Quantitative imaging of lymph function

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Sharma R, Wang W, Rasmussen JC, Joshi A, Houston JP, Adams KE, Cameron A, Ke S, Kwon S, Mawad ME, Sevick-Muraca EM. Quantitative imaging of lymph function. Am J Physiol Heart Circ Physiol 292: H3109–H3118, 2007. First published February 16, 2007; doi:10.1152/ajpheart.01223.2006.—Functional lymphatic imaging was demonstrated in the abdomen and anterior hindlimb of anesthetized, intact Yorkshire swine by using near-infrared (NIR) fluorescence imaging following intradermal administration of 100–200 μl of 32 μM indocyanine green (ICG) and 64 μM hyaluronan NIR imaging conjugate to target the lymph vascular endothelial receptor (LYVE-1) on the lymph endothelium. NIR fluorescence imaging employed illumination of 780 nm excitation light (~2 mW/cm²) and collection of 830 nm fluorescence generated from the imaging agents. Our results show the ability to image the immediate trafficking of ICG from the plexus, through the vessels and lymphangions, and to the superficial mammary, subiliac, and middle iliac lymph nodes, which were located as deep as 3 cm beneath the tissue surface. “Packets” of ICG-transited lymph vessels of 2–16 cm length propelled at frequencies of 0.5–3.3 pulses/min and velocities of 0.23–0.75 cm/s. Lymph propulsion was independent of respiration rate. In the case of the hyaluronan imaging agent, lymph propulsion was absent as the dye progressed immediately through the plexus and stained the lymph vessels and nodes. Lymph imaging required 5.0 and 11.9 μg of ICG and hyaluronan conjugate, respectively. Our results suggest that microgram quantities of NIR optical imaging agents and their conjugates have a potential to image lymph function in patients suffering from lymph-related disorders.

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THE IMPAIRMENT of lymphatic transport capacity occurs due to either 1) lymph vessel damage and subsequent insufficient repair processes, or 2) congenital defects leading to abnormal lymph vessel development. Regardless of the cause, the impairment causes fluid and protein accumulation, which in turn leads to lymphedema. Lymphedema is a lifelong condition progressing from swelling and scarring to immune dysregulation and malnourishment. No curative treatment exists for lymphedema that afflicts 300 million people worldwide (44). Congenital or primary lymphedema affects 1 in every 6,000 newborns (go to http://grants.nih.gov/grants/guide/pa-files/PA-04-071.html for more information) and can also appear at the onset of puberty (10). Acquired or secondary lymphedema is caused by the filaria parasite (in a condition referred to as elephantiasis) or by trauma due to radiation therapy, infiltrating cancer, surgery, or infection. In developing-world countries, 100 million people are afflicted worldwide by filariasis. In Western countries, acquired lymphedema afflicts three to five million people (32a). The etiology for trauma-associated, acquired lymphedema is thought to arise from the interruption of lymph channels coupled with postsurgical infection or radiation-induced skin reaction. The onset of symptoms, however, can occur from days, weeks, to years following the initial trauma, striking at a rate cited between 6 and 62.5% of breast cancer survivors who have undergone axillary lymph node dissection (1, 26, 38), up to 64% of all patients who undergo groin dissections (8), and 25% of all radical hysterectomy patients (4). Little is known about the molecular or functional basis of acquired lymphedema or which persons could be at risk for the condition. There is a paucity of strategies for predicting or managing lymphedema due in part to the lack of diagnostic imaging approaches to noninvasively and routinely measure lymphatic function. Since lymph function is also implicated in diseases of significant prevalence [such as diabetes (16), obesity (13), cancer (28), and asthma (2)], the ability to quantitatively image lymph function could have substantial impact on the health of the world’s population.

In this contribution, we present near-infrared (NIR) fluorescence lymph imaging as a new method to sensitively image lymph vasculature and quantitatively assess lymph function non-invasively within intact subjects. The feasibility of NIR fluorescence lymph imaging is demonstrated in a swine model that mimics the human dermis and lymph plexus architecture. We show that through the intradermal delivery of 100–200 μl of a nonspecific NIR fluorescent dye, we can visualize lymph propulsion across lymph vessels as well as compute the velocity of lymph flow and the frequency of lymph propulsion. In addition, we demonstrate a lymph molecular imaging agent based on hyaluronan, a ligand for lymph vascular endothelial receptor (LYVE-1) (3, 23) to molecularly target the lymphatic vasculature.

