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Proposed pathway and mechanism of vascularized lymph node flaps

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HIGHLIGHTS

- Vascularized submental lymph node flap is effective for limb lymphedema.
- Flaps containing lymph nodes absorbed more fluid than those that did not.
- A mechanism and pathway is proposed based on the results of this study.

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ABSTRACT

Objective. To investigate the pump mechanism and pathway of lymph transit in vascularized lymph node flaps.

Background. Microsurgical treatment of lymphedema with vascularized lymph node transfer can improve signs and symptoms of disease, but the pathways and mechanisms of these flaps warrant further exploration.

Methods. (Animal model) 72 flaps were raised in 18 rats: 36 groin flaps contained lymph nodes (LN), 36 deep inferior epigastric artery perforator flaps did not (non-LN). Indocyanine green (ICG) was added into normal saline (NS), 1%, 3%, 5%, 7% and 10% albumin. Three rats were assigned to each group. LN and non-LN flaps were submerged in solution and surveyed for venous fluorescence. In the 7% albumin and NS groups, volumetric change of solution was measured. (Human model) A similar experiment was performed in humans using five submental LN flaps.

Results. (Animal model) Fluorescence was detected in the venous pedicle of LN flaps submerged in 5%, 7% and 10% albumin, and half of flaps submerged in 3% albumin. Fluorescence was not detected in LN node flaps submerged in ICG-containing NS or 1% albumin solution. Fluorescence was not detected in non-LN flaps. There was greater volume reduction with LN flaps than non-LN flaps ($p < 0.001$). (Human model) Fluorescence was detected in the venous pedicle of all flaps immersed in lymph.

Conclusions. ICG fluorescence was detected in the venous pedicle of rat and human LN flaps submerged in lymph or albumin when the concentration was greater than 3%. Based on these results, a pathway for lymphatic uptake is presented.

1. Introduction

Lymphedema is a complex proliferative process that results from disruptions of the lymphatic circulation, affecting as many as five million Americans, and 200 million worldwide [1–2]. Disease onset may be insidious, with progressive swelling followed by inflammation, fat hypertrophy and fibrosis. Lymphedema may be primary or idiopathic, but more commonly results from lymphadenectomy and radiation. According to studies, patients who had gynecological surgery and lymphadenectomy, as well as those who received pelvic radiation therapy have 10–49% chance of developing lower limb lymphedema. Upper limb lymphedema is also quite common for those who underwent mastectomy, accounting for an estimated 4–62.5% among patients who have received underarm lymphadenectomy and radiotherapy [3].

Preventative strategies are generally more effective than strategies with curative intent. Non-operative management may be mechanical or medical. The most effective surgical strategies are bypass procedures like lymphovenous anastomosis (LVA) or lymphatic tissue autotransplantation (vascularized lymph node transfer, VLN) [1,4–10]. The mechanism of VLN flaps transferred distally to the affected limb was previously proposed: vascularized lymph nodes act as the motor of a

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pump that absorbs interstitial fluids and diverts those fluids to the venous circulation. Fluid is thought to accumulate distally because of gravitational dependency and arm-swinging motion during ambulation (the "catchment effect"). By placing the pump distally where fluid accumulation is greatest, flap efficiency is theoretically maximized [11–12].

VLN flap transfer is feasible for the treatment of mild and severe lymphedema, but the mechanism is not well understood [1]. Interstitial fluid that accumulates in diseased fibrofatty tissue is absorbed by functioning lymphatic tissue and returns to the venous circulation through a network of lymphatic and venous channels that surround the transferred lymph nodes [12–14]. This mechanism is supported by evidence of venous fluorescence detected soon after peripheral intradermal or intra-nodal injection of indocyanine green (ICG) dye [11]. Lymphatic latency time explained why venous uptake was slightly slower after intradermal injection. Contrarians might suggest that the observed phenomenon is better explained by direct uptake by the venous system after injection.

It is thought that the more lymph nodes a flap contains, the better the outcome [15], and that injection of ICG dye into nodes and cutaneous tissue makes its way to the venous circulation [11]. It is premature, however, to assume that lymphatic structures, not veins, mediated venous return of ICG in the aforementioned study. The mechanism for lymphatic or dye uptake is not completely understood. We aim to identify the pathway lymph follows from the extravascular compartment to the intravascular compartment, and enhance the clinical relevance of previous and current data with a simple but carefully-designed set of experiments.

