Optical Coherence Tomography: Real-time Imaging of Bronchial Airways Microstructure and Detection of Inflammatory/Neoplastic Morphologic Changes

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Abstract

Purpose: Current diagnostic imaging modalities for human bronchial airways do not possess sufficient resolution and tissue penetration depth to detect early morphologic changes and to differentiate in real-time neoplastic pathology from nonspecific aberrations. Optical coherence tomography (OCT) possesses the requisite high spatial resolution for reproducible delineation of endobronchial wall profiling.

Experimental Design: To establish whether OCT could differentiate between the composite microstructural layers of the human airways and simultaneously determine in situ morphologic changes, using a bench-top OCT system, we obtained cross-sectional images of bronchi from 15 patients undergoing lung resections for cancer. All scanned sections underwent subsequent detailed histologic analysis, allowing direct comparisons to be made.

Results: OCT imaging enables characterization of the multilayered microstructural anatomy of the airways, with a maximum penetration depth up to 2 to 3 mm and 10-μm spatial resolution. The epithelium, subepithelial components, and cartilage are individually defined. The acquired OCT images closely match histologically defined patterns in terms of structural profiles. Furthermore, OCT identifies in situ morphologic changes associated with inflammatory infiltrates, squamous metaplasia, and tumor presence.

Conclusions: Our results confirm that OCT is a highly feasible optical tool for real-time near-histologic imaging of endobronchial pathology, with potential for lung cancer surveillance applications in diagnosis and treatment.

Current imaging techniques employed for diagnosis of lung disease do not provide sufficient resolution to detect critical early pathologic changes within the bronchial epithelium. Lung cancer is the most common malignancy in the Western world and is the leading cause of cancer-related deaths (1). It is recognized that >85% of lung tumors originate within the bronchial epithelium, with multiscale cellular changes progressing over a relatively long period of time before first presentation of invasive cancer (2). It follows that programs aimed at decreasing lung cancer morbidity/mortality need to invest in development of safe, reliable imaging techniques capable of detecting in situ microinvasive pathologic lesions, to aid early diagnosis and enhance treatment.

There have been tremendous advances in biomedical imaging technologies to assist lung cancer management. These include low-dose spiral computed tomography, positron emission tomography, and magnetic resonance imaging. Although these technologies are significantly better than conventional diagnostic approaches, they are inherently costly, potentially hazardous in terms of radiation dose (3, 4), and limited in availability nationwide. Furthermore, their sheer bulk prohibits use in immediate diagnostic/treatment environments, such as the bronchoscopy or surgical fields. In this respect, autofluorescence bronchoscopy (LIFE-bronchoscopy) enhances identification and diagnosis of in situ mucosal abnormalities, such as early cancerous changes (5); however, this method is still hampered by inadequate image resolution and tissue depth penetration (6). Other bronchoscopic technologies encompassing incorporation of high-frequency ultrasonography, such as EBUS, do achieve deep penetration of the airway tissue, but their spatial resolution is insufficient for clear demarcation of the microstructural profile and morphologic changes (7–9). In addition, due to small differences of acoustic impedance between the healthy and diseased tissues, the imaging contrast offered by EBUS is inherently low.

Optical coherence tomography (OCT) is a rapidly evolving imaging modality, which is noninvasive and noncontact; unlike ultrasonography, OCT does not require a transducing
medium (10). Whereas analogous to B-mode ultrasonography, rather than using a sound signal, OCT delivers near IR light waves to the imaged site through a single optical fiber. This light reflects off the internal microstructural layers within the scanned tissue, allowing micron-scale resolution pick-up of the normal anatomy and any in situ morphologic aberrations. Signal processing involves low coherent interferometry, which is basically the analysis for reflected light waves from the internal tissue microstructures (10, 11). The coherence length of the light source determines the longitudinal resolution. With the appropriate light source, OCT imaging can reliably produce a 5- to 15 μm resolution compared with 150 μm for high-frequency ultrasonography (9). Thus, availability of high-tech OCT beam delivery instruments would constitute a major advance in lung cancer surveillance; importantly, its compactness makes OCT suitable for use in bronchoscopy and during thoracic surgery.

We have already shown OCT can provide near-histologic images of the different microstructural profiles along the tracheobronchial tree and detection of endobronchial foreign body deposition (12, 13). In this present study, we test OCT imaging in freshly excised lungs of patients undergoing resection surgery for lung cancer; we explore OCT’s capability to identify in situ inflammatory and neoplastic aberrations within the bronchial airway and determine whether OCT images reflect conventional histologic analysis.

