Published in final edited form as:

Transl Res. 2013 November ; 162(5): 324–331. doi:10.1016/j.trsl.2013.05.002.

First In-human Intraoperative Imaging of HCC using Fluorescence Goggle System and Transarterial Delivery of Near-infrared Fluorescent Imaging Agent: a Pilot Study

Yang Liu^{#1,2}, Yi-Ming Zhao^{#3}, Walter Akers¹, Zhao-You Tang³, Jia Fan³, Hui-Chuan Sun³, Qing-Hai Ye³, Lu Wang^{3,*}, and Samuel Achilefu^{1,2,4,*}

¹Department of Radiology Washington University, St. Louis, MO 63110, USA

²Department of Biomedical Engineering Washington University, St. Louis, MO 63110, USA

³Department of Liver Surgery, Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, P.R., China

⁴Department of Biochemistry & Molecular Biophysics, Washington University, St. Louis, MO 63110, USA

These authors contributed equally to this work.

Abstract

Surgical resections remain the primary curative interventions for hepatocellular carcinoma (HCC). However, lack of real-time intraoperative image guidance confines surgeons to subjective visual assessment of the surgical bed, leading to poor visualization of small positive nodules and the extension of diffuse HCC. To address this problem, we developed a wearable fluorescence imaging and display system (fluorescence goggle) for intraoperative imaging of HCCs in human patients. In this pilot study, both intravenous (i.v.) and transarterial hepatic (TAH) delivery of indocyanine green (ICG) were explored to facilitate fluorescence goggle-mediated HCC imaging. The results show that all primary tumors in patients (n=4) who received TAH delivery of ICG were successfully identified by the fluorescence goggle. In addition, 6 satellite tumors were also detected by the goggle, 5 of which were neither identifiable in pre-operative MRI and CT images nor by visual inspection and palpation. In the group (n=5) that received ICG by i.v., only 2 out of 6 tumors visible in the pre-operative MRI or CT images were identified with the fluorescence goggle, demonstrating the limitation of this delivery route for a non-tumor selective imaging agent. Comparative analysis shows that the HCC-to-liver fluorescence contrast detected by the goggle was significantly higher in patients that received TAH than i.v. delivery of ICG (P=0.013). This pilot study demonstrates the feasibility of using the fluorescence goggle to identify multifocal lesions and small tumor deposits using TAH ICG delivery in HCC patients.

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*Corresponding authors **Contact Information:** To whom correspondence should be addressed: Samuel Achilefu (achilefu@mir.wustl.edu) OR Lu Wang (wang.lu@zs-hospital.sh.cn).

All authors have read the journal's policy on conflicts of interest and have none to declare.

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Introduction

Hepatocellular carcinoma (HCC) is the third most predominant cause of cancer-related death worldwide [1, 2]. To date, the standard of care for HCC is still far from satisfactory, and prognosis of HCC is poor. Intraoperative ultrasonography (IUS) is currently used in the operating room (OR) to assist surgical resection of HCC. However, IUS is primarily a structural imaging modality that does not provide satisfactory contrast for small tumors and satellite foci [3-6]. The detection of superficially located nodules are particularly difficult to achieve with IUS [7]. In addition, current imaging systems typically rely on graphic display of imaging results on computer monitors, which may distract the surgeon from focusing on surgical site.

To address these challenges and complement current clinical modalities, we have developed a wearable real-time fluorescence imaging and display system, the fluorescence goggle [8]. The fluorescence information of tissues is imaged and directly displayed in the goggle eyepieces worn by a surgeon. The point of view of the goggle is also synchronized with the wearer's head movement and gaze direction [8].

In this pilot study, the fluorescence goggle is specifically designed to work with near infrared (NIR) fluorescent contrast agents. Due to regulatory hurdles in bring tumor-targeted NIR molecular probes to the clinic, most clinical studies have relied on the use of indocyanine green (ICG), which is approved for human use [9-13]. In general, ICG accumulates in the tumor largely by enhanced permeability and retention effect (EPR) or other mechanisms after i.v. administration [9, 10]. Although a small molecule, the binding of ICG to blood proteins increases the net size of the dye, which favors retention around porous vascular regions. Moreover, ICG can be cleared faster from the healthy liver than tumors, which further enhances the contrast. However, the poor tumor specificity, unpredictable pharmacokinetics, and cross-patient variability [10] are major limitations in the use of ICG for cancer imaging.