2. Materials and methods

2.1. Animal experiment

Eighteen male Sprague–Dawley rats (Bio LASCO Taiwan, Taipei, Taiwan) weighing 350 g–400 g were obtained and received care that complied with Chang Gung Memorial Hospital Animal Research Guidelines and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The study was approved by the Chang Gung Institutional Review Board (IRB #1013481B). Care was provided in a pathogen-free animal facility and the animals were provided food and water ad libitum. Rats were anesthetized with 2.0–2.5% isoflurane for all procedures (Aesica Ltd., Queenborough, United Kingdom). In every rat, two lymph node-containing groin flaps (LN, n = 36) and two lymph node-devoid deep inferior epigastric perforator (DIEP) flaps (non-LN, n = 36) were raised with the aid of a Leica M651 surgical microscope (Leica, Wetzlar, Germany) [16].

Bilateral rectangular groin LN flaps were raised in every rat ($n = 36$ total flaps) and their dimensions were recorded. Flap volume was estimated as the product of length, width, and thickness in centimeters. Composite adipose-and-lymph node flaps were pedicled on the superficial circumflex iliac vessels and dissected to the femoral vessels (Fig. 1A). Special care was taken to identify and palpate lymph nodes, but not to skeletonize them, to preserve surrounding lymphatic channels and lymphovenous connections. All flaps contained several palpable nodes and the quantity was documented for each.

Thirty-six quadrangular non-LN DIEP flaps were raised under the same conditions [16]. The flap boundaries were the xiphoid, costal margin, anterior axillary line to the anterior superior iliac spine, and lower abdomen to the midline. Muscle-sparing perforator flaps were dissected from cranial to caudal by identifying the perforators and source vessel, ligating the superior epigastric artery, and capturing as many perforators as feasible until the flap was isolated on its pedicle (Fig. 1B) [11].

An aliquot of 0.5 mL of 0.5% ICG (Sigma Aldrich, St. Louis, Mo.) was added into 25 mL NS (ICG-NS), 1%, 3%, 5%, 7% or 10% albumin solution (ICG-Alb, CSL Behring, King of Prussia, PA.). Three rats ($n = 6$ LN flaps, $n = 6$ non-LN flaps) were assigned to each treatment group. LN (Fig. 1C) and non-LN (Fig. 1D) flaps were immersed into each solution for 15 min. The overhead lights were turned off and the flaps were examined with a custom-made device designed to record invisible ICG fluorescence in real-time by emitting and detecting radiation in the near-infrared spectrum. The detector was a charge-coupled camcorder (Sony HD Handycam CM05, 10.2 megapixels; Sony Corp., New York, N.Y.) that was modified to filter wavelengths less than 820 nm.

The outcome of interest for all groups was detection of ICG fluorescence in the venous pedicle. All rats in the ICG-NS and ICG-Alb (7%) arm were also studied for volume changes in the immersion solution. To achieve this, the weight of fluid before and after immersion for 15 min was recorded. After completion of the experiment, rats were euthanized with intracardiac injection of 1 cm^3 of 2% lidocaine hydrochloride, and specimens were evaluated for the number of lymph nodes they contained. Descriptive statistics were generated using Microsoft Excel 2011 (Microsoft Corp., Redmond, CA). Differences in detectable fluorescence were determined using the Mann–Whitney U-test. Volumetric analysis comparisons were made using the two-tailed Student's *t*-test (SPSS, Inc., Chicago, Ill. Version 20.0). Statistical significance was established at $p < 0.05$.

2.2. Human correlate

Patients undergoing vascularized submental lymph node (VSLN) flap transfer for extremity lymphedema were studied. Between July 2014 and January 2015, four patients who underwent unilateral or bilateral VSLN flap transfer for lower extremity lymphedema were included in a parallel study ($n = 5$ flaps). All procedures were in accordance with the ethical standards of the Chang Gung Memorial Hospital Institutional Review Board (IRB #1013282A3) and national guidelines on human experimentation and with the Helsinki Declaration of 1975 (as revised in 2008). Informed consent was obtained for all patients.

