Real Time Intraoperative Confocal Laser Microscopy-Guided Surgery

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Objective: To assess the potential utility of in vivo histologic surface and subsurface imaging in real-time using the Optiscan confocal laser microscope to detect diseased tissue at the time of surgery.

Summary Background Data: The goal of surgical treatment of diseases such as cancer is complete microscopic resection of diseased tissue; however, current methods for the assessment of extent of disease at the time of surgery are inadequate.

Methods: We assessed the potential of the Optiscan confocal laser microscope, a new device developed for real-time in vivo histologic surface and subsurface imaging during surgery.

Results: Intravenous Fluorescein Sodium contrast enabled visualization of cellular and architectural morphology of intra-abdominal organs with magnification equivalent to light microscopy and enabled differentiation between normal organs and disease.

Conclusions: Real time intraoperative confocal microscopy has significant potential application in detecting disease, and influencing decision-making at the time of surgery.

(*Ann Surg* 2009;249: 735–737)

A fundamental principle in the surgical treatment of diseases such as cancer is complete microscopic extirpation of diseased tissue. A current challenge in the surgical management of intra-abdominal malignancy is the accurate determination of the extent of disease at the time of operation so as to successfully accomplish this goal. Confocal endoscopic microscopy is a technique that currently provides real-time in vivo microscopic images of gastrointestinal tract mucosa during endoscopy,[1,2] and has significant potential clinical utility in colorectal cancer screening,[3] surveillance for cancer in ulcerative colitis,[4] detection of *Helicobacter pylori* infection,[5] diagnosis of gastric cancer,[6] detection of Barrett's associated neoplasia,[7] and in the evaluation of celiac disease.[8] Here, we present the first report of novel technology developed based on endoscopic methodologies, in the surgical management of intra-abdominal malignancy.

The Optiscan intraoperative confocal laser microscope is an adaptation of endoscopic technology to enable real-time in vivo histologic surface and subsurface imaging during surgery. Our aim was to assess the potential utility of this new technology in providing rapid real-time in vivo microscopic histologic information during surgical exploration and resection of intra-abdominal malignancy and assess its potential in assisting the surgeon in decision making at the time of operation, particularly in defining the extent of disease.

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Supported by Cancer Institute New South Wales, The Cancer Council New South Wales, National Health and Medical Research Council of Australia, R. T. Hall Trust.

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ISSN: 0003-4932/09/24905-0735
DOI: 10.1097/SLA.0b013e3181a38f11

METHODS

Ten patients who underwent surgery for intra-abdominal malignancy (gastric adenocarcinoma, pancreatic ductal adenocarcinoma, and small bowel lymphoma) were used in the study. Approval was granted by the Human Research Ethics Committee of the Sydney South West Area Health Service, with written informed consent obtained from all participating patients.

The Optiscan intraoperative confocal laser microscope consists of an optical unit (OU-1000), control unit (CU-1000), monitor, footswitch for depth control, and a rigid imaging probe with a sterilizable and removable rigid outer sheath. The sterile outer sheath has a working length of 320 mm and a diameter of 6.3 mm. Images produced are of an area of 475 μm^2 with 1000× magnification. The device is a clinical grade prototype, and therefore does not have full product certification; however, it is based on confocal technology that Optiscan supplies to Pentax for the ISC-1000 flexible endomicroscopy system and uses identical hardware to the Pentax ISC-1000, which has undergone electrical safety and electromagnetic compatibility testing for Food and Drug Administration Class II, CE, and Therapeutic Goods Administration regulatory approvals.

Once surgical access to the abdominal organ of interest was established, a fluorescent contrast agent (Fluorescein Sodium, 1 mL of 10% solution) is injected intravenously. With sterile water-based lubricating gel applied to the imaging window of the rigid microscope, the tip of the probe is then placed in direct contact with the organ of interest for imaging (Fig. 1A). Footswitch controls are used to adjust the depth of the imaging plane and obtain surface and subsurface microscopic views up to a depth of 200 μm.