Unlike the hepatic parenchyma that receives only 25% of its blood supply through the hepatic artery, the hepatic artery is the primary source of blood supply for HCC [1, 2]. Thus, HCC presents unique pathophysiology that favors selective delivery of non-specific therapeutic and imaging agents to the target malignancies through transarterial hepatic (TAH) injection. This delivery route enables high tumor uptake with attendant reduction in systemic toxicity [1, 2]. In light of this, we hypothesized that transarterial delivery of ICG would facilitate rapid and selective uptake of ICG in HCC, providing high imaging contrast between the tumors and normal hepatic tissue. The fluorescence goggle demonstrated the feasibility of using both routes to image HCC intraoperatively, with the TAH ICG delivery route providing higher contrast in tumors than i.v. method.

MATERIALS AND METHODS

Fluorescence goggle system

The goggle system was developed at Washington University in St. Louis (WUSTL), USA and approved by the WUSTL Human Research Protection Office (FWA00002284) for use at the Liver Cancer Institute (LCI) at Zhongshan Hospital, Fudan University, China. The configuration of the fluorescence goggle used in this study is similar to the previously reported system [8], except the NIR light source now consists of four high-power LEDs (#H2A1-H760, Roithner Lasertechnik) and a short pass emission filter (775 nm; #NT64-615, Edmund Optics) was used in the clinical system. The average irradiance of excitation light on the liver surface is approximately 2.4 mW/cm² at a working distance of

0.5 m, illuminating an area of 12 cm in diameter. The field of view of the goggle system used in the current study is approximately 12 cm × 10 cm at a working distance of 0.5 m.

Patients

The surgical study was carried out according to the principles of the Declaration of Helsinki at LCI and approved by LCI's Institutional Review Board (2011-57). Written informed consent was given by all patients before the enrollment. Nine patients diagnosed with HCC who underwent a curative hepatic resection were enrolled in the study. The patients were randomly assigned into 2 groups (Group 1 and Group 2).

Administration of ICG

For Group 1 patients, ICG (0.5 mg/kg body weight) was injected i.v. within 5 days (mean 2.3 days) before surgery as part of routine ICG liver function test. For Group 2 patients, TAH delivery of ICG was performed intraoperatively. After immobilizing the liver, the surgeons identified branches of the hepatic artery feeding the tumors and threaded small catheters into these branches via gastroduodenal artery. ICG was injected slowly at a dose of 0.2 mg/kg body weight over 2 minutes through the catheter.

Intraoperative imaging with goggle system

The liver was first assessed intraoperatively by conventional methods such as visual inspection and palpation. A surgeon then wore the system for approximately 10 minutes to inspect the lesions under fluorescence imaging (Figure 1A). The fluorescence information obtained was used to guide further assessment of lesions. Contrast-enhanced threshold was set at a tumor-to-surrounding tissue contrast of 1.5 based on the real-time information offered by the fluorescence goggle. Subsequently, standard procedure for liver cancer resection was followed, and the goggle was used to image the surgical site sporadically. In addition to the primary tumors detected by CT or MRI, suspected lesions detected with the help of the fluorescence goggle were resected whenever possible after re-assessment by conventional methods. The average tumor-to-liver contrast was calculated using the ImageJ software. Briefly, the average fluorescence signals in the tumors and surrounding normal liver regions were obtained using ROI analysis. The average tumor signal in tumor region was divided by the average signal in the liver to obtain the average tumor-to-liver contrast.

Histopathological analysis

Standard histopathological analysis was conducted by three skilled pathologists following standard procedures.