**Materials and Methods**

**OCT system.** The bench-top OCT system used in this study is similar to one described previously (12) and was purposely built for us by Prof. Ruikang Wang ( Cranfield University, United Kingdom). Briefly, our OCT system incorporates a fibreoptic Michelson interferometer illuminated by a broad band light source (central wavelength at 1,300 nm with a bandwidth of 52 nm and pigtailed output power ~ 2 mW; Fig. 1). The light source used yields a ~ 10-μm axial resolution in lung tissue (12, 13), with the mean refractive index of bulk airway medium (10). Limited by the numerical aperture of the lens delivering light onto the sample, and the optical frequency of the incident light as in conventional microscopy. The system signal-to-noise ratio measures ~ 100 dB, using a 4 A OD neutral density filter. The scanning rate of the current system is 200 A scans per second.

**Lung OCT imaging.** The study received prior approval from the Shropshire and Staffordshire NHS Health Authority Local Research Ethics Committee. Following informed written consent, excised lungs were obtained from 15 patients undergoing pneumonectomies ($n = 3$), total ($n = 5$), or partial ($n = 7$) lobectomies for lung cancer. Upon retrieval and before histologic processing, visible airways within specimens were carefully exposed longitudinally by blunt dissection to allow precise localization of OCT imaging. The lung sections were kept moist by PBS to avoid dehydration during the scanning process. The exact location and direction of each scanned section was marked using a fine needle and color thread, clearly defining the start-to-finish point of OCT sampling. These markers were left in place when the lung resections were prepared for histologic analysis; thus, microscopic examination of the same anatomic location was mapped to OCT sampling. The position of the probe beam on the scanned tissue was monitored using a visible light guiding beam; the optical probe was never in contact with the sample. OCT scanning was done on the luminal surface of the resected airways, examining longitudinal sections sequentially from macroscopically disease-free airways right up to and including site of tumor. The scanning area length varied from 6 to 12 mm, depending on gross examination of each excised lung specimen; maximizing capture of intermediate morphologic changes between macroscopically healthy to diseased airways. Penetration depth varied, depending on relative refractive index of tissue components and airway generation.

**Histologic analysis.** The scanned tissue sections were then fixed in 10% buffered formalin for 48 hours and subjected to standard paraffin embedding processing. Sections ~5μm thick were cut at the marked tissue sites and stained with standard H&E. Tissue slide examination and micrographs were done with a Nikon Eclipse 80i (Nikon, Melville, NY) and recorded with a Nikon digital Net Camera DN100 on a Nikon Eclipse E600 light microscope (Nikon), respectively. OCT images were directly compared with histologic sections.

**Image and data reproducibility.** To avoid observer bias, analytic comparison of the recorded tomograms and measurement of structural dimensions were carried out independently by two histopathologists (blinded to one another) and the interobserver variability assessed. Each observer separately matched anatomic profiles on OCT tomograms with corresponding histology images. In addition, thickness measurements were done of the epithelium, cartilage plates, and the distance between these two layers in three separate areas of each OCT tomogram and corresponding histologic section; a total of 10 representative images for each patient were analyzed. $P < 0.05$ was accepted as statistically significant.

**Results**

OCT images of normal and diseased bronchial airways from resected lungs of 15 patients undergoing lung cancer surgery were acquired and displayed on a computer screen. OCT displays levels of resolution capable of near-histologic analysis and clearly visualizes marked differences between healthy airway microstructural profile and the presence of inflammation/
malignancy. In accordance with histologic examination, abnormal tissue morphology captured by OCT is reflected in a spectrum of changes from simple variations in microstructural dimensions to actual anatomic distortion. Minimal variations in tissue profiles between some OCT images and comparable histologic sections are noted; these are unavoidable due to sample desiccation after the histologic processing and fixation. To our knowledge, no studies have been reported demonstrating this spectrum of pathologic changes within the airway by OCT in a cohort of lung cancer patients.