VSLN flaps were harvested as described previously by the authors (Fig. 2A) [13]. A composite flap was elevated *en bloc* that included skin, subcutaneous fat, lymph nodes, and the vascular pedicle. During recipient site preparation, interstitial fluid that was presumed to be lymph was collected in a sterile container. A sample of the fluid was studied to determine albumin concentration. In those patients, blood was analyzed for albumin and total protein concentration. An aliquot of 0.5 mL of 0.5% ICG was added to 5 mL of normal saline (ICG-NS) or 5 mL lymph (ICG-Lymph). With the arteriovenous pedicle intact, VSLN flaps were immersed in ICG-NS for 20 min, and followed by ICG-Lymph for 20 min (Fig. 2B).

The overhead lights were turned off and the flaps were examined using an identical ICG fluorescence detector as mentioned. Parameters measured were the number of lymph nodes harvested and the presence of detectable ICG fluorescence in the venous pedicle (as represented in Fig. 2C). Clinical outcomes for these patients were evaluated for circumferential differentiation compared to the unaffected contralateral extremity, and subjective parameters.

3. Results

3.1. Animal experiment

All LN flaps had 2 (n = 25, 69.4%) or 3 (n = 11, 30.6%) visible lymph nodes. Results of this the fluorescence experiment are summarized in Table 1. Fluorescence drained around the pedicle vein in 21 of 36 (58.3%) LN flaps. The venous pedicle fluoresced in 3 of 6 flaps submerged in 3% ICG-Alb and in all LN flaps submerged in 5%, 7% and 10% ICG-Alb. Venous fluorescence was not detected in LN flaps immersed in ICG-NS and1% ICG-Alb. Three of 6pedicles of LN flaps immersed in 3% ICG-Alb did not fluoresce. There was no detectable fluorescence in the venous pedicle of any non-LN flap placed in any solution at 15 min (0%, $p < 0.001$).

Table 1 summarizes the results of fluid volume change after flap immersion in 7% ICG-Alb and ICG-NS. The reduction of volume,

