Novel Indocyanine Green–Phytate Colloid Technique for Sentinel Node Detection in Head and Neck: Mouse Study
Koji Araki, Daisuke Mizokami, Masayuki Tomifuji, Taku Yamashita, Kazunobu Ohnuki, Izumi O. Umeda, Hirofumi Fujii, Shigeru Kosuda and Akihiro Shiotani

Otolaryngology -- Head and Neck Surgery 2014 151: 279 originally published online 14 April 2014
DOI: 10.1177/0194599814530409

The online version of this article can be found at: http://oto.sagepub.com/content/151/2/279
Novel Indocyanine Green–Phytate Colloid Technique for Sentinel Node Detection in Head and Neck: Mouse Study

Koji Araki, MD, PhD¹, Daisuke Mizokami, MD¹, Masayuki Tomifuji, MD, PhD¹, Taku Yamashita, MD, PhD¹, Kazunobu Ohnuki, PhD², Izumi O. Umeda, PhD², Hirofumi Fujii, MD, PhD², Shigeru Kosuda, MD, PhD³, and Akihiro Shiotani, MD, PhD¹

Abstract

Objective. Sentinel node navigation surgery using real-time, near-infrared imaging with indocyanine green is becoming popular by allowing head and neck surgeons to avoid unnecessary neck dissection. The major drawback of this method is its quick migration through the lymphatics, limiting the diagnostic time window and undesirable detection of downstream nodes. We resolved this problem by mixing indocyanine green (ICG) with phytate colloid to retard its migration and demonstrated its feasibility in a nude mouse study.

Study Design. Experimental prospective animal study.

Subjects and Methods. Indocyanine green at 3 concentrations was tested to determine the optimal concentration for sentinel lymph node detection in a mouse model. Effect of indocyanine green with phytate colloid mixture solutions was also analyzed. Indocyanine green or mixture solution at different mixing ratios were injected into the tongue of nude mice and near-infrared fluorescence images were captured sequentially for up to 48 hours. The brightness of fluorescence in the sentinel lymph node and lymph nodes further downstream were assessed.

Results. Indocyanine green concentration >50 μg/mL did not improve sentinel lymph node detection. The addition of phytate colloid to indocyanine green extended the period when sentinel lymph node was detectable. Second echelon lymph nodes were not imaged in mice injected with the mixture, while these were visualized in mice injected with indocyanine green alone.

Conclusion. This novel technique of ICG–phytate colloid mixture allows prolonged diagnostic time window, prevention of downstream subsequent nodes detection, and improved accuracy for the detection of true sentinel lymph nodes.

Keywords

sentinel node navigation surgery, head and neck cancer, indocyanine green, phytate colloid

Received December 3, 2013; revised February 17, 2014; accepted March 14, 2014.

Introduction

Most cancers of the head and neck are squamous cell carcinomas (HNSCC) that arise from the mucosal surfaces of the upper aerodigestive tract. The standard treatment options for HNSCC are surgery and/or radiotherapy, with or without chemotherapy. However, aggressive treatment of HNSCC by these modalities often results in functional disorders such as loss of voice or severe dysphagia. Therefore, there is a need for less invasive treatment for HNSCC that would improve prognosis while maintaining a good quality of life.

HNSCC are known to have high rates of lymphatic metastasis even in early T stages.¹ The control of lymphatic metastasis is one of the most important issues in the treatment of HNSCC, since it has been proved to be the most important prognostic factor in these malignancies. Even occult metastases can become overt if not treated properly, but current imaging tests such as computed tomography

¹Department of Otolaryngology-Head & Neck Surgery, National Defense Medical College, Tokorozawa, Saitama, Japan
²Functional Imaging Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan
³Department of Radiology, National Defense Medical College, Tokorozawa, Saitama, Japan

This article was presented at the 2013 AAO-HNSF Annual Meeting & OTO EXPO; September 29–October 3, 2013; Vancouver, British Columbia, Canada.