Statistical analysis

Statistical analysis was performed with Graphpad Prism software. The tumor-to-liver contrast was reported as mean and standard deviation. To test the difference in fluorescence signal between tumors and corresponding surrounding liver tissues within each group, paired Student's t-test was performed. To test the difference in the tumor-to-liver contrast between Group 1 and Group 2, unpaired Student's t-test was performed. Statistical significance was considered for $P < 0.05$.

RESULTS

Patient characteristics are summarized in Table 1. Intraoperative fluorescence imaging with the fluorescence goggle system was conveniently done without interfering with existing surgical procedures, as the goggle system does not require any physical contact with patients. The surgeon rapidly adapted to the wearable goggle system and eyepiece display

with minimal learning curve. In addition, there was no noticeable motion artifact in the real-time imaging and display of the goggle. Tumor positions, extensions, and potential presence of small lesions were based on the fluorescence information obtained from the goggle system.

For Group 1 patients (n=5), the overall correlation between ICG fluorescence and cancer was low, and tumor-to-liver fluorescence contrast appeared to be highly variable across individuals. In 2 out of the 5 patients, high contrast between the tumors and surrounding liver tissues was observed by the goggle system (Figure 1B-E). Tumors that were not evident to visual inspection were clearly highlighted by the fluorescence goggle (Figure 1B, C). Results of *ex vivo* fluorescence inspection are in accordance with intraoperative imaging (Figure 1D, E) and histopathology was used to confirm HCCs in all patients. However, in the other 3 patients, the tumors did not exhibit higher fluorescence than the non-tumor liver tissue, and the fluorescence contrast was not sufficient to aid surgical resection. In one patient, false positive result was observed, where the healthy liver exhibited higher fluorescence signal than the tumor. In two other patients, tumors as well as surrounding healthy liver showed high fluorescence signal. Although the pilot study does not have statistical data to determine the sensitivity and specificity of the outcomes, it is clear that the non-specific uptake on ICG in tumors will have low specificity. A more tumor-selective molecular probe will overcome this limitation. In this pilot study, there was no significant difference in the fluorescence intensity between tumors and the healthy liver ($P=0.51$) among all 5 patients in Group 1 (6 tumors), and the average tumor-to-liver contrast was 1.93 (± 1.80 s.d.).

For Group 2 patients (n=4), the tumors were readily identified within 3 minutes after TAH delivery of ICG. We observed significantly higher fluorescence intensity than the surrounding healthy liver ($P < 0.0001$). In addition to identifying the primary tumors in all 4 patients (Figure 2), the goggle system also identified 6 satellite tumors. These smaller lesions were not clearly visible on pre-operative MRI and CT scans (Figure 2C, F), and 5 of them (80%) were not evident visually without fluorescence imaging (Figure 2A, D). The goggle clearly highlighted these lesions, which guided the surgeon to further inspect these suspicious areas (Figure 2B, E). Detection of tumor deposits smaller than 2 mm in diameter was possible. For Group 2 patients, the average tumor-to-liver contrast was 4.19 (± 1.37 s.d.) in all 10 tumors identified by the goggle. This is significantly higher than the fluorescence contrast obtained from the Group 1 patients ($P=0.013$). During surgery, the extension of the tumor was also visualized using the goggle, which aided gross surgical margin assessment (Figure 2). The best time window for tumor imaging after TAH delivery is between 2 min and 1 h. The signal in healthy liver gradually increases after the TAH delivery. However, no significant background interference from the healthy liver was observed during the first 2 h after TAH delivery.

The *ex vivo* results further confirmed our *in vivo* findings (Figure 2G-K). Histopathologic analysis validated the malignancy of all suspected fluorescent tissues (Figure 2I).

DISCUSSION

In this pilot study, the potential value of a new multidisciplinary approach was investigated to aid intraoperative HCC imaging. Combination of the fluorescence goggle system and TAH ICG delivery showed the potential for cancer-selective imaging and fluorescence surgical guidance. The fluorescence goggle design features provided real-time imaging of HCC to aid HCC resection in this study with minimal learning curve. The real-time imaging and display capability of the goggle is necessary to avoid motion artifacts. In conjunction with TAH delivery of ICG, the goggle provided large field of view and high tumor-to-liver

contrast. Large field of view allows for rapid and thorough inspection, while high contrast facilitated the detection of small and satellite tumors in the liver.