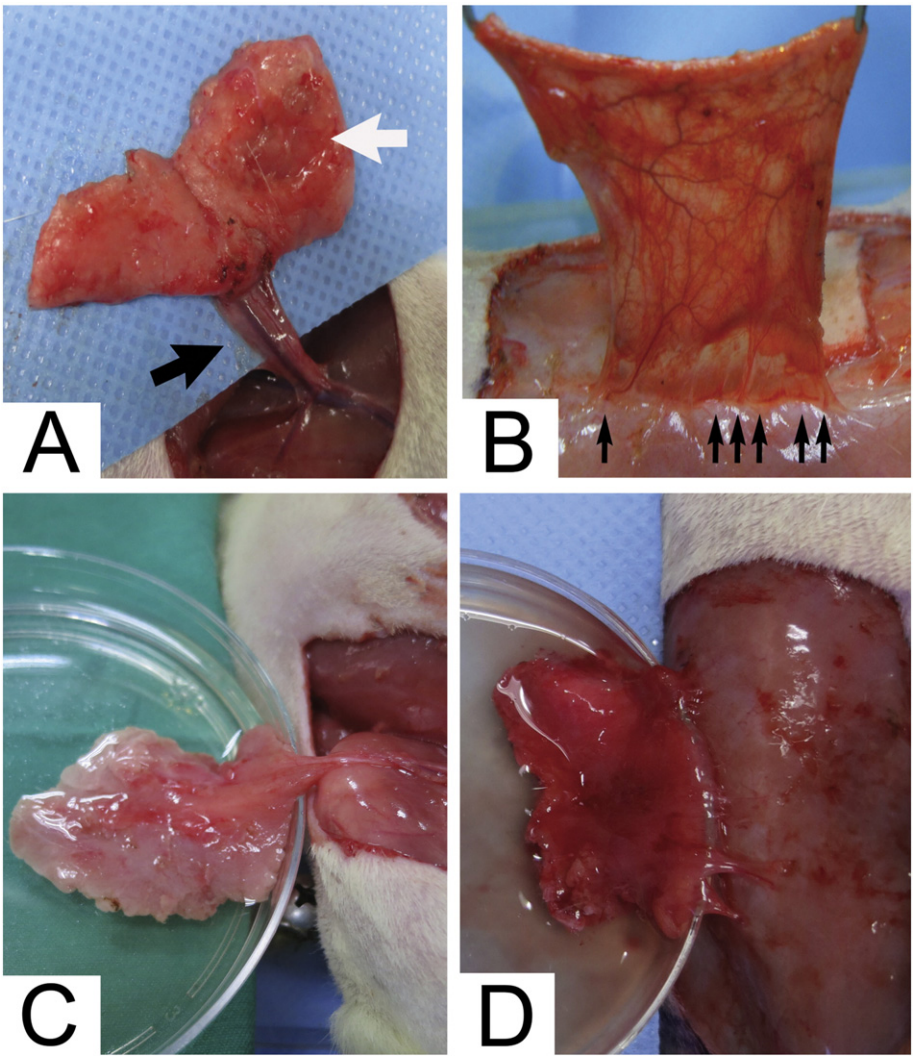

Fig. 1. Flaps studied in the animal experiment. A. The lymph node (*white arrow*) containing groin flap is based on the superficial circumflex iliac vessels (*black arrow*). B. The DIEP flap is perfused by identifiable perforators (*black arrows*) and dissected to the sizeable inferior epigastric vessels (*leftmost arrow*). It contains no lymph nodes. C. A lymph node-containing groin flap immersed in immersion medium before ICG is added. D. DIEP flap that contains no lymph nodes is immersed in immersion medium before ICG is added.

determined by weight change of the immersion fluid, was significantly greater in when LN flaps were bathed (0.72 g reduction) than non-LN flaps (0.34 g reduction, $p < 0.001$). LN flaps were larger (flap volume = 3.4 cm^3) than non-LN flaps (flap volume = 2.9 cm^3; $p = 0.04$). Accounting for this difference, LN flaps still resulted in a greater volume change (0.21 g/cm^3 reduction) than non-LN flaps (0.11 g/cm^3 reduction, $p < 0.001$); this difference occurred in both 7% ICG-Alb and ICG-NS media.

3.2. Human correlate

Results of the human correlate study are listed in Table 2. An 18-year-old woman had unilateral flap transposition for primary lymphedema of her right leg. Two women, 47 and 64-years-old, were treated for right arm lymphedema secondary to axillary node dissection for treatment of breast cancer. The 64-year-old underwent prior radiation therapy. A 60-year old woman underwent bilateral flap harvest for treatment of lymphedema of her left leg secondary to cervical cancer extirpation after radiation; one flap was transposed to her thigh and the other to her ankle. None of the patients had diabetes, a history of current or previous smoking. The average BMI for all patients was 29.7 kg/m^2 (range, 25.5–33.3 kg/m^2). All patients expressed sufficient lymph to allow for flap submersion in ICG-Lymph solution ($n = 5$ flaps). The mean albumin concentration was 3.3 ± 0.3 g/dL in lymph and 3.7 ± 0.6 g/dL in blood. No ICG fluorescence was detected in the pedicle of 5 flaps immersed in ICG-NS after 20 min, but fluorescence was detected in the venous pedicle of all 5 flaps after immersion in ICG-Lymph (see eVideo 1 in the Supplement).The latency time from immersion to detectable venous fluorescence was 13.5 min (range, 10.2–18.3 min). All flaps survived and every patient demonstrated symptomatic improvement at 12 months (see eTable 1 in the Supplement).

4. Discussion

Prior to this study, VLN flaps demonstrated the capacity to divert dye from lymph node parenchyma and cutaneous tissue to the venous circulation [11]. (Table 3) Still, the mechanism of uptake, conduit through which the dye traveled, and clinical significance of the findings remained unclear. A pump mechanism was supported by centripetal transit of peripherally injected dye in a flap [11]. However, in that study the dye was injected into tissues. Transit through circulatory channels might have been driven more by the pressure of injection than other physiologic processes.

We therefore sought a model that more closely represents the conditions that exist during VLN flap transfer by immersing tissues into fluorophore-containing media. Using the model we described, the

