Visualization of fluid drainage pathways in lymphatic vessels and lymph nodes using a mouse model to test a lymphatic drug delivery system

Tetsuya Kodama,1,* Yuriko Hatakeyama,1 Shigeki Kato,1 and Shiro Mori2

1 Graduate School of Biomedical Engineering, Tohoku University, 4-1 Seiryo, Aoba, Sendai, Miyagi 980-8575, Japan
2 Department of Oral and Maxillofacial Surgery, Tohoku University Hospital, 1-1 Seiryo, Aoba, Sendai 980-8575, Japan

*kodama@bme.tohoku.ac.jp

Abstract: Curing/preventing micrometastasis to lymph nodes (LNs) located outside the surgically resected area is essential for improving the morbidity and mortality associated with breast cancer and head and neck cancer. However, no lymphatic therapy system exists that can deliver drugs to LNs located outside the dissection area. Here, we demonstrate proof of concept for a drug delivery system using MXH10/Mo-lpr/lpr mice that exhibit systemic lymphadenopathy, with some peripheral LNs being as large as 10 mm in diameter. We report that a fluorescent solution injected into the subiliac LN (defined as the upstream LN within the dissection area) was delivered successfully to the proper axillary LN (defined as the downstream LN outside the dissection area) through the lymphatic vessels. Our results suggest that this approach could be used before surgical resection to deliver drugs to downstream LNs outside the dissection area. We anticipate that our methodology could be applied clinically, before surgical resection, to cure/prevent micrometastasis in LNs outside the dissection area, using techniques such as ultrasound-guided internal jugular vein catheterization.

©2014 Optical Society of America

OCIS codes: (170.0170) Medical optics and biotechnology; (170.3880) Medical and biological imaging; (170.5380) Physiology.

References and links

1. T. Sato, S. Mori, Y. Arai, and T. Kodama, “The combination of intralymphatic chemotherapy with ultrasound and nano-/microbubbles is efficient in the treatment of experimental tumors in mouse lymph nodes,” Ultrasound Med. Biol. 40(6), 1237–1249 (2014).
2. S. Kato, Y. Shirai, H. Kanzaki, M. Sakamoto, S. Mori, and T. Kodama, “Delivery of molecules to the lymph node via lymphatic vessels using ultrasound and nano/microbubbles,” Ultrasound Med. Biol. in press.
3. L. Shao, S. Mori, Y. Yagishita, T. Okuno, Y. Hatakeyama, T. Sato, and T. Kodama, “Lymphatic mapping of mice with systemic lymphoproliferative disorder: usefulness as an inter-lymph node metastasis model of cancer,” J. Immunol. Methods 389(1-2), 69–78 (2013).
4. T. Okuno, S. Kato, Y. Hatakeyama, J. Okajima, S. Maruyama, M. Sakamoto, S. Mori, and T. Kodama, “Photothermal therapy of tumors in lymph nodes using gold nanorods and near-infrared laser light,” J. Control. Release 172(3), 879–884 (2013).
5. S. Nakatsuru, M. Terada, M. Nishihara, J. Kamogawa, T. Miyazaki, W. M. Qu, K. Morimoto, C. Yazawa, H. Ogasawara, Y. Abe, K. Fukui, G. Ichien, M. R. Ito, S. Mori, Y. Nakamura, and M. Nose, “Genetic dissection of the complex pathological manifestations of collagen disease in MRL/lpr mice,” Pathol. Int. 49(11), 974–982 (1999).
6. M. Nose, H. Komori, T. Miyazaki, and S. Mori, “Genomics of vasculitis: lessons from mouse models,” Ann. Vasc. Dis. 6(1), 16–21 (2013).
7. Y. Tanaka, H. Komori, S. Mori, Y. Soga, T. Tsubaki, M. Terada, T. Miyazaki, T. Fujino, S. Nakamura, H. Kanno, T. Sawasaki, Y. Endo, and M. Nose, “Evaluating the role of rheumatoid factors for the development of rheumatoid arthritis in a mouse model with a newly established ELISA system,” Tohoku J. Exp. Med. 220(3), 199–206 (2010).
When metastasis to a regional LN occurs in breast cancer or head and neck cancer, the use of LN resection is limited to those patients that are in sufficiently good physical condition to tolerate the surgery, and in whom the risk of metastatic foci invading other major organs is minimal. Although the area of dissection is determined by clinicopathological guidelines, there are still no established methods for eliminating metastatic LNs located outside the dissection area. An underlying reason for this is that current drug delivery systems that utilize the hematogenous route without radiation therapy are not able to achieve complete destruction of metastases in LNs both inside and outside the area to be resected.
To address this shortcoming, we have studied lymphatic drainage pathways as proof of concept for a novel lymphatic drug delivery system that could be used to treat/prevent micrometastasis in LNs.