Fluorescence based confocal laser microscopy was developed and used in this study because of its superior resolution compared with non-fluorophore–based systems, and as a consequence fluorescein is essential for imaging. A laser emitted from a light source along the optical fiber of the probe is focused onto the tissue plane of interest. Upon stimulation by the laser light source, an exogenously administered fluorescent agent reflects the light back at a different wavelength. Through movement of the lazer along the x and y axes, a 2-dimensional image is created. The focusing lens, furthermore, can be moved along the z axis to vary the depth of imaging.

The system uses a solid state laser that emits a single visible blue wavelength specifically to excite fluorescein (488 nm). Laser power delivered via the probe to the specimen can be adjusted (using touchscreen controls) during imaging between 0 and 1000 uW (1 mW). Generally, mid laser power (around 500 uW) was used for in vivo imaging of abdominal organs during surgery (the laser power used to capture each individual image can be viewed on the instrument's review mode). The laser power required depends on the intensity of the fluorescent signal coming back from the tissue, and is influenced by the concentration and distribution of the fluorescent agent within the tissue. Immediately after injection, the fluorescent

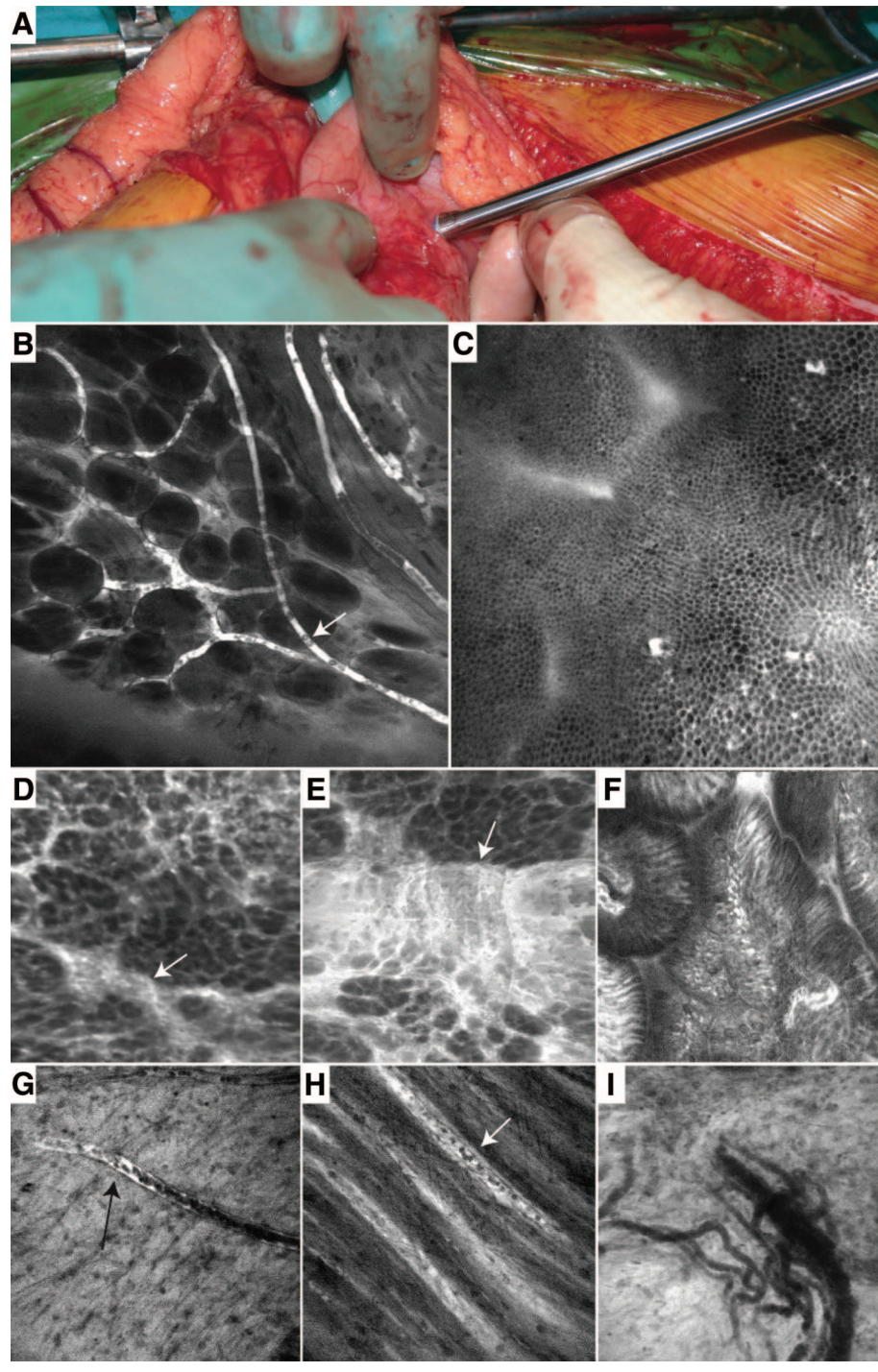

FIGURE 1. A, The application of rigid hand-held confocal microscopy during intraoperative exploration. Confocal images of: B, normal omentum with fat globules within adipocytes and capillaries with visible erythrocytes (arrow); C, normal bile duct epithelium; D, normal pancreas with regular lobular architecture of acini, but with a small amount of fibrosis (arrow); E, chronic pancreatitis with increased fibrous tissue interrupting the normal lobular architecture (arrow); F, normal small intestinal mucosa showing villi and individual epithelial cells; G, normal small intestinal serosa with blood vessel containing erythrocytes (arrow); H, a deeper tissue plane of the same area showing blood vessels in smooth muscle; and I, dilated and tortuous subserosal vessels associated with small intestinal lymphoma.