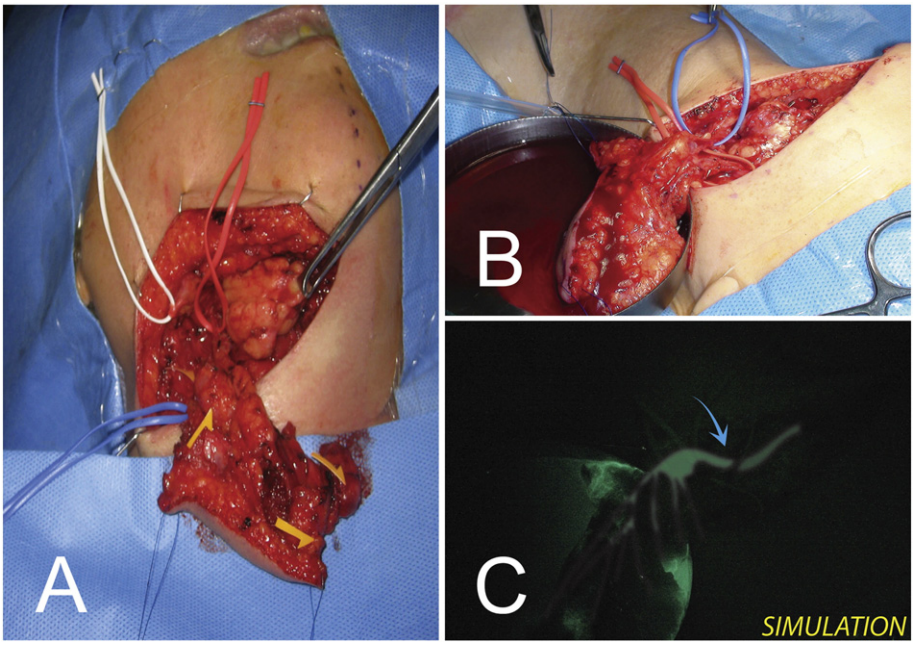

Fig. 2. A. The vascularized submental lymph node (VSLN) flap; the facial artery (*red loop*), facial vein (*blue loop*) and marginal mandibular nerve (*white loop*) are identified. Lymph nodes (*yellow arrows*) are palpable and visible. Care is taken to preserve peri-nodal tissue that contains the network of lymphatic capillaries. B. The flap is submerged in solution. The artery (*red loop*) and vein (*blue loop*) are identified. C. ICG is added and the flap is monitored for near-infrared fluorescence of the venous pedicle (*blue arrow*) in low-light conditions. This is a DIGITALLY-ENHANCED IMAGE. *Please note*: A handheld camcorder with a small LCD viewfinder was used to detect fluorescence; publication-quality still images of near-infrared ICG fluorescence were not obtainable. Instead, high-definition video footage is included as supplemental digital content (eVideo 1).

mean latency period until fluorescence of immersed VLN flaps was 13.5 ± 3.8 min. This was longer than when ICG was injected directly to the subcutaneous tissue in our previous study (5.7 ± 2.2 min) [11]. The results of this study demonstrated: 1) venous fluorescence occurs in both animal and human subjects when LN flaps are soaked in an ICG-Alb medium of a certain concentration. 2) Lymph or albumin-containing media (>3 g/dL concentration) are required for venous fluorescence. 3) Lymph nodes effect increased uptake of the immersion medium.

ICG is only weakly fluorescent in its unbound state [17]. Although unbound-ICG (as with ICG-NS) may make its way to the venous circulation and bind to plasma proteins, it may not be adequately concentrated to be visualized within the venous pedicle. However, when ICG is added to lymph or albumin solution of sufficient concentration, ICG rapidly binds to proteins that are taken up by lymphatic capillaries before reaching the venous circulation. The concentration of ICG by albumin and other proteins then generates visible fluorescence in the venous circulation. This may explain why a concentration threshold appears to exist for fluorescence to occur.

An alternative explanation for the concentration threshold warrants a review of physiology. Briefly, Starling's forces dictate capillary and

Table 1
Results of animal experiment.

Fluorescence study							
Immersion medium	DIEP (non-LN) flap			Groin (LN) flap			*P*-value[a]
	Fluorescence in venous pedicle?						
	Yes, *n*	No, *n*	%	Yes, *n*	No, *n*	%	
ICG-NS	0	6	0	0	6	0	–
ICG-Alb 1%	0	6	0	0	6	0	–
ICG-Alb 3%	0	6	0	3	3	50	0.06
ICG-Alb 5%	0	6	0	6	0	100	0.01
ICG-Alb 7%	0	6	0	6	0	100	0.01
ICG-Alb 10%	0	6	0	6	0	100	0.01
Total	0	36	0	21	15	58.3	<0.001

