (IL)-4, IL-13, IL-17, interferon gamma (IFN-γ), and transforming growth factor (TGF)-β1 negatively regulate lymphangiogenesis. *In vitro*, IL-4, IL-13, and IL-17 have been shown to inhibit LEC proliferation through the downregulation of LEC genes[35]. IL-4, IL-13, IL-17, and TGF-β1 participate in the development of fibrosis-related diseases[4]. IFN-γ and IL-17 activate macrophages through VEGF-C production during lymphangiogenesis in different disease models. IFN-γ and IL-17 can activate macrophages and enhance VEGF-C production during lymphangiogenesis of different disease models[27].

A chronic inflammatory environment initiates diverse lymphangiogenesis processes[32]. Lymphatic vessels do not reform spontaneously, and the remodeling of pre-existing vessels occurs, leading to the dilation of vessels and decreased contractile frequencies[36,37]. It remains unclear how VEGF-C/VEGFR-3 signaling regulates lymphedema-induced lymphangiogenesis.

***Pathological changes of adipose tissue***

A stable lymphatic system is critical for homeostasis, immunity, and lipid reabsorption[33]. Adipocytes are the main parenchymal cells in AT that contribute to energy storage and pathogen defense. Adipocytes are sensitive to pathological changes such as inflammation[36]. The accumulation of lymphatic fluid that contains free fatty acids can lead to fat deposition by activated adipocytes, upregulating fat differentiation genes[24,35,38]. Enhanced lipid storage leads to the hypertrophy and hyperplasia of adipocytes. In AT samples from lymphedema patients, a decrease in elastic fibers and an increase in collagen fibers are observed[30]. Active adipogenesis and fibrosis alter the physiological structure of AT.

ADSCs are multipotent cells with the potential to differentiate into multilineage cells including osteocytes, myocytes, chondrocytes, adipocytes, astrocytes, and endotheliocytes *in vitro* and *in vivo*[39]. However, significantly fewer stem cells exist in pathologic AT compared to normal AT. The differentiation potential of ADSCs to adipocytes can compensate for inadequate lipid storage capacity[40]. Lymphedema leads to the consumption of anti-inflammatory macrophages, which play an important role in the prevention of inflammation and tissue repair. Conversely, inflammatory macrophages are prevalent in hypertrophic AT[34].

**ADSC-BASED THERAPIES**

We searched PubMed, ClinicalTrials.gov, and EMBASE for published articles on lymphedema. “ADSCs”, “stem cell”, “cell therapy”, and “lymphedema” were used as the main search terms. All relevant studies performed from November 2009 to 2019 were selected. In total, five articles reported animal experiments (Table 1) and four reported human experiments (Table 2). In all studies, the location of lymphedema, cell origin, injection methods, evaluation methods, and the results were analyzed.

***Animal studies***

Mice are the only used animal models for lymphedema, which occurs in either the hindlimbs or tails as a result of circumferential incision. Irradiation is used as an auxiliary method with circumferential incision[41,42]. ADSCs are harvested from the AT of the same species from both intraabdominal and inguinal regions. Hwang *et al*[43] used ADSCs isolated from the human body, and complications related to rejection reactions were not observed. VEGF-C hydrogel sheets when applied to the injection site can reduce edema 3 to 4 d post-treatment. The reduction in the circumference and volume at the edema site following the injection of ADSCs occurs within 2-4 wk of treatment.

The dose of cells injected varied from 1 × 104 to 2 × 106, and all showed improved lymphatic function. Yoshida *et al*[42] divided mice into groups injected with 1 × 104, 1 × 105, and 1 × 106 ADSCs. The number of lymphatic vessels significantly increased at 2 wk in a dose dependent manner. Increased LYVE-1 expression with a treatment dose of 1 × 106 cells was significantly higher than that with treatment doses of 1 × 105 and 1 × 104 cells. Likewise, a higher dose of 1 × 105 showed significantly greater LYVE-1 expression than the dose of 1 × 104. Stem cells were subcutaneously injected at the site of lymphedema. Shimizu *et al*[44] showed that ADSCs stimulate lymphangiogenesis by secreting VEGF-C and through the recruitment of lymphatic endothelial progenitor cells. Ackermann *et al*[45] reported that ADSC therapy promotes lymphangiogenesis and lymphedema but to lower levels than platelet-rich plasma (PRP).