Corresponding Author:
Koji Araki, MD, PhD, Department of Otolaryngology-Head & Neck Surgery, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama, 359-8513, Japan.
Email: kojaraki@ndmc.ac.jp
Sentinel lymph node navigation surgery (SNNS) can allow head and neck surgeons to avoid unnecessary neck dissection and reduce the incidence of postoperative morbidities such as facial edema, limited range of motion of the upper limbs, and shoulder pain. Several researchers have reported the successful application of SNNS for the management of HNSCC patients.\(^5\) Since minimally invasive, organ-preserving surgery for the primary lesions of HNSCC has become popular with the development of trans-oral resection procedures such as trans-oral laser microsurgery (TLM),\(^9\) trans-oral robotic surgery (TORS),\(^10\) or trans-oral video-laryngoscopic surgery (TOVS),\(^11\) the combination of these trans-oral surgical procedures with SNNS can provide minimally invasive treatment options with preservation of both organ and function for patients with both primary lesions, as well as those with lymphatic metastasis lesion.

Radionuclide (RN) methods using technetium-99m (\(^{99m}\)Tc)-radiocolloid are the best for accurately identifying sentinel lymph nodes (SLNs). However, RN methods have drawbacks, including radiation exposure and the consequent need to conform to statutory regulations that complicate the performance of SNSS for HNSCC. The high cost of a gamma probe is another impediment to the applicability of RN methods in SLN detection during surgery. In addition, the shine-through phenomenon encountered during the use of RN compounds generally hampers SLN detection, especially for HNSCC where the SLN is often located near the tracer injection site.

Near-infrared fluorescence (NIR) imaging has the potential to address the drawbacks of RN methods by allowing real-time optical detection of the SLN in the midst of surrounding tissues.\(^15\) SNNS techniques using real-time indocyanine green (ICG) fluorescent imaging are widely used in breast and gastric cancer.\(^19\) One of the advantages of these methods is that these can be easily used in the operating room. These enable technically easy and accurate tracer injection, compared to presurgical RN injection techniques into the larynx or hypopharynx under flexible endoscope guidance. Even though some reports have demonstrated improved detection sensitivity with the ICG method,\(^21\) it has a few problems, especially in the head and neck region. SLNs located in deeper tissue are difficult to detect because signal penetration of fluorescent probes is limited by tissue attenuation.\(^22\) Compression of the neck from the outside is one way to resolve this problem.

Another major problem of ICG is its quick migration through lymphatic channels, making it difficult to detect the true SLN. The problem is compounded by the undesirable visualization of other lymph nodes lying downstream.\(^23\) Together, these can lead to serious failure in detecting the true SLN with the help of ICG while performing SNNS in the head and neck region. A novel attempt was made to overcome this disadvantage by modifying the ICG method with the addition of phytate colloid. The feasibility of this method was assessed in a nude mouse study. Phytate colloid has been commercially available for a long time in Japan as a low-price instant-labeling hepatic scanning agent. As phytate colloid can easily form small-sized colloid particles in solutions such as serum that contain calcium ions, it is suitable for SLN detection. The use of this compound for SLN mapping has recently been covered by public health insurance in Japan.

The objective of this study was to assess the SLN detection ability of our novel modified ICG technique in which ICG is mixed with phytate colloid in a mouse model. The optimal ICG concentration for SLN detection in the mouse model was first determined. Subsequently, ICG at the optimal concentration was mixed with phytate colloid to evaluate if the mixture could overcome the problem of quick migration of ICG in the conventional ICG method.

### Materials and Methods

#### Animals

The use of animals in this study was reviewed and approved by the Institutional Animal Care and Use Committee of the National Defense Medical College. Animal experiments were conducted using 5- to 8-week-old athymic BALB/c nu/nu nude mice weighing 20 to 25 grams. All animal procedures were performed under general anesthesia by the intraperitoneal (ip) injection of medetomidine (0.8 mg/kg, ip) and ketamine hydrochloride (40 mg/kg, ip), or inhalation anesthesia with isoflurane. The depth of anesthesia was determined by toe pinch.

#### Preparation of ICG and ICG–Phytate Colloid Mixture

To prepare the stock solution, 25 mg of ICG (Diagnostick for injection, Daiichi-Sankyo Co, Ltd, Tokyo, Japan) was dissolved in 5-mL sterile water. The stock solution was diluted with sterile water to make working solutions of final concentrations of 5000 \(\mu\)g/mL (6400 \(\mu\)M, stock solution), 500 \(\mu\)g/mL (640 \(\mu\)M, 10 times dilution), and 50 \(\mu\)g/mL (64 \(\mu\)M, 100 times dilution).

Phytate colloid solution was prepared according to the manufacturer’s instruction manual of the Techne phytate kit (FUJIFILM RI Pharma Co, Ltd, Tokyo, Japan). ICG–phytate colloid mixture was prepared by mixing ICG with phytate colloid solution with different mixing volume ratios of 1:0 (ICG alone), 1:9, or 1:99. Final concentration of ICG was adjusted to 50 \(\mu\)g/mL (64 \(\mu\)M) in all mixtures.