signal may be very bright especially within the vessels, so a low laser power setting of 200 uW may be adequate. As fluorescein diffuses into the tissue and is gradually cleared over time, the fluorescent signal may attenuate, so laser power can be increased to excite the remaining fluorescein. When trying to image deep structures, however, more laser power will not improve the resolution or image quality (resolution is limited by the light-scattering properties of the tissue and optical capabilities of the confocal system). Too much laser power can result in saturation of the image. Subtle changes in laser power over time are usually sufficient for in vivo imaging, although significant interactive adjustment of laser power can be required if one structure of interest (eg, a pool of fluorescein on a bleeding mucosal surface) is significantly brighter than the underlying structure (eg, intercellular distribution in the epithelium).

The confocal system also has separate image Brightness and Gamma controls that can be adjusted to help highlight a particular feature in the image (each image is made up of a series of pixel intensity measurements). These controls alter the display of the

image (eg, make certain pixels of a certain intensity value brighter or darker), but do not change the raw data collected.

The optical properties of the tissue also influence the depth of imaging possible. Shorter wavelengths (eg, ultraviolet) are highly scattered and do not penetrate deeply into tissue. Use of visible wavelengths (eg, fixed wavelength of 488 nm in this system) allow imaging from the surface to a depth of 200 μm. Longer wavelengths of light (eg, near infrared) interact less with tissue, therefore penetrate deeper, and as a consequence may allow slightly deeper imaging of tissue (subject to an appropriate fluorescent contrast agent being available).

RESULTS

Following intravenous administration of 10% fluorescein, the morphologic features consistent with the expected microscopic appearance of the pancreas, duodenum, small bowel, bile duct, omentum, and stomach were clearly visible (Fig. 1) and consistent between individual patients in the study. The vascular architecture of the pancreas and omentum, columnar epithelium of the bile duct, and villi of the small bowel were visualized, as were individual cells as fluorescein diffused into interstitial spaces. Magnification and resolution was equivalent to 1000× light microscopy with biconcave erythrocytes clearly visible within capillaries (Fig. 1B).