***Human studies***

Peña Quián *et al*[46] provided a case report on a patient with edematous lower limbs resulting from recurrent lymphangitis. Autologous ADSCs (1-2.2 × 109) were injected and a greater number of new lymphatic ramifications and lymph nodes were observed at 6 mo post-treatment *via* lymphoscintigraphy. Toyserkani *et al*[47] used autologous ADSCs in a patient suffering breast cancer–related lymphedema with deformed upper limbs. Fat grafting was performed at the same time. A total of eight axilla injections were performed at a total dosage of 4.0 × 107 cells. The time of follow-up was 4 mo, and positive outcomes were observed. The reduction of arm volume along with the decrease in heaviness and tension led to a lower requirement for compression therapy. In 2017, Toyserkani *et al*[48] enrolled ten patients to explore the feasibility and safety of ADSC therapy. Patients received the same treatment at a slightly higher dose of 5 × 107 cells. However, volume reduction was not significant after 6 mo of follow-up. Up to 50% of the patients reported an alleviation of their discomfort and had a lower requirement for conservative management. In 2019, Toyserkani *et al*[49] performed lymphoscintigraphic evaluations after one year of follow-up. No changes in arm volume and only mild transient complications related to liposuction were noted.

**CONCLUSION**

Stem cells can differentiate into multilineage cells that exist in nearly all tissues and organs. In theory, damaged tissues and organs can recover after stem cell implantation. Secondary lymphedema affects millions with sufferers experiencing persistent, uncomfortable, and dysfunctional extremities. However, existing therapies including conservative and surgical methods fail to improve lymphatic function.

ADSCs can be isolated from ATs by mildly invasive procedures. A prominent characteristic of ADSCs is their low immunogenicity, due to the low levels of expression of major histocompatibility complex (MHC) and costimulatory molecules[50]. ADSCs produce immunomodulatory cytokines including TGF-β that block IFN-γ-induced MHC expression[51]. The downregulation of MHC can avoid immune surveillance, producing immune-privileged cells ADSCs[52]. Complications relating to immune rejection are therefore sparse. ADSCs remain stable over long passages and can differentiate with low rates of apoptosis. As such, ADSC-based therapy may play an important role in secondary lymphedema. ADSCs can differentiate into progenitor cells for lymphangiogenesis and secrete VEGF-C. Both animal and human studies show positive outcomes after the injection of ADSCs with minimal complications. ADSC-based therapy is therefore promising for the treatment of secondary lymphedema.

However, some issues remain for ADSC-based therapy. Suitable animal models are required as pathological changes in acute inflammation differ from those of chronic inflammation, producing variable therapeutic outcomes. Second, a larger number of clinical trials with larger samples and longer follow-up periods are required. In addition, the safety of ADSC-based therapy should be assessed. In lung cancer models[53], ADSCs interact with LLC1 cells through their ability to secrete IL-6 and enhance malignant characteristics *in vitro* and *in vivo*. We believe that ADSC-based therapy is therefore key to the future treatment of secondary lymphedema.

**REFERENCES**

1 **Rochlin DH**, Inchauste S, Zelones J, Nguyen DH. The role of adjunct nanofibrillar collagen scaffold implantation in the surgical management of secondary lymphedema: Review of the literature and summary of initial pilot studies. *J Surg Oncol* 2020; **121**: 121-128 [PMID: 31209884 DOI: 10.1002/jso.25576]

2 **Grada AA**, Phillips TJ. Lymphedema: Pathophysiology and clinical manifestations. *J Am Acad Dermatol* 2017; **77**: 1009-1020 [PMID: 29132848 DOI: 10.1016/j.jaad.2017.03.022]