The pharmacokinetics and biodistribution of a non-specific agent such as ICG is typically unpredictable and may be subject to large cross-patient variations. Thus, there is no consensus yet on the optimal imaging time after i.v. injection [9, 10]. A previous study with stationary imaging system reported that only 51% of HCCs were identified intraoperatively in a clinical study with adequate statistical power (41 HCCs) [10]. Our data for Group 1 patients also shows that intraoperative tumor imaging based on i.v. delivery of ICG is only feasible in a subset of HCCs.

Based on this pilot study, the status of the liver function appears to mediate tumor-to-liver ICG fluorescence contrast in IV-injected patients. Patients who had unsatisfactory tumor-to-liver ICG contrast seemed to have significantly abnormal liver function tests. Probably, normal hepatobiliary excretion in the liver is compromised in patients with impaired liver function, resulting in higher ICG accumulation in the liver with attendant decrease in tumor-to-liver contrast. Thus, i.v. delivery of ICG may be sufficient for patients with less compromised than those with impaired liver function. Unfortunately, most HCC patients have impaired liver function because of the occurrence of many hepatic malignancies such as cirrhosis, hepatitis B, and hepatitis C. This study highlights the need for an alternative method to enhance ICG fluorescence in HCC, regardless of the liver function status. An obvious solution is to develop and use HCC-targeted molecular imaging agents when they become available for human application.

In contrast, we observed high tumor-to-normal liver fluorescence ratio post TAH injection of ICG. This could be attributed to the several factors but it is largely due to the direct perfusion of tumors through the hepatic artery. Unlike i.v. delivery where optimal imaging time is unpredictable and highly variable across individuals, the TAH delivery achieved optimal contrast within 5 minutes post administration. Importantly, the TAH delivery of ICG was conducted intraoperatively where the liver was exposed, allowing rapid determination of the optimal imaging window. The consistency across patients and short wait time are highly desirable for image guidance.

The goggle-aided fluorescence imaging system is best suited for detecting shallow lesions in the liver, where detection sensitivity, spatial resolution, and signal-to-noise ratio (SNR) are high. For the tumors deeply embedded in the liver, the resolution and SNR drop as the photon propagation transitions from ballistic to diffuse regime. Using the current prototype, liver tumors are best imaged at depths within 1.5 cm from the surface. Despite of the encouraging results obtained from this pilot study, there are several limitations. Firstly, the pilot study was designed to demonstrate the feasibility of detecting human tumor tissue during surgery with a small number of patients (n=9). The small patient population is not sufficient to derive reliable diagnostic accuracy, sensitivity and specificity from the method. Moreover, the human protocol requires that the study be conducted within the confines of routine clinical procedure without altering the normal course of diagnosis or treatment. Therefore, any suspicious lesion highlighted by the fluorescence goggle was not resected for histopathology if a surgeon determines that the tissue is not cancerous based on visual inspection and palpation. Clearly, the diagnostic accuracy, sensitivity, and specificity will be addressed in a future early phase human study with the appropriate statistical power and flexible human protocol. Secondly, pre-operative CT and MRI, visual inspection, and palpation were used in this pilot study to compare with goggle-aided fluorescence imaging. In future studies, comparison and correlation between fluorescence imaging and IUS are needed to fully assess the advantage of fluorescence imaging and how well it can complement IUS. Thirdly, the prototype goggle system used in this study is good for pilot

study, but not ergonomically optimized. We are currently developing new goggle systems that are more compact for future clinical studies.

CONCLUSIONS

We have investigated in this proof-of-concept study the potential values of intraoperative HCC imaging with a fluorescence goggle using i.v. and TAH delivery of ICG for contrast enhancement. We demonstrated that transarterial delivery enabled intraoperative HCC-selective imaging with FDA-approved NIR dye, ICG. With fluorescence guidance from the goggle system, successful identification of multifocal lesions and small tumor deposits were achieved.