In MATERIALS AND METHODS, we describe the fluorescent contrast agents employed, animal models, optical imaging instrumentation, and image analysis to quantify lymph function. Dynamic lymph imaging results from the intradermal administration of indocyanine green (ICG), and the hyaluronan conjugate imaging agents are presented with movies presented in the on-line supplemental section. To place our work in context of other lymph imaging modalities, we briefly discuss the state-of-art in clinical and developmental lymph imaging approaches. Finally, we conclude by commenting on the clinical translation of NIR fluorescence enhanced optical imaging for quantifying lymph function in lymphatic diseases.

MATERIALS AND METHODS

Animal Models

Four 2-mo-old, 60 lbs. white Yorkshire swine (K Bar Livestock, HC 69, Box 270, Sabinal, TX) were imaged using protocols that were approved by the Baylor College of Medicine Institutional Animal
Care and Use Committee. The animals were anesthetized, intubated, and maintained with isoflurane. Animal body temperature was maintained at 100°F using a warming blanket. At the end of the procedure, the animals were euthanized, and lymph nodes were resected with fluorescence guidance. Swine were chosen for the lymph mapping study because swine dermis and lymphatic plexus are considered comparable to that of humans (19, 27).

**Imaging Agents**

Indocyanine green and hyaluronan-NIR dye conjugate contrast agents. To study the dynamic lymph flow, solutions of 3 to 32 μM of nonspecific ICG (NDC 17478-701-02, USP, Akorn, IL) were prepared by serial dilution in USP standard 0.9% sodium chloride (NDC 0409-4888-10, lot no. Hospira, Lake Forest, IL).

To molecularly target the endothelial lining of lymph channels, we developed a novel molecular imaging agent. Briefly, the imaging agent consisted of 2 molar percent of a modified IR-783 dye (Sigma-Aldrich Chemical, St. Louis, MO) conjugated to hyaluronic acid (Sigma-Aldrich) through a hexane-1,6-diamine linkage. For simplicity, we refer to the hyaluronan-based agent as HA-NIR. Figure 1 illustrates the chemical structure of the two imaging agents.

The imaging agent was administered using a “research catheter set” with 34-gauge, stainless steel cannula and 1-mm exposed length (Becton Dickinson Technologies Research Triangle, NC). A second intradermal delivery device used was “microcone device” with 34-gauge, stainless steel cannula of 1-mm exposed length (Becton and Dickinson Technologies). Imaging commenced immediately before the administration of agents and lasted for approximately 20 min. After imaging was completed, the lymph nodes were resected under the guidance of fluorescence. Frozen sections of the resected tissues were made at 4-μm thicknesses. Alternative frozen slices were stained with hematoxylin and eosin (H&E) for pathological confirmation of nodal tissues and imaged using a Leica microscope (Germany model number DM600B Leica Microsystems, Ernst-Leitz-Strasse). The NIR fluorescence arising from the dye in the unstained slices was imaged using 785 nm illumination (80 mW, model Sanyo DL7140–201s, Thorlabs, Newton, NJ) and an electron multiplying CCD camera (PhotoMAX, Princeton Instruments, Trenton, NJ) outfitted with the zoom system (Leica Z16 APO). The H&E and NIR fluorescence images were then overlaid to confirm localization of the NIR dye within the lymph node.

**NIR Fluorescence Enhanced Imaging**

Continuous-wave optical imaging of the fluorescent NIR dyes were performed with a home-built intensified charged-coupled device (CCD) described elsewhere (37). Briefly, the device has three principal components: 1) a NIR-sensitive image intensifier (model FS9910C ITT Night Vision, Roanoke, VA); 2) a 16-bit dynamic range, frame transfer CCD camera (Roper Scientific, Tucson, AZ); and 3) the 785-nm laser diode (Thorlabs) used to provide the excitation light for activating ICG and hyaluronan-NIR.