The lymphatic system consists of many valves, and lymphatic flow is unidirectional toward the angulus venosus. Therefore, upstream and downstream lymph nodes can be defined in the lymphatic network. The concept is to inject drugs, before surgical resection, into upstream LNs within the dissection area, so that these drugs are delivered to downstream LNs outside the dissection area. To demonstrate proof of concept, we have developed MXH10/Mo-lpr/lpr (MXH10/Mo/lpr) inbred mice [1–4], and metastatic tumor cell lines able to grow in the mice. The phenotype of the lpr (lymphproliferation) gene is characterized by the accumulation of a large number of polyclonal CD4⁺CD8⁺ T cells in the LNs and spleen [5]; the lpr gene is recognized as a “promoting factor” for collagen disease in MRL mice due to Fas-mediated apoptotic insufficiency, and the causative genes of collagen disease are considered to be the background genes of MRL mice [6]. The MXH10/Mo/lpr mouse strain is a substrain of the recombinant inbred strain of the MXH/lpr mouse [7]. MXH/lpr mice were generated using two different parental inbred strains as progenitors, MRL/lpr (H-2k haplotype) and C3H/lpr (H-2k). MXH10/Mo/lpr mice do not develop severe glomerulonephritis and vasculitis, in contrast to MRL/MpJ-lpr/lpr (MRL/lpr) mice [8], and thus their life span is longer than that of MRL/lpr mice [3]. Conventional mice have LNs with diameters of 1-2 mm, far smaller than those of patients. However, MXH10/Mo/lpr mice aged 2.5-3 months have peripheral LNs that reach up to about 10 mm in diameter, and both the LN size and the time of onset of LN swelling are consistent and predictable. Consequently, breeding and maintenance of MXH10/Mo/lpr mice is easier than that of MRL/lpr mice, and based on these attributes, we determined that MXH10/Mo/lpr mice would be suitable for various studies of murine lymph networks, especially in the field of experimental exploration of LN metastasis [3]. We have identified 22 different LNs situated in the head and neck, limbs, and thoracic and abdominal regions. Furthermore, four peripheral inter-LN vessels have been recognized: from the subiliac LN (SiLN) to the proper axillary LN (PALN); from the parotid LN to the caudal deep cervical LN; and from the popliteal LN to both the sciatic LN and the SiLN. Among these, we consider the lymphatic vessel from the SiLN to the PALN to be superior for accessibility and ease of observation, with the added advantage that it traverses a relatively long distance between the two LNs, with no branching networks. Against the backdrop that the anatomical locations and nomenclatures of murine LNs have often been ignored or even assigned incorrectly, we have used the nomenclature “subiliac LN” instead of “inguinal LN” [3, 9]. In the present study, the SiLN was defined as the upstream LN inside the dissection area and the PALN as the downstream LN outside the dissection area. The fundamental characteristics of the fluid drainage pathways of a lymphatic drug delivery system were investigated, following the injection of a fluorescent solution into the SiLN in order to deliver it to the PALN through the connecting lymphatic vessels.

2. Materials and methods

All experiments were approved by the Institutional Animal Care and Use Committee of Tohoku University.

2.1 Animal model

MXH10/Mo/lpr mice are a substrain of the recombinant inbred mouse strain, MXH/lpr (Fig. 1A) [3]. MXH10/Mo/lpr mice were produced using two different parental inbred strains as progenitors, MRL/Mpj-lpr/lpr and C3H/HeJ-lpr/lpr, followed by an F1 intercross and more than 20 generations of strict brother-sister matings. MXH10/Mo/lpr mice are unique in that most of their peripheral LNs are up to 10 mm in size at 2.5-3 months of age. In addition, MXH10/Mo/lpr mice develop only mild autoimmune diseases. The longitudinal diameters of the SiLN and PALN, measured using a digital caliper, were 10.8 ± 1.9 mm and 10.4 ± 1.6 mm, respectively (mean ± SD; n = 12). The MXH10/Mo/lpr mice were bred under specific

