Ex vivo High-resolution Optical Coherence Tomography (OCT) Imaging of Pleural Reaction after Pleurodesis Using Talc

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The pleura is known as an end target organ of exposure to toxic environmental materials such as fine particulate matter and asbestos. Moreover, long-term exposure to hazardous materials can eventually lead to fatal lung disease such as diffuse pleural fibrosis or mesothelioma. Chest computed tomography (CT) and ultrasound are gold standard imaging modalities for detection of advanced pleural disease. However, a diagnostic tool for early detection of pleural reaction has not been developed yet due to difficulties in imaging ultra-fine structure of the pleura. Optical coherence tomography (OCT), which provides cross-sectional images of micro tissue structures at a resolution of 2-10 μm, can image the mesothelium with a thickness of ~100 μm and therefore enables investigation of the early pleural reaction. In this study, we induced the early pleural reaction according to a time sequence after pleurodesis using talc, which has been widely used in the clinical field. The pleural reaction in talc grouped according to the time sequence (1st, 2nd, 4th weeks) showed a significant thickening (average thickness: 45 ± 7.5 μm, 80 ± 10.7 μm, 90 ± 12.5 μm), while the pleural reaction in sham and normal groups showed pleural change from normal to minimal thickening (average thickness: 16 ± 5.5 μm, 17 ± 4.5 μm, 15 ± 6.5 μm, and 12 ± 7.5 μm, 13 ± 2.5 μm, 12 ± 3.5 μm). The measurement of pleural reaction by pathologic examinations was well-matched with the measurement by OCT images. This is the first study for measuring the thickness of pleural reactions using a biophotonic modality such as OCT. Our results showed that OCT can be useful for evaluating the early pleural reaction.

Keywords: Pleura, Optical coherence tomography, Sclerotherapy, Talc
OCIS codes: (170.6935) Tissue characterization; (170.3880) Medical and biological imaging; (170.1610) Clinical applications; (170.4500) Optical coherence tomography

I. INTRODUCTION

It is widely accepted that chronic exposure to hazardous airborne particles is closely associated with chronic lung disease [1, 2]. The World Health Organization has identified fine particulate matters as 1st-grade carcinogens of lung cancer [3]. Similar to other well-known carcinogens such as asbestos, long-term exposure to fine particulate matter can eventually lead to development of fatal airway diseases such as pleural fibrosis and pleural malignancy, which have been increasing...
in incidence worldwide [4, 5]. Therefore, the development of a diagnostic imaging modality for such exposure has been an essential issue in health care services [6]. Computed tomography (CT) and ultrasound (US) are generally used to detect pleural disease [7, 8]. However, the diagnostic yield of CT and US is not high due to limited resolution [9, 10]. Due to resolution limits, the ultra-fine structure of the pleural layer has been an area of uncertainty for visualization using conventional methods [11, 12]. Recently, optical coherence tomography (OCT) has been developed, which is an imaging modality with non-invasive, high-speed, high-resolution capabilities [13]. In spite of a limited imaging depth, OCT provides real-time cross-sectional images with a micrometer scale. Several OCT studies related to pulmonology have been published [14]. Talc has been commonly used in the chemical, textile, cosmetic, construction industries, and even in the health care industry [15]. There have been many reports regarding potential hazard of industrial talc to induce asbestos-related diseases, including diffuse pleural thickening, interstitial fibrosis, and mesothelioma. In this study [16], we used an animal model of pleurodesis and imaged the pleural reaction induced by talc. Pleurodesis is a medical method used to induce the pleural reaction by infusing a sclerosing agent into the pleural cavity (Fig. 1). Talc is the most commonly used sclerosing agent, with many reports of its effectiveness [17, 18]. The main target of the sclerosing agent is the pleural surface, a regenerative layer for the early pleural reaction after pleurodesis. To the best of our knowledge, this is the first study testing the feasibility of OCT for evaluation of the early pleural reaction based on the time sequence after pleurodesis. In addition, we performed ultrasound examinations for pleural reaction at 28 days after pleurodesis.

II. METHODS

2.1. Animal Preparation

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (DHEW publication NIH 85-23, revised 2010, Office of Science and Health Reports, DRR/NIH, Bethesda, MD). The study was approved by the Animal Care and Use Committee at Kosin University College of Medicine. Male New Zealand white rabbits weighing 3.0 to 3.5 kg were used in this study. The initial intramuscular anesthesia was performed with ketamine 5 mg/kg and xylazine 0.8 mg/kg. We maintained the anesthetic depth by injection of 10 mg/h ketamine and 3 mg/h xylazine. Oxygen saturation was monitored with a pulse oximeter in the ear.