The 785-nm laser diode beam was expanded by using a plano-convex lens and a holographic optical diffuser such that ~0.08 m² of the swine’s body was illuminated. A 785-nm holographic notch band rejection filter (model HNPF-785.0-2.0, Kaiser Optical Systems, Ann Arbor, MI) and an 830-nm image quality bandpass filter (model 830.0 –2.0, Andover, Salem, NH) were placed before the 28-mm Nikkor lens (Nikon) to selectively reject the excitation light and pass the emitted 830-nm wavelength. A total of 400 to 1,000 images with 512 × 512 resolution and 200 ms exposure time were acquired enabling near-real time visualization of ICG trafficking. For image registration, white light images were acquired by replacing the holographic and bandpass filters with a neutral density filter and illuminating the surface of the swine with a low-power lamp.

**Image Analysis**

Images were processed using MATLAB (The Mathworks, Natick, MA), ImageJ (National Institutes of Health, Bethesda, MD), and V++ (Digital Optics, Auckland, New Zealand). ImageJ is an image...

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**Fig. 1.** Structure of contrast agents. A: indocyanine green (ICG). B: hyaluronan conjugated to NIR-783 dye.
analysis and processing program and supports optical imaging file formats. V+ V is the Roper Scientific CCD camera software interface with programmable modules for ICCD operation. MATLAB was used to compute average velocity and frequency of pulsatile lymph flow. To reveal the frequency of the pulsatile flow, which is a characteristic of pumping lymphatics (43), the mean fluorescence intensity from a fixed region of interest on a lymph channel was selected and plotted as a function of imaging time. Average velocity was computed by tracking the position of a “bolus” of ICG pulse moving along the length of a lymph channel.

RESULTS

Real Time Imaging of Lymph Function

Figure 2 represents a typical set of image frames from the movie (abdomen_vessel.mpeg) provided in the supplement section depicting nonspecific ICG trafficking in the lymph vessels of the swine abdomen after four 200-μl injections of 32 μM ICG using the “research catheter set” at the level of the third teats. Each imaged lymph vessel is associated with a single injection site. Lymph propulsion was visualized immediately upon administration of ICG. The vessels 1 and 4 in Fig. 2 drain to the superficial mammary nodes (also known as the inguinal nodes), and the vessels 2 and 3 drain to the subiliac lymph nodes that are located 2.5- to 3-cm deep. As illustrated in the supplemental material, the H&E and NIR fluorescence micrographs of resected fluorescent tissues confirmed dye deposition within the lymph nodes. The still frames are taken at intervals of 36 and 60 s, and the circle in Fig. 2 depicts a typical “packet” of ICG transiting the 12-cm long lymph vessel. Whereas ICG propulsion is not directly evident in all the lymph vessels in the still frames, quantitative analysis of the movie that provided in the supplemental section demonstrates that similar trafficking is seen in the three other lymph vessels.

Figure 3 presents an overlay of white light image of the swine’s anterior hindlimb and a typical fluorescent image frame from the supplemental movie (leg_vessel.mpeg) depicting ICG trafficking in leg lymph vessel. Upon intradermal delivery of 200 μl of 32 μM ICG using a “microcone” device, lymph flow immediately progressed from the site of injection to the middle iliac node and continued for 4 h, the duration for which the animal was anesthetized. In the movie, leg_vessel.avi contained in the supplemental section, the middle iliac node is already fluorescent before the intradermal injection in the hindlimb due to ICG drainage from the subiliac nodes from previous abdominal imaging as shown in Fig. 2. The movie also demonstrates propulsive lymph flow in the hindlimb as seen in the abdominal area. It is noteworthy that while we have performed imaging with ICG concentrations ranging from 3 to 322 μM, we obtained the best results with 32 μM ICG for the Yorkshire swine model.