Volume absorption study			
	DIEP (non-LN) flap	Groin (LN) flap	*P*-value
Experiments, total, *n*[b]	11	11	
Fluid weight reduction, g	0.34 ± 0.11	0.72 ± 0.30	<0.001
Flap volume, cm^3	2.9 ± 0.6	3.4 ± 0.7	0.04
Reduction/volume, g/cm^3			
ICG-Alb, 7% (*n* = 6 experiments)	0.11 ± 0.03	0.18 ± 0.10	0.04
ICG-NS (*n* = 5 experiments)	0.13 ± 0.01	0.25 ± 0.01	<0.001
Any solution	0.11 ± 0.02	0.21 ± 0.07	<0.001

DIEP = deep inferior epigastric artery flap; Non-LN = no lymph nodes in flap; LN = lymph nodes in flap; ICG = indocyanine green; NS = normal saline; Alb = albumin.
[a] Statistical significance is established at $p < 0.05$.
[b] There were 6 experiments using ICG-Alb and 5 using ICG-NS (one specimen in the NS group had spillage of fluid; data was not gathered).

Table 2
Results of human correlate study.

Case	Age, years	LN, *n*	Albumin concentration			Latency in minutes[a]	
			Lymph[b]	Blood		ICG-NS	ICG-Lymph
			Albumin, g/dL	Albumin, g/dL	TP, g/dL		
1	18/F	2	3.16	3.64	7	N/A[d]	10.2
2 (right side)[c]	60/F	4	3.5	3.15	6.5	N/A	12.3
2 (left side)	60/F	4	3.5	3.15	6.5	N/A	10.3
3	47/F	3	2.9	4.19	6.9	N/A	15.4
4	64/F	4	3.2	4.54	7.5	N/A	18.3
	49.8 ± 18.9	3.4 ± 0.9	3.3 ± 0.3	3.7 ± 0.6	6.9 ± 0.4	-	13.5 ± 3.8

LN = lymph nodes in flap; TP = total protein; ICG-NS = normal saline immersion medium; ICG-Lymph = lymph immersion medium.
[a] Time interval between flap immersion and detection of venous fluorescence.
[b] Collected from affected extremity.
[c] Same patient with left and right vascularized submental lymph node flap harvest. One was transferred to the left thigh, the other to the left ankle.
[d] Flourescence was not detected in any flap immersed in normal saline.

Table 3
Summary of published literature related to lymph node transfer for lymphedema treatment.

Year/author	Number of patients	Etiology	Donor site	Recipient site	Method of measurement	Mean follow-up (months)	Outcome	Complications
2006/Becker et al.	24	Secondary lymphedema	Femoral region	Axillary or elbow	Circumference	96	Reduction: 10 patients: normal 10 patients: some reduction 2 patients: no change	Lymphorrhea in 8 patients Infectious in 17 patients One episode of skin infectious disease was recorded in 7 patients
2009/Lin et al.	13	Secondary lymphedema	Groin	Wrist	Circumference	56	Reduction rate: 50.6%	NA
2011/Gharb et al.	21	Secondary lymphedema	Groin	Wrist	Circumference	40	Visual analog scale scores improved after the operation	2 cases of partial flap loss and donor site lymphorrhea
2012/Cheng et al.	6	Secondary lymphedema	Submental	Ankle	Circumference	8.7	Reduction rate: Above knee: 64% Below knee: 64% Above ankle: 67%	One flap required re-exploration for venous congestion but was successfully salvaged
2013/Cheng et al.	10	Secondary lymphedema	Groin	Wrist or elbow	Circumference	39	Reduction rate: 40.4%	NA
2014/Cheng et al.	20	Secondary lymphedema	Groin or submental	Distal recipient site	Circumference	27.3	Reduction rate: 40.5%	NA
2015/Akita et al.	13	Secondary lymphedema	Supraclavicular	Distal region of the thigh or the ankle	Lower extremity lymphedema index	12	Lower extremity lymphedema index: 26.5%	NA
2015/Cheng et al.	Upper-limb: 15 Lower-limb: 10	Secondary lymphedema	Groin or submental	Distal recipient site	Circumference	Upper-limb: 25 Lower-limb: 16	Reduction rate: Upper-limb: 24% Lower-limb: 35%	NA

lymphatic fluid and protein exchange. A strong arteriovenous pressure gradient, and an even stronger intravascular-extravascular pressure gradient, accounts for fluid leakage that accumulates and gives rise to lymph. Lymphatic capillaries absorb accumulating proteins and fluid; they are the predominant site of interstitial protein uptake [18]. Fluid and protein exchange occur until Starling's forces equilibrate and a physiologic lymph/serum albumin ratio is restored. This would favor increased protein uptake with higher concentrations of albumin solution, and hence a threshold exists.