**OCT characterization of disease-free bronchial airway.** We used corresponding histologic sections of the scanned macroscopically disease-free airways for validation of OCT images. Figure 2A shows the anatomy of a human healthy airway with its characteristic multilayered profile. OCT images precisely delineate in cross-section the following anatomic components: epithelium (E), lamina propria (LP), smooth muscle (SM), mucus glands (G) and cartilage (C; Fig. 2B). The transition among these microstructural layers is well defined and closely mirrors the layered appearance profiled on the histology image. The demarcation of the epithelium, mucous gland ducts, and cartilage is specifically mapped by OCT; the lamina propria and submucosal structures, while easily recognizable, at times seem to be less well demarcated. This variation in OCT definition across the different layers of the airway wall could be explained by the presence of a higher nuclear density within structures, such as the epithelium and cartilage, reflected in enhanced reflectivity signals compared with adjacent surrounding tissues. Thus, the relatively higher refractive index of a particular structure results in sharper OCT image interpretation. For example, the comparatively denser extracellular matrix of cartilage decreases scattering of incident light and thus reflects as a dark region on the OCT image. The connective tissue layer, including smooth muscle beneath the epithelium, is also visualized, but we could not differentiate between smooth muscle bundles and blood vessels.

Across all our sections, OCT imaging penetrates the full thickness of the airway wall to at least the outer confines of cartilage plates. Clear imaging is seen to a maximum penetration depth up to 2 to 3 mm, depending on the section scanned, with a spatial resolution of 10 µm and a scanning speed of 200 A scans per second. The actual time to scan a tissue area length of 6 to 12 mm is 30 to 50 seconds. The above findings are consistent across serial sections done on the 15 resected lung specimens (20-30 scans on each lung).

**Identification of in situ inflammatory and neoplastic pathology.** The presence of chronic intense inflammation tends to homogenize tissue and disrupts tissue boundaries histologically (Fig. 3A). Consequently, inflamed tissue results in a less well defined OCT image, where only the epithelium and lamina propria are clearly delineated (Fig. 3B). However, whereas the epithelium is seen to remain intact, the relatively deeper border of lamina propria merges indistinguishably into the submucosa with increasing infiltration from inflammatory cells. Under such circumstances, as relative differences in refractive index between structural components decrease, it becomes increasingly difficult to distinguish cartilage on these OCT images (Fig. 3). In addition, inflamed tissue is more vascular than normal, thereby further reducing the imaging capability due to strong absorption of light at 1,300 nm. However, even at a resolution of 10 µm, OCT imaging captures the remodeling present within inflamed airways as visualized on histologic analysis.

In sections immediately leading to and including site of tumor, OCT images identify tumor presence as destructive growth ignoring and effacing normal tissue boundaries (Fig. 4), similar to histopathology. This featureless OCT image clearly lacks the ordered multilayered appearance of the healthy airway wall seen in Fig. 2. OCT also differentiates between areas of chronic inflammation and invasive malignancy; the clear demarcations of epithelium and lamina propria observed on histologic analysis. This featureless OCT image clearly reflects the remodeling present within inflamed airways as visualized on histologic analysis.

In some lung samples, OCT imaging was able to reflect pathologic changes in keeping with squamous metaplasia. Replacement of the normal single layer of ciliated respiratory epithelium by multilayered squamous epithelium often occurs in smokers and provides a suitable environment for early morphologic changes associated with lung tumor development. Areas of squamous epithelium are thicker and possess different cellular morphology when compared with the appearance of normal respiratory epithelium by light microscopy (Fig. 5A). This increase in epithelium thickness, which characterizes squamous metaplasia, is captured on OCT image and contrasts...
with images of airways lined by a single layer of normal respiratory epithelium (Fig. 5B).

*Image and data reproducibility.* The representative OCT images were consistent across numerous lung sections done on the 15 patients. The coefficient of variability of the differences among the three separate analyses of OCT image to histopathology by both histopathologists was between 3% and 10% and, altogether, that between the two was <5%.

The relative dimensions of structural components on OCT images is comparable with histologic analysis. The measured thickness on OCT images of the epithelium and cartilage is 100 ± 25 and 450 ± 15 μm, respectively, whereas the intervening distance from epithelium to cartilage is 250 ± 28 μm compared with their histologic quantification of 84 ± 21, 378 ± 30, and 210 ± 42 μm, respectively. Quantitative assessment was done on three separate areas of each corresponding OCT tomogram and histology section from macroscopically healthy airways; a total of 10 representative images for each patient were analyzed. There is no significant difference between patients in terms of thickness of healthy bronchial mucosa components (*P* > 0.5). A consistent shrinkage of ≈16% was observed in microstructural component thickness on histologic images compared with OCT. This is due to inevitable post-fixation tissue desiccation. In addition, assigning an average refractive index of 1.4 across the whole airway profile may contribute to a slight overestimation/underestimation of measurements as done on OCT images (e.g., cartilage has an inherent refractive index of 1.51).