Analysis of Lymph Function

To quantify pulsatile lymph flow, a stationary, circular region of interest (ROI) was identified on a fluorescent lymph vessel as shown in Fig. 4A. Figure 4B depicts the fluorescent intensity counts within the ROI as a function of time over 7 min of imaging time. The fluorescence intensity profile illustrates the typical, spontaneous propulsive lymph flow as indicated by the fluorescence intensity peaks repeating on an average of every 46 s when an ICG “packet” was propelled from the injection site toward the lymph node in the second (from left) abdominal afferent lymph vessel. As depicted in Fig. 5A, we identified ROIs across the length of the vessel. Figure 5, B and C, illustrates different views of a three-dimensional plot of fluorescence intensity as a function of vessel length and elapsed time after intradermal injection of 200 μl of 32 μM ICG within the second abdominal lymph vessel as shown in Fig. 2. The plot depicts eight trails of ICG packets (enumerated in Fig. 5, B and C) that were...
propelled from the injection site (at length = 0 cm) to the lymph node (at length = 12.5 cm). For example, at time 225 s, an ICG packet is present at 5 cm from the injection site. Lymphangion contractions propel the ICG forward, and after 4 s, the bolus is at 9.3 cm from the injection site. There is only one ICG packet present at any one time in one segment of the lymph vessel. As can be seen in Fig. 5B, each packet consistently accumulates within a certain segment of the lymph vessel before being emptied into a downstream section of the vessel. Interestingly, we find that at certain ROI locations and at all times, little or no fluorescence intensity is detectable, even though ICG “packets” are transited across the ROI. The red dashed arrow in Fig. 5B illustrates a consistent region of minimal fluorescence intensity for all the intensity trails of propelled ICG “packets.” We take this observation as evidence for probable lymphangions in which fluid accumulates before being propelled to the next sequential lymphangion (43). Figure 5C depicts the top view of Fig. 5B. Figure 5C (shown only for added clarity) illustrates eight ICG packets transiting along the lymph channels as a function of time.

The functional status of lymph channels can also be characterized by measuring velocity of transiting ICG “packets.” Figure 6 plots “snapshots” (from pulse_traffic.mpeg) of ROI fluorescent intensity as a function of vessel length from the site of administration at 28, 30, and 35 s. Superimposed on each plot are the time-averaged ROI fluorescent intensities with standard deviations plotted as a function of vessel length. As depicted in the image frames, a “packet,” as indicated by the dashed curve, could be clearly differentiated from the time-averaged fluorescent intensity, illustrated by the asterisks with error bars. To compute the velocity of propelled packet, the peak of the fluorescent intensity was tracked along the known vessel length that entered the lymph channel at the injection site at time “t” and exited the vessel at the lymph node at time “t + x,” where, x is a known elapsed time. The velocity was calculated as the total length of the vessel divided by the elapsed time x. Pulse frequency was computed by counting the number of peaks transiting a section of vessel in a specified interval of time.

We computed the values of lymph flow velocities and pulse frequencies from imaging on six abdomen and three hindlimb regional lymph vessels. Upon intradermal injection of ICG in the swine’s leg, the leg lymph vessel was observed to immediately take up the dye and propel the ICG “packets” toward the middle iliac lymph node at a frequency ranging from 3.3 to 6.2 pulses of ICG per minute and at a velocity ranging from 0.33 to 0.46 cm/s. Four hours after an injection on the leg, the lymph vessel still depicted active lymphangion contraction by propelling ICG “packets” at a rate of 1.3 pulses/min and at a velocity of 0.62 ± 0.23 cm/s. We also observed a range of velocities from 0.23 to 0.75 cm/s for ICG “packets” transiting in the abdominal lymph vessels, and each bolus of ICG dye or pulse of ICG passed through a fixed location on the vessel at a frequency of 0.5–1.3 pulses/min following 200 µl injection of 32 µM ICG. The rate of propulsion did not correlate to respiration or heart rate.
Staining of Lymph Vessels With HA-NIR Molecule

Figure 7, A and B, represents fluorescent images arising from intradermal injection of 150 μl of 64 μM hyaluronan conjugate (HA-NIR) and 32 μM ICG in the swine hindlimb vessel. Unlike the free, nonspecific ICG dye, HA-NIR “stained” the lymph vessel walls as it filled the vessel. Propulsion of packets of HA-NIR was not observed as was observed for ICG. Instead, HA-NIR uniformly demarcated the lymphatic vessels and lymph nodes for as long as the imaging study was conducted (4 h). With the use of the ROIs indicated on Fig. 7, A and B, the normalized fluorescent intensity values were plotted for the lymph vessels imaged with HA-NIR and ICG. Whereas fluctuation and net reduction of intensity as a function of time was seen for fluorescence owing to ICG lymph trafficking, Fig. 7C shows that the fluorescence intensity due to HA-NIR was almost constant and remained unchanged for the duration of image acquisition 6 min. The observation is consistent with the binding of HA-NIR to LYVE-1 present on the lymph endothelium.