3 **Yang Y**, Yang JT, Chen XH, Qin BG, Li FG, Chen YX, Gu LQ, Zhu JK, Li P. Construction of tissue-engineered lymphatic vessel using human adipose derived stem cells differentiated lymphatic endothelial like cells and decellularized arterial scaffold: A preliminary study. *Biotechnol Appl Biochem* 2018; **65**: 428-434 [PMID: 28981171 DOI: 10.1002/bab.1618]

4 **Dayan JH**, Ly CL, Kataru RP, Mehrara BJ. Lymphedema: Pathogenesis and Novel Therapies. *Annu Rev Med* 2018; **69**: 263-276 [PMID: 28877002 DOI: 10.1146/annurev-med-060116-022900]

5 **Li L**, Yuan L, Chen X, Wang Q, Tian J, Yang K, Zhou E. Current Treatments for Breast Cancer-Related Lymphoedema: A Systematic Review *Asian Pac J Cancer Prev* 2016; **17**: 4875-4883 [PMID: 28030915 DOI: 10.22034/APJCP.2016.17.11.4875]

6 **Keeley V**. Advances in understanding and management of lymphoedema (cancer, primary). *Curr Opin Support Palliat Care* 2017; **11**: 355-360 [PMID: 28984676 DOI: 10.1097/SPC.0000000000000311]

7 **Hadamitzky C**, Zaitseva TS, Bazalova-Carter M, Paukshto MV, Hou L, Strassberg Z, Ferguson J, Matsuura Y, Dash R, Yang PC, Kretchetov S, Vogt PM, Rockson SG, Cooke JP, Huang NF. Aligned nanofibrillar collagen scaffolds - Guiding lymphangiogenesis for treatment of acquired lymphedema. *Biomaterials* 2016; **102**: 259-267 [PMID: 27348849 DOI: 10.1016/j.biomaterials.2016.05.040]

8 **Raju A**, Chang DW. Vascularized lymph node transfer for treatment of lymphedema: a comprehensive literature review. *Ann Surg* 2015; **261**: 1013-1023 [PMID: 24950271 DOI: 10.1097/SLA.0000000000000763]

9 **Toyserkani NM**, Christensen ML, Sheikh SP, Sørensen JA. Stem cells show promising results for lymphoedema treatment--a literature review. *J Plast Surg Hand Surg* 2015; **49**: 65-71 [PMID: 25272309]

10 **Lopez-Santalla M**, Mancheño-Corvo P, Escolano A, Menta R, DelaRosa O, Abad JL, Büscher D, Redondo JM, Bueren JA, Dalemans W, Lombardo E, Garin MI. Biodistribution and Efficacy of Human Adipose-Derived Mesenchymal Stem Cells Following Intranodal Administration in Experimental Colitis. *Front Immunol* 2017; **8**: 638 [PMID: 28642759 DOI: 10.3389/fimmu.2017.00638]

11 **Mazini L**, Rochette L, Amine M, Malka G. Regenerative Capacity of Adipose Derived Stem Cells (ADSCs), Comparison with Mesenchymal Stem Cells (MSCs). *Int J Mol Sci* 2019; **20**: 2523 [PMID: 31121953 DOI: 10.3390/ijms20102523]

12 **Shi YY**, Nacamuli RP, Salim A, Longaker MT. The osteogenic potential of adipose-derived mesenchymal cells is maintained with aging. *Plast Reconstr Surg* 2005; **116**: 1686-1696 [PMID: 16267433 DOI: 10.1097/01.prs.0000185606.03222.a9]

13 **Bower NI**, Vogrin AJ, Le Guen L, Chen H, Stacker SA, Achen MG, Hogan BM. Vegfd modulates both angiogenesis and lymphangiogenesis during zebrafish embryonic development. *Development* 2017; **144**: 507-518 [PMID: 28087639 DOI: 10.1242/dev.146969]