#225349 - $15.00 USD Received 22 Oct 2014; revised 3 Dec 2014; accepted 4 Dec 2014; published 15 Dec 2014 (C) 2014 OSA 1 Jan 2015 | Vol. 6, No. 1 | DOI:10.1364/BOE.6.000124 | BIOMEDICAL OPTICS EXPRESS 126
2.2 Detection of LNs and lymphatic vessels

Two kinds of dyes were used; 0.5% Evans blue (MW: 961; Sigma-Aldrich Japan, Tokyo, Japan) and 0.5 mM 5(6)-carboxyfluorescein (MW: 376; excitation: 492 nm; emission: 517 nm; Sigma-Aldrich Japan, Tokyo, Japan). The former was used to identify visually two LNs in the axillary area, namely the PALN and the accessory axillary LN (AALN), while the latter was used to visualize dynamically the flow from the SiLN to the axillary area. A 1-mL syringe connected to a 27-gauge standard double-wing needle (Terumo Co., Tokyo, Japan) was filled with the appropriate solution and fixed onto a syringe pump (Legato100, KD Scientific, Inc, Holliston, MA, USA). To gain access to the LNs, a skin incision was performed under deep general anesthesia (2.5% isoflurane in oxygen). The solution (120 μL) was injected near the center of the LN at a velocity of 50 μL/min using a driven syringe pump. Images were obtained immediately after the injection of the fluorescent solution with the aid of a fluorescence stereomicroscope (M165-FC; fluorescent filter: GFP2; excitation: 460-500 nm; emission: > 510 nm; Leica, Bensheim, Germany) connected to a high-speed camera (Cool SNAP HQ2; Photometrics, Tokyo, Japan).

3. Results

There are two LNs in the axillary area, namely the PALN and AALN. First, we investigated the downstream lymphatic route from the SiLN to both the PALN and AALN by injecting Evans blue solution into the SiLN (Fig. 1(B)). Evans blue solution reached the PALN but not the AALN. Some solution flowed into the veins near the SiLN, depending on the elapsed time after the injection and injection volume.

![Fig. 1. The MXH10/Mo/lpr mouse and the lymphatic route. A. MXH10/Mo/lpr mouse. AALN: accessory axillary lymph node; PALN: proper axillary lymph node; SiLN: subiliac lymph node. B. 0.5% Evans blue solution was injected into the SiLN of an MXH10/Mo/lpr mouse to trace the downstream lymphatic route to the axillary region. The solution reached the PALN but not the AALN. Some solution flowed into the vein near the SiLN. LV: lymphatic vessel. Arrows: flow direction.](image-url)
Fig. 2. Sequential frames from the Media 1. Fluorescence solution flowed into the 1st lymphangion (b) and reached the 32nd lymphangion (i). (a) 1 sec, (b) 30 sec, (c) 39 sec, (d) 44 sec, (e) 47 sec, (f) 52 sec, (g) 58 sec, (h) 64 sec, (i) 68 sec after the start of recording.

Fig. 3. Lymphatic route from the SiLN to the PALN. A. Overall view of the lymphatic route from the SiLN to the PALN. The lymphatic vessel consisted of many lymphangions (cf. Media 1). B. Afferent lymphatic vessel (ALV) in the PALN. C and D. A valve in a lymphangion. C is depicted in unicolor, D in pseudocolor. The valve acted as a one-way valve. A vein running parallel to the lymphatic vessel (LV) is evident in D.

Next, we investigated flow in the lymphatic vessel and the potential route from the SiLN to the PALN by injecting fluorescent solution into the SiLN. Media 1 and its sequential frame images (Fig. 2) show the flow of the fluorescent solution in the SiLN and lymphatic vessel. The fluorescent solution that filled the SiLN entered the efferent lymphatic vessel and flowed...
into the PALN through more than 32 lymphangions (400-2000 μm in length), each of which represents the functional unit of a lymphatic vessel and lies between two semilunar valves. The average velocity of the fluorescent solution was approximately 838 μm/s, and the lymphatic length from the SiLN to the 32nd lymphangion was measured to be about 32 mm. The average velocity was obtained by dividing the total length of the lymphatic vessel (31,861 μm; 1st to 32nd lymphangion) by time (the time interval between (i) and (b) was 38 sec; (b) shows that the fluorescence solution flowed into the efferent lymphatic vessel, defined here as the 1st lymphangion; (i) shows that the solution flowed into the 32nd lymphangion).