#### Effect of ICG Concentration for SLN Detection

The effect of ICG concentration was first evaluated to find out its optimum concentration in the mouse model. Twelve nude mice were assigned to 3 groups of 4 mice each. Ten \(\mu\)L of ICG of the following concentrations (5000, 500, and 50 \(\mu\)g/mL) was injected with a 29 gauge needle into the tongue of nude mice of each group, respectively. SLN was detected as a fluorescent spot shining through the skin under a near-infrared

---

22 Compression of the neck from the outside is one way to resolve this problem.

23 Together, these can lead to serious failure in detecting the true SLN with the help of ICG while performing SNNS in the head and neck region. A novel attempt was made to overcome this disadvantage by modifying the ICG method with the addition of phytate colloid. The feasibility of this method was assessed in a nude mouse study. Phytate colloid has been commercially available for a long time in Japan as a low-price instant-labeling hepatic scanning agent. As phytate colloid can easily form small-sized colloid particles in solutions such as serum that contain calcium ions, it is suitable for SLN detection. The use of this compound for SLN mapping has recently been covered by public health insurance in Japan.

The objective of this study was to assess the SLN detection ability of our novel modified ICG technique in which ICG is mixed with phytate colloid in a mouse model. The optimal ICG concentration for SLN detection in the mouse model was first determined. Subsequently, ICG at the optimal concentration was mixed with phytate colloid to evaluate if the mixture could overcome the problem of quick migration of ICG in the conventional ICG method.

### Materials and Methods

#### Animals

The use of animals in this study was reviewed and approved by the Institutional Animal Care and Use Committee of the National Defense Medical College. Animal experiments were conducted using 5- to 8-week-old athymic BALB/c nu/nu nude mice weighing 20 to 25 grams. All animal procedures were performed under general anesthesia by the intraperitoneal (ip) injection of medetomidine (0.8 mg/kg, ip) and ketamine hydrochloride (40 mg/kg, ip), or inhalation anesthesia with isoflurane. The depth of anesthesia was determined by toe pinch.

#### Preparation of ICG and ICG–Phytate Colloid Mixture

To prepare the stock solution, 25 mg of ICG (Diagnostick for injection, Daiichi-Sankyo Co, Ltd, Tokyo, Japan) was dissolved in 5-mL sterile water. The stock solution was diluted with sterile water to make working solutions of final concentrations of 5000 \(\mu\)g/mL (6400 \(\mu\)M, stock solution), 500 \(\mu\)g/mL (640 \(\mu\)M, 10 times dilution), and 50 \(\mu\)g/mL (64 \(\mu\)M, 100 times dilution).

Phytate colloid solution was prepared according to the manufacturer’s instruction manual of the Techne phytate kit (FUJIFILM RI Pharma Co, Ltd, Tokyo, Japan). ICG–phytate colloid mixture was prepared by mixing ICG with phytate colloid solution with different mixing volume ratios of 1:0 (ICG alone), 1:9, or 1:99. Final concentration of ICG was adjusted to 50 \(\mu\)g/mL (64 \(\mu\)M) in all mixtures.

#### Effect of ICG Concentration for SLN Detection

The effect of ICG concentration was first evaluated to find out its optimum concentration in the mouse model. Twelve nude mice were assigned to 3 groups of 4 mice each. Ten \(\mu\)L of ICG of the following concentrations (5000, 500, and 50 \(\mu\)g/mL) was injected with a 29 gauge needle into the tongue of nude mice of each group, respectively. SLN was detected as a fluorescent spot shining through the skin under a near-infrared
fluorescence imaging system (Photodynamic eye, Hamamatsu Photonics K.K., Hamamatsu, Japan). Fluorescent images were photographed sequentially at 10 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours after ICG injection. The first fluorescent spot was defined as the SLN; fluorescent spots detected afterwards on the same side were defined as second echelon lymph nodes. The relative brightness of the SLN was determined by measuring the signal-to-background ratio (SBR).24

**Effect of ICG–Phytate Colloid Mixture on SLN Detection**

Twelve mice were assigned to 3 groups of 4 mice each to evaluate the effect of different mixing ratios of ICG–phytate colloid on SLN detection. Ten μl of the ICG–phytate colloid mixture solution of different mixing ratios (ICG alone, 1:9 or 1 part of ICG in 9 parts of phytate colloid solution, and 1:99 or 1 part of ICG in 99 parts of phytate colloid solution) was injected into the tongue of the mice with a 29 gauge needle. ICG concentration was 50 μg/mL in all solutions (settled by the results of previous experiment).