The volume study of the animal experiment demonstrated that a flap's failure to fluoresce was neither an indicator nor a predictor of fluid uptake. In contrast, there was a trend toward increased per-cm^3-volume change when flaps were immersed in normal saline medium than hypertonic albumin, even though none of those flaps fluoresced. In every experiment, we manipulated the oncotic pressure of immersion medium and the balance of free-versus-bound ICG. Previous studies have shown lymphatic flow increases when the interstitial oncotic pressure approaches plasma concentrations, and our findings are consistent [19].

Venous fluorescence implies avenues exist that allow for protein to enter the venous circulation. Lymphatic channels exist in both lymph node-containing and deficient flaps, but fluorescence cannot occur without lymph nodes. Therefore, the exchange is thought to occur at the level of the lymph node. The two structures that exist in a LN flap that are most likely to permit protein transit are low-resistance blind-ended termini of lymphatic capillaries [20–21] (ingress) and the high-endothelial venules (HEV, egress) within the lymph node itself [22–24]. An updated schematic of the proposed mechanism of, and pathway for, lymph uptake in VLN flaps is presented in Fig. 3.

Results of the animal fluorescence study were reproduced in humans, who at one year demonstrated improvement in symptoms and limb circumference. We designed the experimental model to closely represent conditions that occur with VLN flap transfer, however, we did not control for hydrostatic pressure. We expect that the turgor of a congested, lymphedematous extremity will favor early fluid uptake by the flap because hydrostatic pressure of the interstitium is increased. As the interstitial pressure normalizes, interstitial hydrostatic pressure, and therefore lymphatic uptake may decrease. This may explain a pattern of rapid initial decongestion, followed by a plateau, after procedures like LVA [25] and LVN flap transfer [26].

The results of this study support our previous conclusion that vascularized tissue containing lymph nodes is capable of delivering ICG from the extracellular milieu to the venous circulation [27]. We demonstrated that lymph nodes are necessary for venous fluorescence to be detected, ruling out the possibility that venular uptake was contributory to venous fluorescence. Although lymphovenous connections (LVCs) were proposed half a decade ago and studied in human and animals since [22–24,28–32], Shao et al. [33] only recently demonstrated in a mouse model that LVCs exist at the level of the lymph node. We imagine intra-nodal LVCs explain VLN effectiveness, and why quantity of lymph nodes correlates with improved lymphatic drainage [15].

This study identified three requirements for VLN flap function: the presence of vascularized lymph nodes (including afferent lymphatic channels), favorable Starling forces, and intact arteriovenous anastomoses. We recognize the importance of [34–36], but have yet to identify clinical relevance of maintaining as many afferent lymphatic channels in the flap as possible. Since lymphatic capillaries are the predominant sites of lymph ingress, maximizing contiguous lymphatics within that lymphosome [37] might optimize flap efficiency. It is also possible that lymphovenous shunting is the rate-limiting step, not lymphatic uptake, so including more lymphatic capillaries might be futile, perhaps “flooding” the pump. In our experience, preserving a rind of perinodal tissue alone is sufficient to achieve clinical improvement. Further study will demonstrate whether inclusion of a more expansive lymphosomal network or “dragnet” would enhance flap efficiency and outcomes. The authors emphasize that pedicle fluorescence provides insight to the mechanism of function, but it does not predict fluid uptake. For example, we demonstrated that in all immersion media, groin flaps absorbed more volume per cm^3 than DIEP flaps. This was true for groin flaps immersed in NS that did not fluoresce, yet demonstrated a greater rate of fluid uptake (0.25 g/cm^3) than DIEP flaps (0.13 g/cm^3).