Confocal image data were collected at a scan rate of 1.6 frames per second (1024 × 1024 pixels). At each site of interest, the depth of imaging was varied using the "z" control that allowed for surface imaging down to a depth of up to 200μm. Approximately 7 to 10 images at different depths were captured from each site after stabilization of the probe perpendicular to the organ surface and applying light pressure. The depth control can be operated rapidly and each site could be fully evaluated from the surface down to the subsurface in less than 1 minute and in total added only 15.7 ± 4.3 minutes (range: 7–20 minutes) to each operation. A total of 2420 confocal images were obtained from 10 patients, with a mean of 242 ± 77 images for each patient, and an average of 40 per organ examined. There were no adverse effects related to the procedure, in particular there was no staining of the tissues with Fluorescein, and there was no tissue injury from the probe.

When applied to the surgical transection margins of the pancreas for pancreatic cancer, confocal examination differentiated between the morphologic appearances of chronic pancreatitis and normal pancreas (Figs. 1D, E). In normal pancreas, the regular lobular architecture of pancreatic acini is preserved, but interrupted by fibrous septae in chronic pancreatitis. Subsequent histopathological assessment of the resected specimen using light microscopy identified similar appearances of chronic pancreatitis. In an effort to identify features that differentiated diseased from normal small intestine, we examined the confocal microscopic appearance of the serosal surface of macroscopically normal small bowel, which demonstrated normal vasculature (Figs. 1G, H). When the confocal microscope was applied to the region suspected to harbor either inflammation or malignancy, which was the cause of the patients' anemia, distended and tortuous vasculature was visible (Fig. 1I). This area was resected and demonstrated a small intestinal lymphoma on histopathology. Histopathological examination of diseased and normal organs was representative on confocal images in all cases; however, the exact microscopic sites were not compared. Although surgical margins were examined for tumor at the time of resection, all resection margins were clear of tumor. Hollow viscera were not opened for confocal examination of the tumor at the time of surgery.

Specific hurdles to obtaining good quality images included stability of the probe and a good seal to the organ of interest, which required application of a water-soluble lubricant. Stabilization of the probe and the organ of interest bimanually by the operator minimized movement artifact. Organs with thick capsules such as the liver, or a layer of adipose tissue such as the pancreas limited imaging due to the depth of penetration being insufficient for parenchymal imaging.

DISCUSSION

This first assessment of intraoperative confocal microscopy for intraoperative examination of abdominal organs suggests that it is feasible to use confocal microscopy to rapidly assess the in vivo histology of various abdominal organs, with recognizable microscopic architecture and cellular features of each organ. Furthermore, the device is ergonomic for use during surgery, not time-consuming, and safe.

This new technology has potential applications that would substantially alter current surgical practice and optimize intraoperative management, particularly for cancer therapy. A central principle in the operative management of malignancy is the complete or maximal extirpation of diseased tissue. Occult metastatic disease is difficult to detect, and the extent of resection is often guided by the probability of achieving clearance, with knowledge concerning resection margins only determined some days after surgery. Examples include assessment of metastatic disease for cancer of the upper gastrointestinal tract, pancreatectomy either for pancreatic cancer, or precancerous lesions such as intraductal papillary mucinous neoplasms, debulking of peritoneal disease in ovarian cancer and resection of liver metastases. Intraoperative sampling with histologic frozen-section examination is commonly used, is often suboptimal and can be time-consuming, adding up to 60 minutes onto operating times. A better understanding of the morphologic appearances on confocal microscopy and correlation with histopathological appearances on light microscopy is required to define its applicability. Foreseeable future applications include the selective targeting of cancer cells using fluorophore-labeled ligands that would selectively bind to the surface of cancer cells and more accurately detect malignancy. Different fluorophores for deeper penetration using longer wavelength, and an ability to differentially label cell nuclei would also facilitate the advancement of this technology.

In conclusion, real-time "virtual histologic" examination during intra-abdominal surgery using the intraoperative confocal laser microscope is feasible, reproducible and safe. The ability of this technique to differentiate normal and diseased tissue has significant potential in guiding and assisting surgeons in deciding the type as well as the extent of surgery. The potentially significant benefits of this new technology warrant further investigation to define its role in the surgical treatment of cancer and other diseases.

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