Acknowledgments

This study is based upon work supported by the Mallinckrodt Institute of Radiology Development Fund, and in part by the US National Institutes of Health (NIH) grants R01 CA171651 and U54 CA136398 (Network for Translational Research). Yang Liu was supported by the US Department of Defense (DoD) Breast Cancer Research Program Predoctoral Award (W81XWH-11-1-0059). Walter Akers was supported by Award Number K01RR026095 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH and the DoD.

List of Abbreviations

HCC	Hepatocellular carcinoma
CT	computed tomography
MRI	magnetic resonance imaging
PET	positron emission tomography
IUS	intraoperative ultrasonography
NIR	near infrared
ICG	indocyanine green
FDA	Food and Drug Administration
EPR	enhanced permeability and retention effect
TAH	transarterial hepatic
H&E	Hematoxylin and eosin
WUSTL	Washington University in St. Louis
LCI	Liver Cancer Institute

References

- [1]. Akriviadis EA, Llovet JM, Efremidis SC, Shouval D, Canelo R, Ringe B, et al. Hepatocellular carcinoma. *Br J Surg*. 1998; 85:1319–31. [PubMed: 9782009]
- [2]. Rahbari NN, Mehrabi A, Mollberg NM, Muller SA, Koch M, Buchler MW, et al. Hepatocellular carcinoma: current management and perspectives for the future. *Ann Surg*. 253:453–69. [PubMed: 21263310]
- [3]. Arai S, Tanaka S, Mitsunori Y, Nakamura N, Kudo A, Noguchi N, et al. Surgical strategies for hepatocellular carcinoma with special reference to anatomical hepatic resection and intraoperative contrast-enhanced ultrasonography. *Oncology*. 2010; 78(Suppl 1):125–30. [PubMed: 20616594]

- [4]. van Vledder MG, Torbenson MS, Pawlik TM, Boctor EM, Hamper UM, Olino K, et al. The effect of steatosis on echogenicity of colorectal liver metastases on intraoperative ultrasonography. *Arch Surg.* 2010; 145:661–7. [PubMed: 20644129]
- [5]. Ukimura O, Okihara K, Kamoi K, Naya Y, Ochiai A, Miki T. Intraoperative ultrasonography in an era of minimally invasive urology. *International journal of urology : official journal of the Japanese Urological Association.* 2008; 15:673–80. [PubMed: 18564203]
- [6]. Buell JF, Cherqui D, Geller DA, O'Rourke N, Iannitti D, Dagher I, et al. The international position on laparoscopic liver surgery: The Louisville Statement, 2008. *Ann Surg.* 2009; 250:825–30. [PubMed: 19916210]
- [7]. van der Vorst JR, Hutteman M, Mieog JS, de Rooij KE, Kaijzel EL, Lowik CW, et al. Near-infrared fluorescence imaging of liver metastases in rats using indocyanine green. *J Surg Res.* 174:266–71. [PubMed: 21396660]
- [8]. Liu Y, Bauer AQ, Akers WJ, Sudlow G, Liang K, Shen D, et al. Hands-free, wireless goggles for near-infrared fluorescence and real-time image-guided surgery. *Surgery.* 149:689–98. [PubMed: 21496565]
- [9]. Gotoh K, Yamada T, Ishikawa O, Takahashi H, Eguchi H, Yano M, et al. A novel image-guided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. *J Surg Oncol.* 2009; 100:75–9. [PubMed: 19301311]
- [10]. Ishizawa T, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, et al. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. *Cancer.* 2009; 115:2491–504. [PubMed: 19326450]
- [11]. Sampath L, Wang W, Sevick-Muraca EM. Near infrared fluorescent optical imaging for nodal staging. *J Biomed Opt.* 2008; 13:041312. [PubMed: 19021320]
- [12]. Schaafsma BE, Mieog JS, Hutteman M, van der Vorst JR, Kuppen PJ, Lowik CW, et al. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. *J Surg Oncol.* 104:323–32. [PubMed: 21495033]
- [13]. Troyan SL, Kianzad V, Gibbs-Strauss SL, Gioux S, Matsui A, Oketokoun R, et al. The FLARE intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping. *Ann Surg Oncol.* 2009; 16:2943–52. [PubMed: 19582506]

Figure 1.