There are important limitations to this study. Most importantly, we relied on macroscopic and grossly measurable parameters like volume change and visible venous pedicle fluorescence to investigate a process that occurs at the molecular and cellular level. The pathway described herein is therefore theoretical. The collective global experience

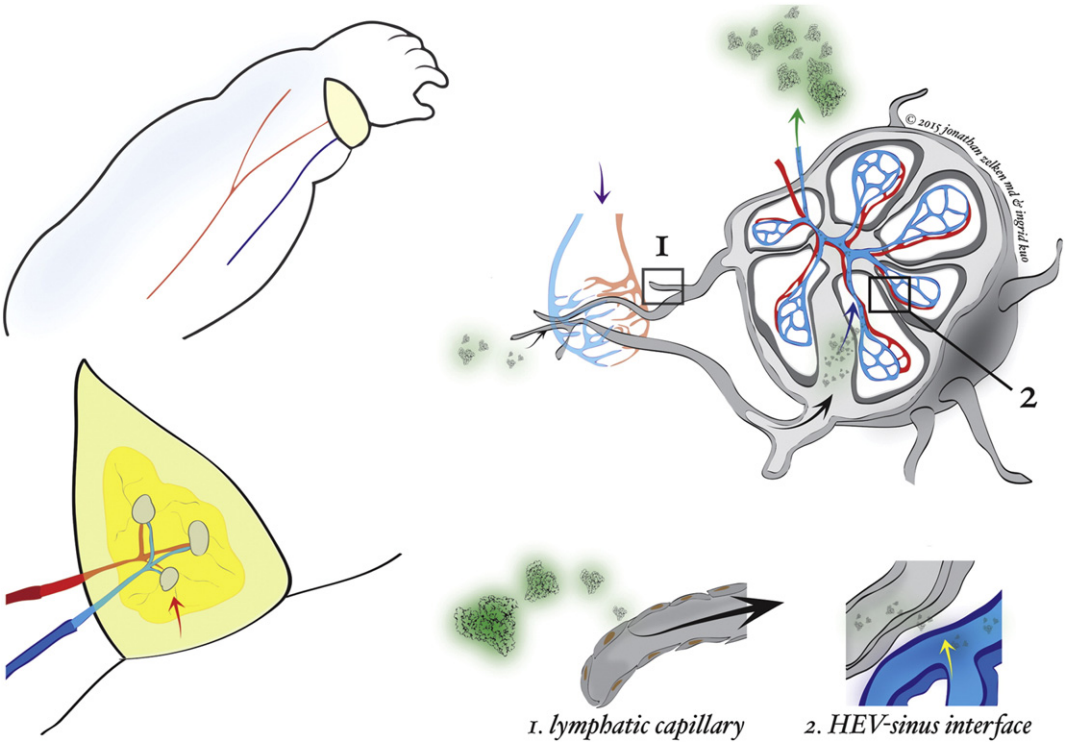

Fig. 3. Schematic of updated pump mechanism. *Left upper*, a lymphedematous arm is represented and the vascularized submental lymph node (VSLN) flap (*yellow*) is placed distally to exploit the catchment effect. *Left lower*, a close-up view of a VSLN flap anastomosed to the radial artery and cephalic vein includes several lymph nodes (*red arrow*) in continuity with the vascular pedicle and peri-nodal fat. *Right*, peri-nodal fat contains a network of lymphatic capillaries surrounded by capillary beds (*purple arrow*). Starling forces, established by arteriovenous pressure gradients at this level, dictate protein uptake, which occurs at the level of blind lymphatic capillary endings (*black arrow*, 1). Albumin-bound ICG (*green structures*) concentrates in the lymph node (*gray mass*) then translocates via high endothelial venules (*gray arrow*, 2) to the venous circulation (*green arrow*).

evaluating outcomes of VLN flap transfer is an emerging science. Although it has been shown that VLN flaps have the capacity to uptake fluid, we can only assume this process is responsible for limb circumference reduction observed in clinical practice. Long-term change and flap efficacy has not been thoroughly studied, nor has the clinical significance of limb circumference reduction. In addition, we do not know whether a history of radiation has an influence on outcomes or the influence of preoperative lymphedema stage on outcomes after flap transfer.

This study provides a better understanding of the pump mechanism of the VLN flap. The conclusions drawn have clinical relevance by allowing us to exploit various aspects of the mechanism to enhance pump efficiency and outcomes. For example, future study may examine the role of the surrounding soft tissue network to identify whether a lymphatic dragnet phenomenon exists or whether de-epithelializing and burying flaps improves outcomes by exposing lymphatic channels. Other possibilities to improve pump efficiency might include venous afterload reduction (i.e., with sildenafil), ligation of lymph node efferent to avoid "spillage" of lymph, and adjunctive compression therapy when extremity turgor reaches normal or subnormal values.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2016.01.007.

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