Probing pancreatic disease using tissue optical spectroscopy

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Abstract. Pancreatic adenocarcinoma, one of the leading causes of cancer death in the United States, has a five-year survival rate of only 4%. Present detection methods do not provide accurate diagnosis in the disease’s early stages. To investigate whether optical spectroscopy could potentially aid in early diagnosis and improve survival rates, reflectance and fluorescence spectroscopies were employed for the first time in a limited pilot study to probe freshly excised human pancreatic tissues (normal, pancreatitis, and adenocarcinoma) and in vivo human pancreatic cancer xenografts in nude mice. In human pancreatic tissues, measurements were associated with endogenous fluorophores NAD(P)H and collagen, as well as tissue optical properties, with larger relative collagen content detected in adenocarcinoma and pancreatitis than normal. Good correspondence was observed between spectra from adenocarcinoma and cancer xenograft tissues. Reflectance data indicated that adenocarcinoma had higher reflectance in the 430- to 500-nm range compared to normal and pancreatitis tissues. The observed significant differences between the fluorescence and reflectance properties of normal, pancreatitis, and adenocarcinoma tissues present an opportunity for future statistical validation on a larger patient pool and indicate a potential application of multimodal optical spectroscopy to differentiate between diseased and normal pancreatic tissue states. © 2007 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.2818029]

Keywords: fluorescence; reflectance; tissue spectroscopy; carcinoma; pancreas; time resolved.

Paper 07258LR received Jul. 19, 2007; revised manuscript received Aug. 30, 2007; accepted for publication Sep. 3, 2007; published online Dec. 14, 2007.

Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the United States.1 This dismal prognosis results from most symptomatic patients having unresectable disease at clinical evaluation, leading to a five-year survival rate of 4%.1,2 Current diagnostic methods, including computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS), have not been able to provide accurate diagnosis in early stage disease,3 either by failing to identify small lesions or accurately differentiating masses as either adenocarcinoma or pancreatitis (inflammation of the pancreas). These challenges occur due to the relative inaccessibility of the pancreas given its anatomical location, the non-specific nature of symptoms (pain and weight loss), as well as the characteristic stromal reaction with intense fibrosis associated with both adenocarcinoma and pancreatitis.3 This significantly complicates attempts to differentiate the two similar appearing lesions by imaging, even with cytological evaluation of needle aspirates.4 Consequently, patients may undergo major surgery to reveal only benign or inflammatory disease on pathologic examination. One study reported that as many as 9.2% of surgeries were negative for neoplastic disease, even though they had been performed for a clinical suspicion of malignancy.5 Thus, in vivo detection of the cancer at an early stage could greatly improve the chances of patient survival by meeting a critical unmet need of accurately differentiating masses from pancreatitis. Optical spectroscopy could be employed via fiber-optic probes guided by imaging such as EUS to provide incremental biochemical and morphological information about tissue to improve diagnostic accuracy.

Reflectance spectroscopy can provide information about tissue morphology (including cell size and density), while endogenous fluorescence can shed light on a tissue’s biochemical and morphological framework. These two modes of optical spectroscopy can thus provide complementary information that can be used to study disease in living tissue. Few optical studies have been reported for human pancreatic tissue: optical coherence tomography (OCT) has been employed to identify the neoplastic and non-neoplastic main pancreatic duct layer structures ex vivo,6 and a microendoscope has been developed to provide white light reflectance and fluorescence images.7 In this report, reflectance and fluorescence spectroscopy were applied to detect and interpret differences between pancreatic tissue types by studying freshly excised human tissues, as well as in vivo mouse xenograft models. To the best of our knowledge, this is the first multimodal approach that incorporates reflectance and fluorescence spectroscopy to distinguish between normal, pancreatitis, and adenocarcinoma tissues using endogenous contrast.

The most common treatment for pancreatic cancer is the Whipple resection, a surgical procedure that involves removing the diseased proximal portion of the pancreas. The procedure can last 7 h owing to the inaccessibility of the pancreas. Fluorescence and reflectance data were obtained from pancreatic tissues of two patients undergoing Whipple surgery, within 15 min of resection [Fig. 1(a)]. Due to an inherent difficulty in visually differentiating tumor and other tissue types in resected pancreatic tissue, the resected specimen typically contained normal and pancreatitis tissue along with the adenocarcinoma (tumor) tissue. Post-spectroscopy pathology indicated that in patient 1 (2), within a resected sample of 6.5 × 5 × 4 (6.5 × 5.5 × 3) cm3, the tumor was approximately 2 (4) cm in diameter. On each resected specimen, optical measurements were obtained from five sites. Measurements were repeated to test reproducibility, after which tissue (0.5 cm thick) was removed from each site and sent for histological analysis. Pathology indicated that three sites measured on the first patient were pancreatitis, while the other two were normal tissue sites. All five sites measured on the second patient
were adenocarcinoma tissue sites. The study was approved by the Institutional Review Board, and written consent was obtained from the patients.