SLN and second echelon LNs were detected with a near-infrared fluorescence imaging system the same way as in the experiment on determining optimal ICG concentration. Fluorescent images were captured sequentially 10 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours after ICG–phytate colloid mixture solution injection. Relative brightness of SLN was determined by measuring SBR.

**Statistical Analysis**

Statistical analysis was performed with the nonparametric Mann-Whitney U test. P values < .05 were considered statistically significant. All values were expressed as the mean ± standard error.

**Results**

**Effect of ICG Concentration for SLN Detection**

Figure 1 shows the results after the injection of different concentrations of ICG. SLN was visible just after injection in all groups. The fluorescent intensity of SLN reached its maximum level around 3 to 6 hours after injection, then slowly decreased, and practically disappeared 24 hours after injection in all groups. Second echelon LNs were detected after 30 to 60 minutes in many mice. No obvious toxicity was found in all mice.

![Figure 1](image1.png)

**Figure 1.** Effect of different indocyanine green (ICG) concentrations. Sentinel lymph nodes (SLN) fluorescence was immediately visible, peaked at 3 to 6 hours, and generally disappeared by 24 hours. Second echelon LNs often appeared after 30 to 60 minutes. White arrow: second echelon LN.

**Effect of ICG–Phytate Colloid Mixture on SLN Detection**

Figure 2 shows the change in SBRs measured over time. Signal intensity increased right after injection, peaked after around 3 to 6 hours, and decreased in all groups. No significant difference was observed in the trend of change in fluorescent intensity among the groups. Therefore, we concluded that an ICG concentration more than 50 μg/mL (64 μM, 100× dilution of the stock solution) did not improve SLN detection; it was therefore decided to settle for an ICG concentration of 50 μg/mL (64 μM) in the next experiment.

![Figure 2](image2.png)

**Figure 2.** Effect of different indocyanine green (ICG) concentrations on sentinel lymph nodes (SLN) fluorescence brightness. No significant difference was observed in SLN fluorescence brightness among the groups injected with different concentrations of ICG.

**Effect of ICG–Phytate Colloid Mixture on SLN Detection**

Figure 3 shows the results of injecting ICG–phytate colloid mixture of different mixing volume ratios. SLN was visible in all groups. The fluorescent intensity of SLN reached its maximum level around 3 to 6 hours after injection, then slowly decreased, and practically disappeared 24 hours after injection in all groups. Second echelon LNs were detected after 30 to 60 minutes in many mice. No obvious toxicity was found in all mice.

![Figure 3](image3.png)

**Figure 3.** Shows the results of injecting ICG–phytate colloid mixture of different mixing volume ratios. SLN was visible.
30 minutes after injection in mice injected with ICG–phytate colloid mixture, while SLN was visible right after injection in mice injected with ICG alone. The fluorescent intensity reached its maximum level around 6 to 12 hours after injection. Some SLNs were detectable even after 24 hours, and no second echelon LN was observed in mice injected with ICG–phytate colloid mixture. No obvious toxicity was found in all mice.

**Figure 3.** Effect of indocyanine green (ICG)–phytate colloid mixture. Sentinel lymph nodes (SLN) appeared within 30 minutes, peaked at 6 to 12 hours, and remained detectable after 24 hours, with no visualization of second echelon lymph nodes.

30 minutes after injection in mice injected with ICG–phytate colloid mixture, while SLN was visible right after injection in mice injected with ICG alone. The fluorescent intensity reached its maximum level around 6 to 12 hours after injection. Some SLNs were detectable even after 24 hours, and no second echelon LN was observed in mice injected with ICG–phytate colloid mixture. No obvious toxicity was found in all mice.

**Figure 4.** Effect of different ratios of indocyanine green (ICG) and phytate colloid on sentinel lymph nodes (SLN) fluorescence. Fluorescence with ICG–phytate mixtures of both ratios differed significantly from that of ICG at 10 minutes, 30 minutes and 24 hours. *P < .05 when compared with ICG alone and ICG:phytate colloid 1:9, **P < .05 when compared with ICG alone and ICG:phytate colloid 1:99.