**Discussion**

In this study, we compare OCT images of immediately exposed airways within lungs resected from patients undergoing surgery for lung cancer with gold standard histologic analysis. Our data establish that OCT is a feasible optical biopsy method, imaging 2 to 3 mm into airway tissue and defining the highly organized multilayered architecture of healthy bronchi to near-histologic analysis. Even at a spatial resolution of 10 μm, OCT can identify morphologic changes associated with *in situ* presence of inflammation and neoplasia.

OCT is analogous to optical “ultrasound,” delivering near IR light waves (rather than sound) to the imaged tissue through a single optical fiber. As such, OCT represents a technological shift from another current methods employed in lung cancer screening. By using variations in optical backscatter within the imaged tissue, OCT can effectively differentiate normal airway profiles from regions bearing *in situ* pathologic aberrations. As the ordered multilayered anatomy of the healthy airway is disrupted by structural remodeling consequent on development of inflammation and/or neoplasia, OCT tomograms reflect
bland images, which ignore the normal microstructural boundaries. Inflammation is characterized by a loss of depth beyond the epithelial and lamina propria layers on the OCT image. This partly results from the presence of infiltrating inflammatory cells, such as lymphocytes, which have a high nuclear density, thereby modulating the refractive index of the imaged bulk tissue. In addition, enhanced vascular supply within the inflamed region contributes to reduced imaging capability, as whole blood induces strong attenuation of light. This originates from the absorption of light by hemoglobin and the scattering properties of RBC (15). In contrast, invasive neoplastic changes seem as featureless images on OCT, reflecting total loss of local architecture characteristic of tumor infiltration seen on subsequent histologic examination. Similar images have been reported in the gut tumors (16, 17).

Continuing developments in OCT engineering will include measures to overcome these pitfalls in future as differential tissue contrast information is gleaned from imaging various states of human airway pathology.

We acknowledge our current OCT system is limited in its ability to define individual cellular profiles, and that this would be a significant step forward to enable OCT imaging to revolutionize lung cancer surveillance. Developments will need to include faster data acquisition and processing for in vivo real-time recording and enhancement of resolution by use of broad-band light sources, OCT-multiphoton microscopy, or polarization-sensitive OCT (18). Inevitably, such developments will need to be finely balanced against constraints placed by the particular in vivo milieu in which OCT is being used and the complex tissue profile. Thus, whereas improvements in resolution <2 µm have already been achieved (19), it may be difficult to enhance imaging depth beyond 2 to 3 mm within the same OCT system. We are encouraged by the ability of our current OCT system to identify areas of squamous metaplasia along an airway; specifically as dysplastic changes, which precede invasive neoplasia often by years, show a progressive increase in the thickness of epithelium, that usually parallels the grade of dysplasia. Aside from neoplastic changes, the bronchial airway epithelium can also undergo remodeling in thickness and morphology associated with development of chronic airways diseases (20). Thus, the availability of such “optical pathologic fingerprints” through improvements in OCT technology would enable diagnosis of the early distinct cellular hyperplastic/metaplastic changes associated with lung cancer and chronic airway diseases.

Although the clinical use of OCT imaging has been tested in a variety of biological tissues (21–24), its use in lung screening is still in its early stages. We foresee OCT imaging proving invaluable within two scenarios in thoracic medicine. The urgent need for better visualization of the endobronchial tree to monitor in situ mucosal aberrations and early diagnosis of lung cancer necessitate the exploitation of an OCT catheter design for incorporation into conventional bronchoscopy. Lessons may be obtained from other medical fields; recent reports of a small OCT catheter consisting of a rotary optical fiber joint for intravascular imaging (25) and the MEMS-based OCT endoscope for imaging urinary tracts (26) could be exploited in future designs of an OCT bronchoscopic system for routine in vivo screening. Obviously, engineering of such a flexible system would need to overcome the intricate changing anatomic profile of the human tracheobronchial tree and other issues, such as blood flow changes. However, an immediate potential for in vivo surface and near-surface imaging application for our OCT system is within thoracic surgery. As complete lung tumor removal offers best long-term survival for cancer patients, there is an urgent need for safe noninvasive tools for use at operation, to enable real-time near-histologic information on early cancerous changes within the lung/thoracic cavity and to determine precisely resection margins. Currently, tissue disease status at operation is done by gross examination and, in some cases, cytologic/histologic assessments involving significant laboratory time, expense, and wait delay. Optimizing the current OCT system for feasible use during lung cancer surgery would enhance patient operability, preserve tumor-free lung in the compromised patient, and guide implementation of any adjunct interventions (27, 28).

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References

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