To better display the differences between HA-NIR and ICG, Fig. 8, C and D, shows the three-dimensional plots of fluorescent intensity as a function of vessel length and time for HA-NIR dye and the nonspecific ICG dye in swine lymph vessels. For HA-NIR, the intensity remains constant with time but diminishes along the length of the vessel at increasing distances away from the injection site. This observation may be explained by either the variation in the depth of the vessel as it drains into the lymph node or due to the binding of HA-NIR to LYVE-1 and subsequent depletion of unbound HA-NIR as it transits along the lymph vessel away from the site of injection. On the other hand, the fluorescence intensity due to ICG varies with time and length along the vessel, showing “spikes” associated perhaps with the spontaneous lymphangion contractions and a reduction of intensity owing to its exit from the lymph channel.

DISCUSSION

In this study, we have presented a NIR fluorescence-enhanced optical imaging method to stain the lymph vessel and assess lymph function using ICG and a novel hyaluronan conjugate in anesthetized swine. Our results demonstrate that we can noninvasively image ICG trafficking in lymph channels, up to 16 cm in length, running from the lymphatic plexus through to lymph nodes.

Currently, there are two approaches used to image lymph within intact subjects: lymphangiography and lymphoscintigraphy. The earliest reports of lymphangiography are from Kinmonth (21), who used the selective uptake of blue dye administered intradermally to find and cannulate lymph vessels for infusion of oily, radiopaque contrast agents. Infection, lung embolization, pulmonary edema, respiratory distress, damage to the lymphatics, and the need for large volumes of radiopaque-imaging agents prevent lymphangiography from mapping lymph architecture in humans (40). Lymphangiography following intradermal uptake of iodinated and gadolinium-based contrast agents for computed tomography (CT) (7, 8)
and magnetic resonance (MR) (3, 13) imaging of lymph architecture has been demonstrated, but the techniques require intradermal administration of milliliter volumes of dextran-bound fluorescein to map local superficial lymph architecture with field of views <1 cm in diameter. Owing to the limited tissue penetration depth of the visible light used to excite fluorescein, it is unable to evaluate the lymph architecture spanning pre- and postcollectors through vessels and nodes as we demonstrate herein.

Other techniques similar to lymphangiography include the use of particles for imaging. When administered intravenously, particles at diameters up to 150 nm are removed from the circulation via liver endothelial filtration, and when particles are smaller than 10 nm, they are removed via blood vessels in the lymphoid parenchyma for ultimate deposition within lymph nodes (32). When administered intravenously, iron oxide particles of 4–5 nm in diameter are taken up by macrophages to provide negative enhancement of tumor-positive nodes for imaging via MR imaging. Cutaneous injection of gadolinium-laden dendrimer agents of 6–12 nm diameter (22) and submicron-diameter microbubbles (3, 24) have also been demonstrated preclinically to image the lymphatics of mice using MR and ultrasound imaging. Cutaneous and parenchymal administration of 15- to 20-nm diameter type II quantum dots have been used to determine lymph architecture intraoperatively using fluorescence optical imaging techniques (34, 35, 41, 42).

Regardless, none of these techniques offer temporal resolution and sensitivity for imaging lymph function noninvasively within intact subjects.

Lymphoscintigraphy is currently the “gold-standard” imaging technique to assess lymph drainage and consists of intradermal or intraparenchymal injection of filtered 99mTc-sulfur colloid (40–100 nm in diameter) or 99mTc-albumin nanocolloid (5–80 nm in diameter) for planar gamma scintigraphy to map the regional lymph system. Lymphoscintigraphy can also be performed using soluble, radiolabeled proteins such as 99mTc-labeled albumin (29), human immunoglobin (33), and mannosyl-dextran (9, 47) with subcutaneous and intradermal injections performed with varying degrees of reported success (33). As a diagnostic technique, lymphoscintigraphy has been utilized to guide and assess lymphedema treatment efficacy based on rates of lymphatic clearance, dermal backflow, and ratios or radioactivity in regions associated with axillary or inguinal nodal basins (42).

Yet, the lack of sensitivity of scintigraphy techniques necessitates long gamma camera integration times and prevents dynamic imaging needed to quantitatively assess lymph function. Table 1 provides a comparison of the imaging modalities used to assess lymph architecture and drainage.