14 **Le Guen L**, Karpanen T, Schulte D, Harris NC, Koltowska K, Roukens G, Bower NI, van Impel A, Stacker SA, Achen MG, Schulte-Merker S, Hogan BM. Ccbe1 regulates Vegfc-mediated induction of Vegfr3 signaling during embryonic lymphangiogenesis. *Development* 2014; **141**: 1239-1249 [PMID: 24523457 DOI: 10.1242/dev.100495]

15 **Watanabe C**, Matsushita J, Azami T, Tsukiyama-Fujii S, Tsukiyama T, Mizuno S, Takahashi S, Ema M. Generating Vegfr3 reporter transgenic mouse expressing membrane-tagged Venus for visualization of VEGFR3 expression in vascular and lymphatic endothelial cells. *PLoS One* 2019; **14**: e0210060 [PMID: 30601868 DOI: 10.1371/journal.pone.0210060]

16 **Kivelä R**, Salmela I, Nguyen YH, Petrova TV, Koistinen HA, Wiener Z, Alitalo K. The transcription factor Prox1 is essential for satellite cell differentiation and muscle fibre-type regulation. *Nat Commun* 2016; **7**: 13124 [PMID: 27731315 DOI: 10.1038/ncomms13124]

17 **François M**, Caprini A, Hosking B, Orsenigo F, Wilhelm D, Browne C, Paavonen K, Karnezis T, Shayan R, Downes M, Davidson T, Tutt D, Cheah KS, Stacker SA, Muscat GE, Achen MG, Dejana E, Koopman P. Sox18 induces development of the lymphatic vasculature in mice. *Nature* 2008; **456**: 643-647 [PMID: 18931657 DOI: 10.1038/nature07391]

18 **Deng J**, Dai T, Sun Y, Zhang Q, Jiang Z, Li S, Cao W. Overexpression of Prox1 Induces the Differentiation of Human Adipose-Derived Stem Cells into Lymphatic Endothelial-Like Cells In Vitro. *Cell Reprogram* 2017; **19**: 54-63 [PMID: 28055225 DOI: 10.1089/cell.2016.0038]

19 **Semo J**, Nicenboim J, Yaniv K. Development of the lymphatic system: new questions and paradigms. *Development* 2016; **143**: 924-935 [PMID: 26980792 DOI: 10.1242/dev.132431]

20 **Murtomaki A**, Uh MK, Choi YK, Kitajewski C, Borisenko V, Kitajewski J, Shawber CJ. Notch1 functions as a negative regulator of lymphatic endothelial cell differentiation in the venous endothelium. *Development* 2013; **140**: 2365-2376 [PMID: 23615281 DOI: 10.1242/dev.083865]

21 **Gauvrit S**, Villasenor A, Strilic B, Kitchen P, Collins MM, Marín-Juez R, Guenther S, Maischein HM, Fukuda N, Canham MA, Brickman JM, Bogue CW, Jayaraman PS, Stainier DYR. HHEX is a transcriptional regulator of the VEGFC/FLT4/PROX1 signaling axis during vascular development. *Nat Commun* 2018; **9**: 2704 [PMID: 30006544 DOI: 10.1038/s41467-018-05039-1]

22 **Urner S**, Planas-Paz L, Hilger LS, Henning C, Branopolski A, Kelly-Goss M, Stanczuk L, Pitter B, Montanez E, Peirce SM, Mäkinen T, Lammert E. Identification of ILK as a critical regulator of VEGFR3 signalling and lymphatic vascular growth. *EMBO J* 2019; **38**: e99322 [PMID: 30518533 DOI: 10.15252/embj.201899322]

23 **Vittet D**. Lymphatic collecting vessel maturation and valve morphogenesis. *Microvasc Res* 2014; **96**: 31-37 [PMID: 25020266 DOI: 10.1016/j.mvr.2014.07.001]

24 **Jiang X**, Nicolls MR, Tian W, Rockson SG. Lymphatic Dysfunction, Leukotrienes, and Lymphedema. *Annu Rev Physiol* 2018; **80**: 49-70 [PMID: 29029593 DOI: 10.1146/annurev-physiol-022516-034008]