**Figure 4.** Effect of different ratios of indocyanine green (ICG) and phytate colloid on sentinel lymph nodes (SLN) fluorescence. Fluorescence with ICG–phytate mixtures of both ratios differed significantly from that of ICG at 10 minutes, 30 minutes and 24 hours. *P < .05 when compared with ICG alone and ICG:phytate colloid 1:9, **P < .05 when compared with ICG alone and ICG:phytate colloid 1:99.

The initial detection of SLN was delayed in mice injected with ICG–phytate colloid mixture, but the SLN remained detectable for much longer than in mice injected with plain ICG. These results indicate the advantageous prolongation of the detection time window of SLN with our modified ICG technique.

**Detection of Second Echelon Lymph Nodes**

**Table 1** showed the average number of second echelon LNs detected per mouse. Second echelon LNs were visualized only in mice injected with ICG alone, but not in mice injected with ICG–phytate colloid. These downstream nodes are not visualized with our novel ICG modified technique, which is an advantage because it improves the accuracy of detection of true SLNs.

**Discussion**

Though SNNS for head and neck region are considered as a technique for mainly melanoma and cutaneous SCC, recent reports for aerodigestive tract HNSCC supports the value of this procedure. Yamauchi et al\(^{25}\) demonstrated the results of meta-analysis in early HNSCC, in which a total of 16 studies (987 patients) were included. The identification rate, sensitivity, false-negative rate, negative predictive value, and accuracy were 95.2%, 86.3%, 13.7%, 94.2%, and 95.0%, respectively. We have also reported satisfactory results in a retrospective multicenter analysis of 177 patients with oral and laryngopharyngeal SCC in Japan.\(^{26}\) Fan et al\(^{27}\) reported the prognosis of cT1-2N0 oral tongue SCC treated with SNNS or elective neck dissection. They concluded that the extent of neck dissection can be reduced for SLN negative patients, owing to the non-inferiority in prognosis associated with SNNS. We have developed trans-oral video-laryngoscopic surgery that enables almost same resection with TORS for oropharynx, hypopharynx, and supraglottis cancer.\(^{11-14}\) Our experience with 60 patients demonstrated that more than 60% were clinically N0.\(^{15}\) Therefore SNNS that allows head and neck surgeons to avoid unnecessary neck dissection has great potential.
for establishment of minimally invasive and individualized treat-
ment for HNSCC.

This mouse study showed that our innovative technique of mixing ICG with phytate colloid successfully prolonged the SLN diagnostic time window and prevented downstream nodes from being visualized (Table 2). These features of the technique are expected to contribute to true SLN detection, especially in the head and neck region. ICG is injected around the tumor under rigid laryngoscope guidance when SNNS is planned at the same time as trans-oral resection of the primary tumor. Afterwards, the skin is incised over the fluorescent signal, and an attempt is made to detect and biopsy the SLN. These procedures take time, with the consequent risk of losing the true SLN in the midst of many downstream nodes that also fluoresce because of quick migration of ICG. In a preliminary report in patients with cancer of the oral cavity or oropharynx, Van der Vorst et al reported that the SLN could be identified within 5 minutes of injection in 7 out of 10 patients and within 30 minutes in all patients. They also commented that the average number of fluorescent lymph nodes significantly increased over time. In this context, our technique has the potential to prevent the quick migration of ICG and enable true SLN detection with greater assurance.

Phytate colloid is now available as an instant labeling kit in Japan. The simple addition of normal saline containing 99mTc pertechnetate to this labeling kit yields 99mTc-phytate colloid with diameters between 100 and 200 nm; the size of colloidal particles grows further upon reaction with ionized calcium in vivo, and these enlarged colloidal particles are preferentially phagocytosed by macrophages. Therefore, 99mTc-labeled phytate colloids are the tracers of choice for hepatosplenic scintigraphy. It has been reported that the ideal size of tracer particles for SNNS is between 20 and 500 nm. 99mTc-labeled phytate colloids are therefore used for SNNS in Japan. Small particles of 99mTc-phytate migrate right after injection to SLNs where these combine with calcium ions and increase in size. These larger particles of 99mTc-phytate with calcium are retained in SLNs for much longer periods without passing through to downstream non-SLN nodes; this property of phytate colloids makes them very suitable for SNNS. A report has demonstrated that colloidal particles tagged to radioactive and non-radioactive labels have a similar distribution in the body. Although we have not confirmed the detailed particle profile of phytate colloid mixed with ICG, our current experimental results suggest that ICG is likely to interact with phytate colloids and that ICG–phytate colloid should be distributed in the similar way as 99mTc-phytate colloid.