Optical measurements were also made on human pancreatic cancer xenografts in nude mice. Experiments on mouse models provided a controlled access and reproducibility not possible in human clinical studies and helped in interpreting the data from human pancreatic tissues. Human pancreatic cancer cells were cultured and injected into the pancreas of nonobese diabetic/severe combined immunodeficiency mice. After 3 weeks, two nonobese diabetic/severe combined immunodeficiency cancer cells were cultured and injected into the pancreas of mice. After 3 weeks, two nonobese diabetic/severe combined immunodeficiency cancer cells were cultured and injected into the pancreas of mice.

Data was obtained using a reflectance and fluorescence lifetime spectrometer (RFLS), which was described previously and recently modified. The plot in Fig. 2(b) shows the average of all normalized reflectance spectra from human pancreatic normal (blue line, \( n = 4 \)), pancreatitis (orange dotted-dashed line, \( n = 6 \)), and adenocarcinoma (red dashed line, \( n = 9 \)) tissues (color online only). Tissue spectra revealed cellular NAD(P)H (emission around 460 nm) and extracellular matrix collagen (emission peak around 400 nm). The collagen emission from adenocarcinoma and pancreatitis tissues was larger than that from normal tissues, as anticipated from the increase in fibrosis in the diseased tissues. The relative excess of collagen emission from pancreatitis versus normal tissue corresponded well with the increase in fluorescence decay time measured for all pancreatitis tissues versus all normal tissues, which was attributed to the longer excited-state lifetime associated with collagen compared to NAD(P)H.

The measurement was repeated in a second experiment. The plot in Fig. 2(c) shows the average of all normalized reflectance spectra measured from human pancreatic normal (blue line, \( n = 4 \)), pancreatitis (orange dotted-dashed line, \( n = 6 \)), and adenocarcinoma (red dashed line, \( n = 9 \)) tissues. Tissue spectra revealed cellular NAD(P)H (emission around 460 nm) and extracellular matrix collagen (emission peak around 400 nm). The collagen emission from adenocarcinoma and pancreatitis tissues was larger than that from normal tissues, as anticipated from the increase in fibrosis in the diseased tissues. The relative excess of collagen emission from pancreatitis versus normal tissue corresponded well with the increase in fluorescence decay time measured for all pancreatitis tissues versus all normal tissues, which was attributed to the longer excited-state lifetime associated with collagen compared to NAD(P)H. The gray dotted line shows in vivo fluorescence measured from a human pancreatic cancer xenograft grown in one of the mice. The measured fluorescence peaked around 460 nm, as was expected, since the mice were immunodeficient and so the expected fibrosis usually associated with tumor tissue was absent. Thus, the measured xenograft spectrum could be associated purely with the cellular component of the grown tumor [mainly NAD(P)H] and corresponded well with the fluorescence attributed to the cellular signal in the human study.

The plot in Fig. 2(d) shows the average of all normalized reflectance spectra from human pancreatic normal (blue line), pancreatitis (orange dotted-dashed line), and adenocarcinoma (red dashed line) tissues. Features in the range from 400 to 440 and 540 to 580 nm were attributed to hemoglobin absorption. The adenocarcinoma sites showed a higher reflectance than pancreatitis and normal tissue sites in the 430 to 500 nm range. Such tissue reflectance features have been associated with both the density and size of cellular scatterers (e.g., nuclei and organelles). Indeed, this reflectance feature was observed in the data obtained from xenograft tumor in vivo.
mice (gray dotted line) and corresponded well with measurements made on human adenocarcinoma.

Figure 3 quantifies some of the major differences observed among spectra by plotting the mean wavelength integrated fluorescence intensity versus mean ratio of reflectance at 470 nm to that at 650 nm ($R_{470}/R_{650}$) can be used to distinguish between human pancreatic tissue types (normal—blue squares; pancreatitis—orange circles; and adenocarcinoma—red triangles). The error bars indicate the standard error. (Color online only.)

survival rates in the management of pancreatic cancer.

Acknowledgments

This work was supported by a grant from the University of Michigan Medical School Translational Research Program (to M.-A. M. and J. S.).

References