25 **Park HS**, Jung IM, Choi GH, Hahn S, Yoo YS, Lee T. Modification of a rodent hindlimb model of secondary lymphedema: surgical radicality versus radiotherapeutic ablation. *Biomed Res Int* 2013; **2013**: 208912 [PMID: 24350251 DOI: 10.1155/2013/208912]

26 **Hadrian R**, Palmes D. Animal Models of Secondary Lymphedema: New Approaches in the Search for Therapeutic Options. *Lymphat Res Biol* 2017; **15**: 2-16 [PMID: 28128668 DOI: 10.1089/lrb.2016.0015]

27 **Ogata F**, Fujiu K, Matsumoto S, Nakayama Y, Shibata M, Oike Y, Koshima I, Watabe T, Nagai R, Manabe I. Excess Lymphangiogenesis Cooperatively Induced by Macrophages and CD4(+) T Cells Drives the Pathogenesis of Lymphedema. *J Invest Dermatol* 2016; **136**: 706-714 [PMID: 27015456 DOI: 10.1016/j.jid.2015.12.001]

28 **Chen CE**, Chiang NJ, Perng CK, Ma H, Lin CH. Review of preclinical and clinical studies of using cell-based therapy for secondary lymphedema. *J Surg Oncol* 2020; **121**: 109-120 [PMID: 31385308 DOI: 10.1002/jso.25661]

29 **Sierla R**, Dylke ES, Kilbreath S. A Systematic Review of the Outcomes Used to Assess Upper Body Lymphedema. *Cancer Invest* 2018; **36**: 458-473 [PMID: 30289283 DOI: 10.1080/07357907.2018.1517362]

30 **Tashiro K**, Feng J, Wu SH, Mashiko T, Kanayama K, Narushima M, Uda H, Miyamoto S, Koshima I, Yoshimura K. Pathological changes of adipose tissue in secondary lymphoedema. *Br J Dermatol* 2017; **177**: 158-167 [PMID: 28000916 DOI: 10.1111/bjd.15238]

31 **Gardenier JC**, Kataru RP, Hespe GE, Savetsky IL, Torrisi JS, Nores GD, Jowhar DK, Nitti MD, Schofield RC, Carlow DC, Mehrara BJ. Topical tacrolimus for the treatment of secondary lymphedema. *Nat Commun* 2017; **8**: 14345 [PMID: 28186091 DOI: 10.1038/ncomms14345]

32 **Liao S**, von der Weid PY. Inflammation-induced lymphangiogenesis and lymphatic dysfunction. *Angiogenesis* 2014; **17**: 325-334 [PMID: 24449090 DOI: 10.1007/s10456-014-9416-7]

33 **Ly CL**, Kataru RP, Mehrara BJ. Inflammatory Manifestations of Lymphedema. *Int J Mol Sci* 2017; **18**: 171 [PMID: 28106728 DOI: 10.3390/ijms18010171]

34 **Mishima T**, Ito Y, Nishizawa N, Amano H, Tsujikawa K, Miyaji K, Watanabe M, Majima M. RAMP1 signaling improves lymphedema and promotes lymphangiogenesis in mice. *J Surg Res* 2017; **219**: 50-60 [PMID: 29078910 DOI: 10.1016/j.jss.2017.05.124]

35 **Hespe GE**, Nores GG, Huang JJ, Mehrara BJ. Pathophysiology of lymphedema-Is there a chance for medication treatment? *J Surg Oncol* 2017; **115**: 96-98 [PMID: 27566412 DOI: 10.1002/jso.24414]

36 **Cucchi F**, Rossmeislova L, Simonsen L, Jensen MR, Bülow J. A vicious circle in chronic lymphoedema pathophysiology? An adipocentric view. *Obes Rev* 2017; **18**: 1159-1169 [PMID: 28660651 DOI: 10.1111/obr.12565]

