A novel laparoscopic near-infrared fluorescence spectrum system with indocyanine green fluorescence overcomes limitations of near-infrared fluorescence image-guided surgery

Yuma Ebihara1,2, Liming Li3, Takehiro Noji1, Yo Kurashima1, Soichi Murakami1, Toshiaki Shichinohe1, Satoshi Hirano1

1Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan, 2Division of Minimally Invasive Surgery, Hokkaido University Hospital, Sapporo, Hokkaido, Japan, 3Department of Bio-material, Graduate School of Photonics Science, Chitose Institute of Science and Technology, Chitose, Hokkaido, Japan

Abstract

Background: Near-infrared (NIR) fluorescence image-guided surgery (FIGS) introduces a revolutionary new approach to address this basic challenge in minimally invasive surgery. However, current FIGS systems have some limitations – the infrared rays cannot detect and visualise thick tissues with low concentrations of the fluorescent agent. We established a novel laparoscopic fluorescence spectrum (LFS) system using indocyanine green (ICG) fluorescence to overcome these limitations.

Materials and Methods: Bovine serum albumin (BSA) was conjugated to ICG, and the mixtures were serially diluted at $5 \times 10^{-8} - 5 \times 10^{-1}$ mg/mL. We used the LFS system and a NIR camera system (NLS; SHINKO OPTICAL CO., LTD Tokyo, Japan) to determine the optical dilution for the fluorescence detection. BSA was conjugated to ICG ($5.0 \times 10^{-2}$ mg/mL) and used to coat the clips. We attempted to identify the fluorescence-coated clip from the serosal side of the cadaveric porcine stomach tissues using the LFS system and the NIR camera system. We measured the depth of the cadaveric porcine stomach wall at the thickest part that could be confirmed.

Results: We could not visualise fluorescence concentrations $<2.5 \times 10^{-3}$ mg/mL using the NIR camera system. The spectrum was detected at a concentration $<2.5 \times 10^{-3}$ mg/mL. We were able to identify the spectrum of ICG (829 nm) to a 13-mm depth of cadaveric porcine stomach wall by using the LFS system but could not identify the same with the NIR camera system regardless of wall thickness.

Conclusions: The novel LFS system with NIR fluorescence imaging in this ex vivo and cadaveric porcine model was confirmed useful at deeper depths and lower concentrations. Based on these findings, we anticipate that the LFS system can be integrated and routinely used in minimally invasive surgery.

Keywords: Indocyanine green, laparoscopic fluorescence spectrum system, near-infrared fluorescence imaging
INTRODUCTION

Near-infrared (NIR) fluorescence imaging has recently experienced rapid expansion on a global scale. With real-time intra-operative NIR fluorescence imaging of tumours and sentinel lymph nodes (SLN), the technique promises to guide the oncologic surgeon towards optimal radical resection and clinical results.\(^1\) NIR fluorescence image-guided surgery (FIGS) introduces a revolutionary new approach to address this basic challenge in minimally invasive surgery. The feasibility of laparoscopic NIR fluorescence imaging systems using indocyanine green (ICG) has recently been reported for the purpose of SLN mapping in various solid cancers.\(^2-4\) However, currently these systems have some limitations, including that the infrared rays cannot detect tumours within thick tissue using low concentrations of the fluorescent agent. We established a novel laparoscopic fluorescence imaging and spectrum (LFS) system to overcome these limitations.

MATERIALS AND METHODS

Novel laparoscopic near-infrared fluorescence spectrum system

The laser diode (wavelength, 785 nm; maximum output power, 5 mW) was used as the exciting light source. The laser beam for irradiation was focused on the central part of the fused silica coaxial fibre, which has a Y-type configuration [Figure 1]. A notch filter was used to filter out the reflected laser signal exiting the collecting fibre; thereafter, only the fluorescence signal was detected using the photonic multichannel analyser. The NIR fluorescence excited from the ICG sample was collected through the outer part of the coaxial fibre and measured by spectroscopy. Because the centre wavelength of fluorescence occurs at about 840 nm, which is close to the exciting wavelength of 785 nm, a narrow-band notch filter was used at the front of the input slit of the spectrocope to cut off the signal from the exciting light source. For details about the novel system, see our previous report\(^5\) [Figure 1].

Near-infrared camera system

We used a NIR camera system (NLS; SHINKO OPTICAL CO., LTD Tokyo, Japan) that activates ICG with emitted light (wavelength, 760 nm).