The high sensitivity of fluorescence optical imaging arises due to the theoretically larger number of fluorescent photons available for imaging over the available gamma photons emitted in scintigraphy. Unlike a radiotracer, fluorophores can be safely reactivated within tissues by tissue-penetrating NIR excitation light. For a fluorophore with a lifetime of 1 ns and quantum yield of 10%, the maximum number of emitted photons for imaging is 10^8 photons per second per molecule as opposed to a radiotracer, of which its maximum output is one photon imaging event per tracer molecule. Indeed, the sensitivity of NIR optical imaging over scintigraphy techniques has been previously demonstrated with a dual-labeled optical and nuclear imaging agent (14).
In the past, researchers have utilized fluorescence in vivo microscopy to visualize superficial lymphatics and have reported lymph flow velocities of lymph capillaries to be 3 \( \mu \text{m/s} \) in tail skin of mice, 11 \( \mu \text{m/s} \) in frog lung, and 10 \( \mu \text{m/s} \) in superficial human skin (43). To the best of our knowledge, our results provide the first time report of lymph flow velocities in an intact animal, and they are three orders of magnitude greater than the lymph velocity in lymph capillaries. Previous measurements were performed using in vivo fluorescence microscopy to evaluate the flux of visible fluorescent dyes, such as FITC dextran, in the network of lymphatic capillaries 0.2–20 mm in length that were present in the plexus. These lymphatic capillaries have incompetent valves and illustrate two-way movement of lymph fluid that is finally drained into deeper lymph vessels that could not be viewed in the previous studies owing to the poor penetration depth of the visible light.

In contrast, in our studies, afferent lymph vessels up to 16 cm in length were studied. Our computed velocities suggest ICG that is propelled by lymphangion contractions in afferent lymph vessels that transport interstitial fluid unidirectionally from the lymphatic plexus to the lymph nodes.

Overall, fluorescence imaging of lymph is not new; microlymphangiography has been performed for a decade or more but has been limited to the cutaneous lymphatics owing to the poor penetration depth of the visible light. Previously, we imaged reactive lymph nodes within a canine population with spontaneous mammary disease following intravenous injection of a modified photodynamic agent and ICG (12, 44). McGreevy et al. (27) intradermally injected a red excitable imaging agent Cy5 conjugated to vitamin B12 to target lymph nodes with tumor cells that exhibit enhanced sequestration of the vitamin. Frangioni and coworkers have employed 15- to 20-nm diameter type II quantum dots injected within the foot pad of rodents (20), within the intraparenchymal space (34, 42), submucosal esophageal space (34, 35), and colonic mesentery of swine (41) to enable intraoperative detection of lymph nodes for resection and subsequent biopsy. Owing to the high quantum efficiency of quantum dots, the nodes could be detected 1 cm deep within the intraoperative surgical field but not within the intact swine. Nonetheless, none of these previous imaging studies demonstrated the ability to image lymph dynamically for assessment of function. Although the use of quantum dots may offer potential advantages, the concerns of the fate and toxicity of quantum dots due to their size (hydrodynamic diameter after pegylation) and the heavy metals they contain may hinder their translation into clinic for intraoperative lymph node mapping.
In 1999, LYVE-1 was identified as a lymph-specific receptor for hyaluronan. LYVE-1 is expressed on lymph endothelial cells as well as liver sinusoids, tubular epithelial cells of kidney, and cells from adrenal glands and pancreas (3, 6, 17). Whereas very recent controversy challenges the lymph-specific marker on the basis that macrophages also express LYVE-1 (39), the role of LYVE-1 in the lymphatics remains unclear, although it is thought to shuttle hyaluronan from tissue to lymph across the lymph endothelium via transcytosis. Hyaluronan itself is an extracellular matrix glycosaminoglycan that is an abundant component of skin and mesenchymal tissues where it functions to facilitate cell migration during wound