37 **Mauri C**, Wang G, Schulte-Merker S. From fish embryos to human patients: lymphangiogenesis in development and disease. *Curr Opin Immunol* 2018; **53**: 167-172 [PMID: 29800868 DOI: 10.1016/j.coi.2018.05.003]

38 **Chakraborty A**, Barajas S, Lammoglia GM, Reyna AJ, Morley TS, Johnson JA, Scherer PE, Rutkowski JM. Vascular Endothelial Growth Factor-D (VEGF-D) Overexpression and Lymphatic Expansion in Murine Adipose Tissue Improves Metabolism in Obesity. *Am J Pathol* 2019; **189**: 924-939 [PMID: 30878136 DOI: 10.1016/j.ajpath.2018.12.008]

39 **Megaloikonomos PD**, Panagopoulos GN, Bami M, Igoumenou VG, Dimopoulos L, Milonaki A, Kyriakidou M, Mitsiokapa E, Anastassopoulou J, Mavrogenis AF. Harvesting, Isolation and Differentiation of Rat Adipose-Derived Stem Cells. *Curr Pharm Biotechnol* 2018; **19**: 19-29 [PMID: 29667552 DOI: 10.2174/1389201019666180418101323]

40 **Liu L**, Mei M, Yang S, Li Q. Roles of chronic low-grade inflammation in the development of ectopic fat deposition. *Mediators Inflamm* 2014; **2014**: 418185 [PMID: 25143667 DOI: 10.1155/2014/418185]

41 **Hayashida K**, Yoshida S, Yoshimoto H, Fujioka M, Saijo H, Migita K, Kumaya M, Akita S. Adipose-Derived Stem Cells and Vascularized Lymph Node Transfers Successfully Treat Mouse Hindlimb Secondary Lymphedema by Early Reconnection of the Lymphatic System and Lymphangiogenesis. *Plast Reconstr Surg* 2017; **139**: 639-651 [PMID: 28234840 DOI: 10.1097/PRS.0000000000003110]

42 **Yoshida S**, Hamuy R, Hamada Y, Yoshimoto H, Hirano A, Akita S. Adipose-derived stem cell transplantation for therapeutic lymphangiogenesis in a mouse secondary lymphedema model. *Regen Med* 2015; **10**: 549-562 [PMID: 26237700 DOI: 10.2217/rme.15.24]

43 **Hwang JH**, Kim IG, Lee JY, Piao S, Lee DS, Lee TS, Ra JC, Lee JY. Therapeutic lymphangiogenesis using stem cell and VEGF-C hydrogel. *Biomaterials* 2011; **32**: 4415-4423 [PMID: 21421266 DOI: 10.1016/j.biomaterials.2011.02.051]

44 **Shimizu Y**, Shibata R, Shintani S, Ishii M, Murohara T. Therapeutic lymphangiogenesis with implantation of adipose-derived regenerative cells. *J Am Heart Assoc* 2012; **1**: e000877 [PMID: 23130156 DOI: 10.1161/JAHA.112.000877]

45 **Ackermann M**, Wettstein R, Senaldi C, Kalbermatten DF, Konerding MA, Raffoul W, Erba P. Impact of platelet rich plasma and adipose stem cells on lymphangiogenesis in a murine tail lymphedema model. *Microvasc Res* 2015; **102**: 78-85 [PMID: 26365474 DOI: 10.1016/j.mvr.2015.09.001]

46 **Peña Quián Y**, Hernández Ramirez P, Batista Cuellar JF, Perera Pintado A, Coca Pérez MA. Lymphoscintigraphy for the assessment of autologous stem cell implantation in chronic lymphedema. *Clin Nucl Med* 2015; **40**: 217-219 [PMID: 25549344 DOI: 10.1097/RLU.0000000000000688]

47 **Toyserkani NM**, Jensen CH, Sheikh SP, Sørensen JA. Cell-Assisted Lipotransfer Using Autologous Adipose-Derived Stromal Cells for Alleviation of Breast Cancer-Related Lymphedema. *Stem Cells Transl Med* 2016; **5**: 857-859 [PMID: 27151914 DOI: 10.5966/sctm.2015-0357]