Fluorescent reagent

ICG, which was purchased from Diichi-Sankyo (Tokyo, Japan), is a popular diagnostic regent approved clinically for the examination of hepatic and circulatory function.\(^6,7\) ICG is a sterile anionic water-soluble but relatively hydrophobic tricarbocyanine molecule with a molecular mass of 776 Daltons. The ICG dye was developed for NIR photography by the Kodak research laboratories in 1955 and approved for clinical use in 1956 by the United States Food and Drug Administration. On being intravenously injected, ICG rapidly binds to plasma proteins, especially lipoproteins, with minimal leakage into the interstitium. An absorption peak at 780 nm and emission peak at 830 nm were observed.\(^8\) The absorption wavelength of water and haemoglobin containing an abundance of living tissue was <600 nm; thus, it was possible to excite the ICG.\(^9\)

Ex vivo indocyanine green detectability test

Bovine serum albumin (BSA) powder was purchased from FUJIFILM Wako Pure Chemical (Osaka, JAPAN) and dissolved in distilled water to achieve concentrations of \(5 \times 10^{-4} \text{–} 5 \times 10^{-1} \text{mg/mL}\). The BSA solution was added to the dye solution for the mixed solutions, and the mixtures were serially diluted in BSA solution to achieve 50- to 50,000-fold dilutions. To determine the optimal dilution for fluorescence detection, 100 \(\mu\text{L}\) of each dilution was added to individual wells in a 96-well plate and the plate was examined using the LFS system. This study was repeated thrice. Light emission detection by the LFS system was defined by a diphasic spectral graph with two peaks at approximately 780 nm. Our novel LFS system and a NIR camera system were used to emit excitation light from 10 mm away.

Indocyanine green detectability test with cadaveric porcine stomach

Endoscopic clips (model HX-610-135) were purchased from Olympus Medical System Corp. The front part of each clip was coated with BSA-conjugated ICG (5.0 \(\times\) \(10^{-2}\) mg/mL; the brightest concentration in ex vivo ICG detectability test) [Figure 2]. The distance was measured between the LFS system/NIR camera system and

![Figure 1: Novel laparoscopic near-infrared fluorescence spectrum system](image-url)
the clip positioned at the mucosal surface of the stomach wall. Our novel LFS system and the NIR camera system were used to emit excitation light from 10 cm away.

RESULTS

Serial dilutions of ICG conjugated to 0.05–0.75 g/mL BSA were imaged using the LFS system and NIR camera system. The concentration of $5.0 \times 10^{-2}$ mg/mL was brightest on the NIR camera system. We could not visualise fluorescence concentrations of $<2.5 \times 10^{-3}$ mg/mL using the NIR camera system. However, the spectrum was detected at the concentration of $<2.5 \times 10^{-3}$ mg/mL on the LFS system [Figure 3]. In the ICG detectability test with cadaveric porcine stomach, we were able to identify the spectrum of ICG (829 nm) to a 13-mm depth of the cadaveric porcine stomach wall using the LFS system [Figure 4] but not with the NIR camera system regardless of wall thickness.

DISCUSSION

FIGS is rapidly emerging as a complementary technique to conventional white-light reflectance imaging. Although several devices for FIGS have been described to date, only a few have progressed to clinical application. ICG is a popular diagnostic reagent that was approved clinically for the examination of hepatic and circulatory function.\(^{10}\)

The injected ICG binds rapidly to albumin and is carried more specifically through the vessels than indigo carmine or Evans blue.\(^{11}\) ICG-related allergic reactions are less common than those of blue dyes such as isosulfan blue.\(^{12}\) ICG has an absorption peak of 800 nm in vivo and is detected as a green colour. Detection by absorption spectroscopy is more sensitive than colour perception, and some surgeons reported that FIGS with ICG injection is useful for SLN detection.\(^{13,14}\)

In terms of ICG dye method limitations, such the loss of visibility in dense fat and rapid transit, some novel ICG-based techniques such as FIGS and ICG fluorescence imaging have been reported as convenient and reliable detection methods. However, we sometimes are unable to detect SLN using the NIR camera system due to a lack of clear delineation between the SLN and the surrounding fat tissue. Ishikawa et al.\(^{13}\) reported an obese patient with a false-negative SLN by laparoscopic SLN navigation using the NIR fluorescence laparoscopic system despite infrared rays being able to penetrate fatty tissue up to a depth of 3 mm. Kitai et al.\(^{15}\) noted in their preliminary report of ICG fluorescence imaging in breast cancer surgery that the sensitivity of fluorescence spectroscopy is much greater than that of absorption spectroscopy. They also reported that fluorescence was observed from an ICG solution embedded 1-cm deep in the material with optical properties compatible with human tissue in a preliminary study using a phantom. However, these systems have some limitations, including that the infrared rays cannot detect tumours within thick tissue or low concentrations of the

Figure 2: Representative near-infrared images of fluorescence-coated clip. The front part of clip was coated with bovine serum albumin-conjugated indocyanine green. (a) White light image. (b) Fluorescence imaging. (c) Spectograph. Arrows indicate the indocyanine green coated portion

Figure 3: Ex vivo indocyanine green detectability test. (a) Near-infrared system; (b) novel laparoscopic near-infrared fluorescence spectrum system. Serial dilutions of indocyanine green conjugated to 5–75 mg/mL bovine serum albumin were imaged using the laparoscopic fluorescence spectrum system and a near-infrared camera system. We could not visualize the fluorescence at concentrations of $<5.0 \times 10^{-3}$ mg/mL using the near-infrared camera system but were able to confirm fluorescence concentrations of $<2.5 \times 10^{-2}$ mg/mL in the laparoscopic fluorescence spectrum system.
Ebihara, et al.: Novel laparoscopic near-infrared fluorescence imaging and spectrum system