Table 1. Lymph imaging modalities

<table>
<thead>
<tr>
<th>Modalities</th>
<th>Imaging</th>
<th>Contrast Agent</th>
<th>Route</th>
<th>Volume</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray</td>
<td>Lymphangiography</td>
<td>Lymph vessels and nodes</td>
<td>Ethidol, Lipiodol</td>
<td>5–8 ml (human)</td>
<td>15, 30, 45, 46</td>
</tr>
<tr>
<td></td>
<td>Computed tomography</td>
<td>Lymph vessels and nodes</td>
<td>PFOB*, iodinated particles, (200–300 nm)</td>
<td>0.1–0.5 ml (human)</td>
<td>7, 18, 45, 46, 49</td>
</tr>
<tr>
<td>MR-lymphography</td>
<td>Lymph nodes and vessels</td>
<td>Gadolinium chelate (12 nm), USPIO‡, SPIO‡(4–5 nm)</td>
<td>Interstitial or intravenous injection</td>
<td>0.5 ml (human)</td>
<td>7, 24, 31, 45, 49</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Lymph nodes</td>
<td>Technitium TC-99m sulfur (40–100 nm) or albumin (5–80 nm)</td>
<td>Intradermal or subcutaneous injection</td>
<td>&lt;1 ml (human)</td>
<td>24, 44, 49</td>
</tr>
<tr>
<td>Nuclear medicine</td>
<td>Lymphoscintigraphy</td>
<td>Lymph nodes and vessels</td>
<td>Microbubbles, (≈2 μm)</td>
<td>0.5–2 ml (dog)</td>
<td>1, 7, 48</td>
</tr>
<tr>
<td>PET</td>
<td>Optical imaging</td>
<td>Lymph nodes</td>
<td>FDG-PET</td>
<td>5–10 ml (human)</td>
<td>25</td>
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<td>Microlymphangiography</td>
<td>Superficial lymphatics</td>
<td>Interstitial injection</td>
<td>10 μl (human)</td>
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<tr>
<td>NIR-particle imaging</td>
<td>Intraoperative lymph imaging</td>
<td>Quantum dots, 15–20 nm ICG</td>
<td>Cutaneous and parenchymal</td>
<td>100–200 μl (pig)</td>
<td>20, 34, 35, 41, 42</td>
</tr>
<tr>
<td>NIR-molecular imaging</td>
<td>Lymph nodes</td>
<td>Quantum dots, 15–20 nm ICG</td>
<td>Intradermal</td>
<td>100–200 μl (dog)</td>
<td>12, 36</td>
</tr>
</tbody>
</table>

PFOB, perfluorobromine; USPIO, ultrasmall superparamagnetic iron oxide; SPIO, superparamagnetic iron oxide; MR, magnetic resonance; PET, positron electron topography; NIR, nuclear infrared; ICG, indocyanine green.

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healing, inflammation, and embryonic morphogenesis. Effectively, HA forms a pericellular matrix around epithelial cells and fibroblasts reducing the level of intracellular adhesion. Hyaluronic acid is mobilized from tissue injury sites through the lymphatic vessels to lymph nodes where it is 90% degraded before entering the circulation with subsequent uptake by the liver. Levels of hyaluronic acid and its degradation products are known to be elevated during tissue injury, sepsis, and lymphedema. Hyaluronic acid is approved by the Food and Drug Administration and is commercially available for joint fluid therapy in osteoarthritis patients and is also being employed for correction of facial wrinkles. When administered into the lymphatic space, the hyaluronan-conjugated imaging agent may provide an efficient marker of lymph endothelium allowing noninvasive visualization of lymphatic channels as demonstrated herein, or for intraoperative lymph mapping. Currently, we are exploring the use of molecular specificity of hyaluronan to lymph endothelium and LYVE-1 in particular and are designating “smart” imaging agents, which report upon its degradation to study the roles of LYVE-1 and hyaluronan in the lymphatic system. By administering hyaluronan imaging conjugate intra-dermally for uptake into the lymphatic space, we can target the LYVE-1 expression in the lymphatics. Owing to the minute quantities administered and its degradation in the lymphatic system, the background that would otherwise occur with systemic administration is not observed in our studies.

Herein, our results suggest that the combination of low dose, rapidly cleared, soluble organic dyes and their conjugates could be used for noninvasive imaging of lymph function. ICG has been used safely for several years and is currently approved for use to evaluate hepatic function and retinopathy with doses ranging from 5 to 25 mg intravenously with as many as five repeated administrations. Upon combining intradermal administration with a sensitive NIR fluorescence detection system such as used herein, opportunities to use trace amounts of ICG or other NIR excitable agents for functional imaging may enable diagnosis of lymph impairment as well as its improvement after manual decongestive therapy. Other potential treatment strategies, such as vascular endothelial growth factor C gene therapy, could also be better evaluated with the ability to directly image lymph function in patients.

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