Fluorescence goggle system and goggle-aided fluorescence imaging with i.v. delivery of ICG. (A) Color picture of surgeon wearing fluorescence goggle during human HCC resection. Real time fluorescence information is imaged and displayed in the goggle eyepiece. (B-E) Successful cases of intraoperative imaging of HCCs with fluorescence goggle and iv delivery of ICG (Group 1). In 2 patients, tumors were successfully identified under fluorescence imaging with goggle. Intraoperative (B) Color and (C) fluorescence images of a patient. Intraoperative imaging with goggle showed the primary tumor and extension of tumor which were non-obvious to naked eye inspection and palpation. (D) Color and (E) fluorescence images of ex vivo inspection are also shown. In (B-E), yellow arrows indicate primary tumors.

Figure 2.

Intraoperative imaging of HCCs with fluorescence goggle and trans-arterial delivery of ICG (Group 1). (A) Color, (B) fluorescence and (C) pre-operative MRI images of a patient are shown. Multifocal tumors with non-defined boundaries were found on MRI. Intraoperative imaging with goggle showed the primary tumor, satellite tumors and extension of tumor which are non-obvious to naked eye inspection and palpation. In (A) and (B), blue arrows indicate the primary tumor and yellow circles outline satellite tumors. In (C), yellow arrow indicates the multifocal tumors. (D) Color, (E) fluorescence and (F) pre-operative CT images of another patient are shown. This patient suffered from multifocal tumors with non-defined boundaries. In (D) and (E), yellow circles outline the primary tumor, while green and yellow arrows indicate satellite tumors. In (F), yellow arrow indicates the multifocal tumors. Ex vivo (G) Color and (J) fluorescence image of tissues resected are shown. (H) and (K) are magnified images of an area of interest outlined by green rectangles in (G) and (J). Primary tumors (blue arrows) as well as satellite tumors (green rectangles) can be detected. Detection of tumor deposits fewer than 2 mm in diameter was possible. (I) A representative histological image of tumors detected by the fluorescence goggle.

Table 1

Patient characteristics.

Group Number	ICG Delivery Method	Patient Number	Gender	Histo-pathology	Position, Size and Embedded Depth of Primary HCC	In vivo Tumor-to-liver Fluorescence Contrast	Primary Tumor Detected in vivo by Fluorescence Goggle	Number of Satellite Tumors and Metastases Detected by Fluorescence Goggle
Group 1 (intravenous)	Intravenous delivery within 5 days prior to the surgery	5	M	HCC	Right lobe 4.9 × 4.4cm <0.5cm from surface	-	No	0
		6	M	HCC	Right lobe 6.1 × 4.2cm <0.5cm from surface	-	No	0
		7	M	HCC	Right lobe 5.3 × 4.9cm <0.5cm from surface	++	Yes	0
		8	M	HCC	Right lobe 1.5 × 1.5cm <1.5cm from surface	-	No	0
		9	M	HCC	Left lobe 2.8 × 2.5cm <0.5cm from surface	++	Yes	0
Group 2 (trans-arterial)	Trans-arterial delivery intraoperatively	1	M	HCC	Right lobe 3.1 × 3.3cm <0.5cm from surface	++	Yes	2
		2	M	HCC	Right lobe 12 × 10cm <0.5cm from surface	++	Yes	0
		3	M	HCC	Left lobe 5.2 × 4.8cm <1cm from surface	+	Yes	0
		4	M	HCC and Cholangio-carcinoma	Left lobe 4.3 × 4.8cm <0.5cm from surface	++	Yes	4

HCC: hepatocellular carcinoma; ++: strong tumor-to-liver fluorescence contrast; +: moderate tumor-to-liver fluorescence contrast; -: no tumor-to-liver fluorescence contrast.