48 **Toyserkani NM**, Jensen CH, Andersen DC, Sheikh SP, Sørensen JA. Treatment of Breast Cancer-Related Lymphedema with Adipose-Derived Regenerative Cells and Fat Grafts: A Feasibility and Safety Study. *Stem Cells Transl Med* 2017; **6**: 1666-1672 [PMID: 28653440 DOI: 10.1002/sctm.17-0037]

49 **Toyserkani NM**, Jensen CH, Tabatabaeifar S, Jørgensen MG, Hvidsten S, Simonsen JA, Andersen DC, Sheikh SP, Sørensen JA. Adipose-derived regenerative cells and fat grafting for treating breast cancer-related lymphedema: Lymphoscintigraphic evaluation with 1 year of follow-up. *J Plast Reconstr Aesthet Surg* 2019; **72**: 71-77 [PMID: 30293963 DOI: 10.1016/j.bjps.2018.09.007]

50 **De Miguel MP**, Fuentes-Julián S, Blázquez-Martínez A, Pascual CY, Aller MA, Arias J, Arnalich-Montiel F. Immunosuppressive properties of mesenchymal stem cells: advances and applications. *Curr Mol Med* 2012; **12**: 574-591 [PMID: 22515979 DOI: 10.2174/156652412800619950]

51 **Berglund AK**, Fortier LA, Antczak DF, Schnabel LV. Immunoprivileged no more: measuring the immunogenicity of allogeneic adult mesenchymal stem cells. *Stem Cell Res Ther* 2017; **8**: 288 [PMID: 29273086 DOI: 10.1186/s13287-017-0742-8]

52 **Berglund AK**, Fisher MB, Cameron KA, Poole EJ, Schnabel LV. Transforming Growth Factor-β2 Downregulates Major Histocompatibility Complex (MHC) I and MHC II Surface Expression on Equine Bone Marrow-Derived Mesenchymal Stem Cells Without Altering Other Phenotypic Cell Surface Markers. *Front Vet Sci* 2017; **4**: 84 [PMID: 28660198 DOI: 10.3389/fvets.2017.00084]

53 **Jian M**, Qingfu Z, Yanduo J, Guocheng J, Xueshan Q. Anti-lymphangiogenesis effects of a specific anti-interleukin 7 receptor antibody in lung cancer model in vivo. *Mol Carcinog* 2015; **54**: 148-155 [PMID: 24115038 DOI: 10.1002/mc.22082]

**Footnotes**

**Conflict-of-interest statement:** Theauthors declare no conflict of interests for this article.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Corresponding Author's Membership in Professional Societies:** Vice Chairman of the Dental and Alveolar Surgery Professional Committee, Chinese Stomatological Association.

**Peer-review started:** January 3, 2020

**First decision:** February 19, 2020

**Article in press:**

**Specialty type:** Cell and tissue engineering

**Country/Territory of origin:** China

**Peer-review report’s scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P- Reviewer:** Grawish M, Tanabe S **S- Editor:** Wang JL **L- Editor:** Wang TQ **E- Editor:**