Fluorescent agent. Therefore, we developed the LFS system that detects the fluorescence spectrum as a solution to these problems. Spectrum analysis is one method to overcome the imprecision that results from a subjective assessment. It is a method that consists of separating fluorescent light into a spectrum and measuring the peak fluorescence value. The novel LFS system enables to measure the fluorescent light spectrum laparoscopically, which also makes it possible to detect the weak fluorescent light of ICG for the invisible lesion. Here, we validated the usefulness of a novel LFS system in ex vivo and cadaveric porcine stomach models. We found that the novel LFS system successfully overcomes the limitations of NIR FIGS.

CONCLUSIONS

The novel LFS system successfully detected ICG on the NIR fluorescence spectrum. Our findings suggest that the LFS system can be integrated and routinely used in minimally invasive surgery.

Acknowledgements

This study was supported by a grant from Kakken (25461938). The authors declare they have no competing interests relevant to this article. We would like to thank Editage (WWW.editage.jp) for English language editing.

Financial support and sponsorship

Nil.

Conflicts of interest

Division of Minimally Invasive Surgery, Hokkaido University Hospital is an endowment department, supported with an unrestricted grant from Advantest Corporation.

REFERENCES

1. Namikawa T, Sato T, Hanazaki K. Recent advances in near-infrared fluorescence-guided imaging surgery using indocyanine green. Surg Today 2015;45:1467-74.
2. Kusano M, Tajima Y, Yamazaki K, Kato M, Watanabe M, Miwa M. Sentinel node mapping guided by indocyanine green fluorescence imaging: A new method for sentinel node navigation surgery in gastrointestinal cancer. Dig Surg 2008;25:103-8.
3. Micog JS, Troyan SL, Hutteman M, Donohoo KJ, van der Vorst JR, Stockdale A, et al. Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescent sentinel lymph node mapping in breast cancer. Ann Surg Oncol 2011;18:2483-91.
4. Rocha A, Domínguez AM, Lécuru F, Bourel N. Indocyanine green and infrared fluorescence in detection of sentinel lymph nodes in endometrial and cervical cancer staging – A systematic review. Eur J Obstet Gynecol Reprod Biol 2016;206:213-9.
5. Li L, Ebihara Y, Shirogane R, Saito M. Near infrared fluorescence imaging and spectrum of indocyanine green for laparoscopy diagnosis in gastric cancer, Chin. Opt. Lett. 10 2012:S21003–S321005, https://doi.org/10.3788/COL201210. S21003.
6. Leevy CM, Bender J. Physiology of dye extraction by the liver: Comparative studies of sulfobromophthalein and indocyanine green. Ann N Y Acad Sci 1963;111:161-76.
7. Goresky CA. Initial distribution and rate of uptake of sulfobromophthalein in the liver. Ann J Physiol 1964;207:13-26.
8. Alander JT, Kaartinen I, Laaksö A, Pätilä T, Spellmann T, Tuchin VV, et al. A review of indocyanine green fluorescent imaging in surgery. Int J Biomed Imaging 2012;2012:940585.
9. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. Nat Med 2003;9:123-8.
10. Caesar J, Shaldon S, Chiandussi L, Guevara L, Sherlock S. The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. Clin Sci 1961;21:43-57.
11. Takayama S, Furuhama K, Ohura K, Onodera T, Akimoto T. Experimental studies on the usefulness of indocyanine green (ICG) as a lymphatic vital dye. Oyo Yakuri Pharmacometries 1980;19:603-14 (in Japanese).
12. Cimmino VM, Brown AC, Szocik JF, Pass HA, Moline S, De SK, et al. Allergic reactions to isosulfan blue during sentinel node biopsy – A common event. Surgery 2001;130:439-42.
13. Ishikawa K, Yasuda K, Shiromizu A, Etoh T, Shiraiishi N, Kitano S. Laparoscopic sentinel node navigation achieved by infrared ray electronic endoscopy system in patients with gastric cancer. Surg Endosc 2007;21:1131-4.
14. Nimura H, Naitomiya N, Mitsumori N, Yamazaki Y, Yanaga K, Urashima M. Infrared ray electronic endoscopy combined with indocyanine green injection for detection of sentinel nodes of patients with gastric cancer. Br J Surg 2004;91:575-9.
15. Kitai T, Inomoto T, Miwa M, Shikayama T. Fluorescence navigation with indocyanine green for detecting sentinel lymph nodes in breast cancer. Breast Cancer 2005;12:211-5.

Figure 4: Indocyanine green detectability test with cadaveric porcine stomach. We were able to identify the spectrum of indocyanine green (829 nm) to a 13-mm depth of the cadaveric porcine stomach wall using the laparoscopic near-infrared fluorescence spectrum system but were unable to identify it with the near-infrared camera system regardless of wall thickness. Negative control: Non-fluorescence-coated clip.