Figure 3(A) shows an overall view of the lymphatic route from the SiLN to the PALN in one mouse, and Fig. 3(B) provides a magnified view of the afferent lymphatic vessel in the PALN. Figure 3(C) and 3(D) show the valve in the lymphangion depicted in unicolor and pseudocolor, respectively. Unidirectional flow from the SiLN to the PALN was observed in all mice, with each lymphatic valve acting as a one-way valve. The valve leaflets formed a needle-like tip with a half-angle of 27°.

Figure 4 shows representative bifurcation patterns for the lymphatic vessel running between the SiLN and PALN. Figure 4A(a-c) shows images of a lymphatic vessel with one bifurcation; the lymph flowed in a single direction from the SiLN to accumulate in the PALN, and a vein is seen running parallel to the lymphatic vessel. The bifurcation patterns could be classified into four types (Fig. 4B; n = 20); (a) no bifurcation, i.e., a single vessel (2/20); (b) one bifurcation (8/20); (c) two bifurcations (4/20); and (d) three bifurcations (6/20). In most cases the lymphatic vessel had at least one bifurcation, i.e., a single lymphatic vessel was relatively uncommon.

Figure 5 shows the fluid drainage pathways in the SiLN after injection of the fluorescent solution. Approximately half of the SiLN was filled with the fluorescent solution 10 sec after injection (Fig. 5A(a)), and the solution began to flow into the efferent lymphatic vessel 20 sec after injection (Fig. 5A(b)). At 40 sec after injection, the SiLN was entirely filled with the fluorescent solution (Fig. 5A(c)). The mean fluorescence intensities in the regions of the SiLN and efferent lymphatic vessel versus time elapsed after injection are shown in Fig. 5(B). The fluorescence intensity reached a maximum value 50 sec after injection, after about 42 μL of fluorescent solution had been injected. Since the mean fluorescence intensity remained relatively stable after 50 sec, the maximum capacity of the SiLN was about 40 μL. Volumes of solution greater than this value most likely drained into the blood vessels of the SiLN.

We then investigated the fluid drainage pathways in the PALN. Figure 6(A) shows the fluid drainage pathways in the PALN after injection of fluorescent dye into the SiLN. At time 0 sec (i.e., the moment that injection commenced), there was no detectable signal in the PALN. However, entry of fluorescent solution into the afferent lymphatic vessel (ALV) and lymphatic channel (LC) was evident at 60 sec. After 120 sec, two afferent lymphatic vessels were detected, and the lymphatic channel was filled with fluorescent solution. Figure 6(B) shows the relationship between the mean fluorescence intensity and the time elapsed following injection. The mean fluorescence intensity was constant up to 60 sec after the injection was started and then gradually increased to reach a maximum value at around 110 sec; approximately 40 μL of fluorescent solution had entered the PALN during the 50 sec period between these time points. Thereafter, the fluorescence intensity remained constant until the termination time (144 sec). The fluid drainage pathway in the PALN of a mouse after termination of the injection (i.e., 144 sec after the injection was started) is shown in Fig. 6(C). The fluorescent solution derived from the two afferent lymphatic vessels emptied into the periphery of the PALN (Fig. 6C(a)). The fluorescent solution followed a circuitous route (toward the right side of the image) to flow into the marginal sinus (MS). The center was filled with fluorescent solution derived from three lymphatic channels. Figure 6C(b) shows a magnified view of Fig. 6C(a). The center was filled with fluorescent solution derived from the lymphatic channels. There was no flow from the PALN to the AALN, as shown in Fig. 3.
Fig. 4. Bifurcation patterns for the lymphatic vessel that lies between the SiLN and the PALN. A. Representative images showing one bifurcation: (a) unicolor; (b) gold; and (c) pseudocolor. The lymph flowed in a single direction from the SiLN to accumulate in the PALN. A vein ran parallel to the lymphatic vessel (LV). B. Classification of the bifurcation patterns into four types ($n = 20$): (a) no bifurcation, i.e., a single vessel (2/20); (b) one bifurcation (8/20); (c) two bifurcations (4/20); and (d) three bifurcations (6/20).