**Table 1 Adipose-derived stem cell-based therapy for secondary lymphedema in animals**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Year** | **Ref.** | **Animal number** | **Edema site** | **Lymphoedema modeling** | **ADSC origin** | **Cell dose** | **Injection method** | **Auxiliary treatment**  | **Valuation criteria** | **Result(s)** |
|
| 2011 | Hwang *et al*[43] | *n* = 5 (5 groups) | Hindlimb | Circumferential incision | Human | NM | Injected subcutaneously at the site of the damaged lymphatic vessels  | VEGF-C hydrogel (A VEGF-C hydrogel sheet was applied to the injection site and was sutured into the injured dermal junction) | (1) Circumference; and (2) IHC staining (LYVE-1, PKH-26) | (1) A significant decrease in dermal edema at 3-4 wk; and (2) significant lymphatic vessel regeneration |
|
| 2012 | Shimizu *et al*[44] | *n* = 12 (3 groups) | Tail | Circumferential incision | Allogeneic | 2 × 106  | Injected at two different points of the lymphedematous skin flap  | N | Circumference | A decrease in lymphedema |
|
| 2015 | Ackermann *et al*[45] | *n* = 10 (3 groups) | Tail | Circumferential incision | Allogeneic | NM | NM | N | (1) Circumference; (2) IHC staining (LYV-1); and (3) real-time laser Doppler imaging for wound perfusion | (1) ADSCs affected lymphangiogenesis and lymphedema development; and (2) PRP affected more significantly than ADSCs |
| 2015 | Yoshida *et al*[42] | *n* = 20 (5 groups) | Hindlimb  | Circumferential incision | Allogeneic | 1 × 104; 1 × 15; 1 × 106 | NM | N | (1) Circumference; and (2) IHC (LYVE, VEGF-C,VEGFR, EGFP) | The number of lymphatic vessels significantly increased at 2 wk, which was dose-dependent of implanted ADSCs. |
| 2017 | Hayashida *et al*[41] | *n* = 5 (4 groups) | Hindlimb  | Circumferential incision | Allogeneic | 1 × 106 | Injected subcutaneously at proximal and distal limb | N | (1) Hind-paw edema volume; and (2) IHC (LYVE-1, VEGF-C) | Increased the number of lymphatic vessels; induced the lymphatic flow drainage to the circulatory system. |
|

ADSCs: Adipose-derived stem cells; IHC: Immunohistochemistry; LYVE-1: Lymphatic vascular hyaluronan receptor-1; Prox-1: Prospero homeobox protein 1; PRP: Platelet-rich plasma; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; NM: Not mentioned; N: None.

**Table 2 Adipose-derived stem cells-based therapy for secondary lymphedema in human**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Year** | **Ref.** | **Study type** | **Patient number** | **Edema site** |  **Etiology** | **ADSC origin** | **Cell dose** | **Injection method** | **Auxiliary treatment**  | **Valuation criteria** | **Result(s)** | **Follow-up** |
| 2015 | Peña Quián *et al*[46] | Case report | 1 | Lower limb | Recurrent lymphangitis  | Autologous  | 1-2.2 × 109 | NM | N | Lymphoscintigraphy  | The presence of new lymphatic ramifications and a greater number of lymph nodes | 6 mo |
| 2016 | Toyserkani *et al*[47] | Nonramdomized clinical trial  | 1 | Upper limb | BCRL | Autologous  | 4.07 × 107 | Injected at 8 different points of axilla | Fat grafting (10 mL) | (1) Circumference; (2) DXA scans; and (3) discomfort (infection, pain, and swelling) | (1) A reduction in volume; (2) relief of symptoms (heaviness and tension); (3) a reduction of compression therapy; and (4) no complication | 1 and 4 mo |
| 2017 | Toyserkanl *et al*[48] | Nonramdomized clinical trial  | 10 | Upper limb | BCRL | Autologous  | 5 × 107  | Injected at 8 different points of axilla | Fat grafting (28 mL) | (1) Volume assessment; (2) DXA scans; and (3) discomfort (redness, swelling, itching, pain, and infection) | No significant reduction in volume; reduction of conservative managements (50%); reduction of symptoms | 1, 3, 6, and 12 mo |
| 2019 | Toyserkanl *et al*[49] | Nonramdomized clinical trial  | 10 | Upper limb | BCRL | Autologous  | 5 × 107  | Injected at 8 different points of axilla | Fat grafting (30 mL) | (1)Volume assessment; (2) DXA scans; and (3) discomfort (redness, swelling, itching, pain, and infection) | No significant reduction in volume; reduction of conservative managements (50%) | 1, 3, 6, and 12 mo |

ADSCs: Adipose-derived stem cells; BCRL: Breast cancer–related lymphedema; NM: Not mentioned; N: None.