The preparation of ICG–phytate colloid for our technique entails nothing but mixing ICG solution with phytate colloid, a process that is simple and easy. Neither ICG nor phytate colloids are expensive; besides, both have been used with no ethical and safety issues in clinical practice in Japan for a long time. Therefore, ICG–phytate colloid mixture has a high potential for clinical application and it is hoped that attempts will be made to use this technique in a clinical setting in the near future.

It is also planned to demonstrate the use of hybrid ICG-99mTc-phytate colloid as a fluorescent and radioactive

<table>
<thead>
<tr>
<th>Table 1. Detection of Second Echelon Lymph Nodes at Various Intervals after Tracer Injection (Number of Second Echelon Lymph Nodes Per Mouse).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indocyanine green (ICG) alone (5000 μg/mL)</td>
</tr>
<tr>
<td>ICG alone (500 μg/mL)</td>
</tr>
<tr>
<td>ICG alone (50 μg/mL)</td>
</tr>
<tr>
<td>ICG:phytate 1:9</td>
</tr>
<tr>
<td>ICG:phytate 1:99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Comparison of Plain Indocyanine Green (ICG) with ICG–Phytate Colloid Mixture.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of sentinel lymph nodes (SLN) detection</td>
</tr>
<tr>
<td>Time of maximum brightness of SLN fluorescence</td>
</tr>
<tr>
<td>SLN detection after 24 h</td>
</tr>
<tr>
<td>Visualization of second echelon lymph nodes</td>
</tr>
</tbody>
</table>
tracer at the same time. A similar hybrid tracer technique using nanocolloid for SNNS in the head and neck region has been reported by researchers from the Netherlands who demonstrated that the lymphatic drainage pattern of hybrid ICG-99mTc nanocolloid was identical to that of 99mTc-nanocolloid with no adverse reactions.35,36 These findings warrant further evaluation of hybrid ICG-RN tracer techniques for SNNS to harness the convenience of intraoperative fluorescent guidance after initial RN localization. Our technique using phytate colloid instead of nanocolloid promises to offer the same advantages with the ICG-99mTc-phytate colloid conjugate migrating slowly through lymphatic channels, resulting in prolonged SLN detection with no visualization of second echelon LNs. Moreover, our technique is cheaper and might possibly be safer because phytate colloids are not proteinaceous in nature, unlike nanocolloids. This novel hybrid technique might therefore have a high potential for clinical application. To the best of our knowledge, there have been no basic studies on hybrid tracers of this kind, and the findings from such research could enhance the role of tracers for SNNS.

Furthermore, we are designing a new treatment strategy to target SLN. ICG is not only a tracer for NIR fluorescence imaging, but also as a sensitizer for photodynamic therapy (PDT) with an absorption maximum of 810 nm.57 The efficacy of ICG-enhanced PDT has been studied in various fields; however, the strong binding of ICG to plasma proteins leads to rapid clearance in the liver and minimizes selective toxicity for tumors. Side effects related to photosensitivity are also seen after systemic administration.58 In this context, our technique of conjugating ICG to phytate colloid could enable SLN-specific, targeted PDT by allowing a longer retention time of ICG in the SLN and preventing its migration to lymph nodes further downstream. For example, HNSCC patients without clinically evident lymph node metastasis (N0) undergoing chemotherapy or radiotherapy, can be offered SLN-targeted PDT by injecting ICG–phytate colloid around the tumor and exposing the site of the SLN repeatedly with near-infrared radiation of 810 nm wavelength to take advantage of the prolonged retention of ICG in the SLN. SLN-targeted therapy of this kind should have fewer systemic side effects and is expected to be effective in controlling lymph node metastasis in the head and neck region.

Conclusion
Our modification of the ICG technique for SNNS, consisting of the addition of phytate colloid to ICG, was able to prolong the diagnostic time window of SLNs and prevent the visualization of LNs lying further downstream. This simple and easy technique has the potential to overcome the disadvantage of quick migration of ICG and offer greater assurance in the detection of the true SLN. It also has the advantage of being ready for clinical application without ethical and safety issues. We hope to elucidate and assess the basic mechanism of this technique, apply it in a clinical setting, refine it further as a hybrid fluorescent-RN method, and utilize its potential in SLN-targeted PDT.