Fig. 5. Fluid drainage pathways in the SiLN after injection of fluorescent solution. A. Internal diffusion of fluorescent solution in the SiLN after injection. (a) 10 sec, (b) 20 sec, (c) 40 sec. B. Change in the mean fluorescence intensity in the SiLN and in the efferent lymphatic vessel (ELV). The fluorescence intensity in the SiLN became saturated at 40 sec, while the intensity in the region of the ELV began to increase sharply at 20 sec, and then increased steadily up to 40 sec. A 120 μL volume of fluorescent solution was injected into the center of the SiLN at a velocity of 50 μL/min, using a syringe pump.
Fig. 6. Fluid drainage pathways in the PALN after injection of fluorescent solution into the SiLN. A. Change in the fluorescence intensity in the PALN. (a) 0 sec, (b) 60 sec, (c) 120 sec after injection. ALV: afferent lymphatic vessel; LC: lymphatic channel. Scale: 1 mm. B. Relationship between the mean fluorescence intensity in the PALN and the time elapsed from the start of the injection. C. Fluid drainage pathways in the PALN in another mouse after the injection was terminated (after 144 sec). (a) The fluorescent solution derived from the two afferent lymphatic vessels emptied into the PALN around the periphery. The fluorescent solution took a circuitous route to the right-hand side and passed into the marginal sinus (MS). Scale: 1 mm. (b) The center was filled with fluorescent solution derived from the lymphatic channels and MS. Scale: 1 mm. The fluorescent solution (120 μL) was injected into the center of the SiLN at a velocity of 50 μL/min, using a driven syringe pump. PALN: proper axillary lymph node; ALV: afferent lymphatic vessel.

Next, we investigated the flow from the AALN to the PALN after injection of fluorescent solution into the AALN (Fig. 7). The inside of the AALN was filled with fluorescent solution 30 sec after the injection (Fig. 7Ab). The PALN began to fill with fluorescent solution after 60 sec (Fig. 7Ac), and filling continued up to 120 sec after the start of the injection (Fig. 7A(c-e)). Figure 7(B) shows the relationship between the mean fluorescence intensity and the time that had elapsed from the start of the injection; the plot labeled ‘none’ represents the area outside the PALN and AALN. The increase in fluorescence intensity of the AALN preceded that of the PALN; the fluorescence signal in the PALN increased 50 sec after injection. There was no increase in fluorescence in the area outside the PALN and AALN. From the data provided in Figs. 5 and 6, it may be seen that there was no lymphatic network from the SiLN to the AALN; however, there was lymphatic flow from the AALN to the PALN.

4. Discussion

The methodology of intranodal injection has been performed in humans for intranodal lymphangiography [10] as well as for therapeutic intervention [11]. However, the present paper is the first to show the concept of a using a lymphatic drug delivery system for injecting drugs, before surgical resection, into upstream LNs within the dissection area so that these
drugs are delivered to downstream LNs outside the dissection area via the lymphatic network. In MXH10/Mo/lpr mice that develop systemic swelling of LNs to sizes similar to those in humans, we show that the fluid drainage pathways in the SiLN, PALN and lymphatic vessels can be visualized in real time by injecting fluorescent solution into the SiLN. The results obtained can serve as the basis for the development of a lymphatic drug delivery system that could be used, before surgical resection, to convey drugs to LNs located outside the dissection area.

The SiLNs and PALNs of the MXH10/Mo/lpr mice used here had longitudinal diameters of 10-11 mm. When 120 μL of fluorescent solution was injected into the SiLN, the SiLN was filled by about 40 μL of this solution (Fig. 5), with the excess draining into the efferent lymphatic vessel to reach the PALN. The PALN was also filled by about 40 μL of solution (Fig. 6(B)). The remaining 40 μL of the injected solution likely drained into the lymphatic vessels/veins towards the liver/lungs via the blood circulation. In fact, metastasis can be induced in these organs by the injection of tumor cells into the SiLN [12]. We identified several patterns of branching and recombination in the lymphatic vessels extending from the SiLN to the PALN, and found that a single lymphatic vessel was relatively uncommon (Fig. 4(B)). The fluorescent solution flowed only in one direction from the SiLN to the PALN; this one-way flow is maintained by the operation of lymphatic valves (Fig. 3(C), 3(D)) [13]. Both the PALN and AALN are located in the axillary region. Although there was no lymphatic drainage from the PALN to the AALN (Fig. 6), a lymphatic network could be detected from the AALN to the PALN (Fig. 7).

In normal mice, with LN diameters of only 1-2 mm, it is difficult to develop techniques for injecting drugs/tracers directly into upstream LNs, thereby delivering them to downstream LNs [14]. Thus, in most previous papers, fluid drainage pathways in LNs and lymphatic vessels has been studied using interstitial/intradermal injections: from the chin to the neck and cervical LNs [15]; from the middle phalanges of the upper extremities to the axillary LN [16]; from the footpad to the popliteal LN [17, 18]; from the tissue around the exposed SiLN to the PALN [19]; from the hindfoot to the PALN [20]; from the tail to the ischial nodes [21]; and from the footpad to the the axillary node [21]. Using these approaches, it would take a considerable time to capture the filling of entire lymphatic vessels with tracers because the lymph velocity is less than 10 μm/s [22]. Thus, tracers might leak from the lymphatic vessels before they reached the end points the researchers wished to study, resulting in vaguely outlined lymphatic vessels. In the present paper, we succeeded in using microscopy and imaging techniques to identify LNs, lymphatic vessels and lymphatic valves after the injection of fluorescent solution. The clarity and detail of our findings may be due to the short time period between injection of the tracer and the recording of images. In Media 1, the flow velocity of the fluorescent solution was calculated to be 838 μm/sec, which is much faster than that of normal lymph flow by a factor of 800.

Many types of contrast media, such as albumin, dextran and liposomes, have been developed for use in lymphatic imaging following interstitial administration [23]. Once the technique for direct injection of drugs/tracers into lymphatic vessels is further established, it is hoped that new types of drugs/tracers will be developed with longer residence times in LNs [24]. In addition, technological improvements will be needed to allow the detection of micrometastases in LNs outside the dissection area. Currently, a LN size greater than 10 mm in the short-axis diameter, determined by CT, MRI or ultrasound, is considered to be the most important radiologic criterion for LN metastasis. However, this size-based characterization of LN metastasis frequently leads to erroneous diagnosis, for example, when metastatic LNs are of normal size, or when abnormally enlarged LNs are solely the consequence of reactive swelling.
Fig. 7. Fluid drainage pathways in the AALN and PALN after injection of fluorescent solution into the AALN. A. Fluid drainage pathways in the AALN and PALN. Fluorescent solution was injected into the AALN. Flow was detected from the AALN to the PALN. (a) 0 sec, (b) 30 sec, (c) 60 sec, (d) 90 sec, (e) 120 sec after injection (n = 2). B. Relationship between mean fluorescence intensity and the time elapsed from the start of the injection. PALN: proper axillary lymph node; AALN: accessory axillary lymph node; none: regions outside the PALN and AALN. The fluorescent solution (120 μL) was injected into the center of the AALN at a velocity of 50 μL/min, using a driven syringe pump.

To improve diagnostic accuracy, it will be vital to establish a new set of diagnostic criteria for LN metastasis based on the development and use of high-resolution imaging techniques and specific contrast agents. Recently, we have demonstrated, using a novel contrast-enhanced high-frequency ultrasound system, that alterations in blood vessel volume and density precede alterations in LN size in the early stages of LN metastasis [12, 25]. Detection of these changes by ultrasonography may offer new criteria for the early diagnosis of LN metastasis. Therefore, in the near future, it may be possible to prevent/cure early-stage metastasis to LNs outside the dissection area using a combination of a lymphatic drug delivery system and imaging techniques such as contrast-enhanced high frequency ultrasound imaging. Such a therapeutic approach could greatly improve the morbidity and mortality associated with breast cancer and head and neck cancer. The lymphatic drug delivery system described here is based on the hypothesis that the lymphatic network is accessible within and without the dissection area. It is well known that lymphangiogenesis occurs when cancer metastases block lymph flow and re-route lymph drainage. Indeed, Kwon et al. [26] have reported that lymphatic drainage can occur across the midline of the animal in response to cancer metastasis. In humans, SPECT/CT imaging has shown lymphatic re-routing in response to cancer metastasis [27]. Recently it has been reported that functional intraluminal valves prevent fluid communication between the lymphatic vessels and draining LN [28]. When we consider a lymphatic drug delivery system that uses therapeutic reagents such as liposomes and nanoparticles, the interaction of valves and reagents will be an important factor to be considered. Thus, the methodology of the drug delivery system will need to be optimized for various conditions.
5. Conclusion

Fluid drainage pathways in the SiLN, PALN and lymphatic vessels were monitored by direct injection of a fluorescent solution into the SiLN. Our results suggest that it will be possible to develop a lymphatic drug delivery system for use before surgical resection: micrometastases in downstream LNs outside the dissection area could be cured/prevented by injecting drugs (via the lymphatics) into upstream LNs within the dissection area. In the clinic, techniques such as ultrasound-guided internal jugular vein catheterization have already been established. Therefore, the lymphatic drug delivery system presented here should represent a feasible method for use in the near future.

Acknowledgments

This study was supported in part by JSPS KAKENHI Grant Numbers 26293425(SM), 26670856 (SM), 26242051(TK), and 24650286(TK).