2.2. Pleurodesis

Figure 1 describes a brief concept of pleurodesis and Fig. 2 shows a procedure of pleurodesis of this study. The rabbits were fixed on the operation table in a lateral position for preparation and image acquisition. The right chest was shaved, and a 1.0-cm skin incision was made midway between the spine and the scapular tip. A 16-gauge plastic catheter was placed percutaneously into the right pleural space. To compare the effects of the agents used for pleurodesis, rabbits were divided into three groups treated intrapleurally: normal (control group, $n = 5$), 400 mg/kg talc (talc group, $n = 15$ (day 7, $N = 5$; day 14, $N = 5$; and day 28, $N = 5$)), and 10 ml normal saline (sham control group, $n = 15$ (day 7, $N = 5$; day 14, $N=5$; and day 28, $N = 5$)). Before starting pleurodesis procedures, we selected 400 mg/kg of talc because it was the lowest dose that produced recognizable changes in the previous study [19]. The talc slurry was instilled into the right thoracic cavity through the 16-gauge plastic catheter.

2.3. Follow-up after Pleurodesis

After the procedure, the rabbits were monitored daily for weight-loss, inappetance, moribund state, infection, and organ system dysfunction. All efforts were made to minimize suffering.

Animals were euthanized using CO$_2$ gas after an intramuscular injection of ketamine at day 7 ($N = 5$), day 14th ($N = 5$),

FIG. 1. Illustration of (a) normal chest x-ray, (b) pleurodesis, which is a medical procedure in which the pleural space is artificially obliterated by infusion of sclerosing agents, and (c) abnormal chest x-ray with pleural reaction, which is the pleural thickening caused by inflammatory response of mesothelial cell after pleurodesis.
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FIG. 2. Illustration of animal experiment sequence. (a) Step 1; the right chest was shaved and a 1.0 cm skin incision was made midway between the spine and the scapular tip, (b) Step 2; a 16-gauge plastic catheter was placed percutaneously into the right pleural space, (c) Step 3; tale 1 mg/ml was installed into pleural cavity, and (d) Step 4; resection of chest wall after the rabbit was euthanized with CO₂ gas, and OCT imaging was performed. (e) chest x-ray after procedure which showed the 16-gauge plastic catheter (arrows) in the thoracic cavity.

2.4. OCT System
Two lab-made spectral-domain optical coherence tomography (SD-OCT) systems were used to measure thickness of pleura from the basement membrane to the top of granulation in real-time. We used an 850 nm OCT system with a better resolution for imaging the early pleural reaction at the first week, while using a 1310 nm OCT system with a better penetration for imaging the advanced pleural reaction at the second and fourth weeks.

FIG. 3. Schematic diagram of our SD-OCT based on a Michelson interferometer. A broadband light source sent a beam to a fiber based 2-by-2 beam splitter through optical fiber and the split beams were directed to reference arm and sample arm. The light source has a center wavelength of 850 nm (BroadLighter D855, Superlum, Ireland) or 1310 nm (SLED Butterfly, EXALOS, Schlieren, Switzerland) and a full width at half maximum (FWHM) of 100 nm or 80 nm, respectively. After that, beams were reflected at reference mirror and samples, respectively. The reflected beams were coupled into each optical fiber and delivered to the 2-by-2 beam splitter. The merged beam was diffracted by a grating (1800 lines/mm for the 850 nm OCT system or 1145 lines/mm for the 1310 nm OCT system, Wasatch Photonics, NC, USA), depending on wavelength. The diffracted interference pattern was detected by a CMOS camera with 4096 pixels (Sprint spL4096-140km, Basler, PA, USA) at a line rate of 140 kHz for the 850 nm OCT system or an InGaAs camera with 1024 pixels (SU1024-LDH2, Goodrich, Princeton NJ, USA) at a line rate of 92 kHz for the 1310 nm OCT system. A scan unit with two galvanometers (6220H, Cambridge Technology, MA, USA) allowed to scan specimens in two axes with 5 by 5 mm² scanning area. To obtain specifications of the OCT system, we measured the point spread function. The 850 nm OCT system has a depth resolution of 3 μm in air, a roll-off distance of 1 mm at 12 dB power drop, and a dynamic range of 103 dB. The 1310 nm OCT system has a depth resolution of 11.629 μm in air, a roll-off distance of 1 mm at 5.65 dB power drop, and a dynamic range of 106 dB.

2.5. US System
We used a commercial US system with a linear transducer (10 M Hz) (Sonix Touch, Ultrasonix, British Columbia, Canada). The US system provided 93 μm of spatial resolution for x and y directions.

2.6. Pathologic Examination
Ex vivo samples were selected from the parietal pleura
FIG. 4. Normal chest wall and parietal pleura; (a) gross image, (b and e) 1310 nm OCT images; yellow arrow means the top surface of granulation and yellow head means the basement membrane at the pleural layer, (c and f) pathology (H-E stain) magnified to 40 times, and (d) thickness measured at A, B and C and arithmetically averaged.

FIG. 5. Chest wall and mild pleural thickening a week later after pleurodesis; (a and e) gross images, (b and f) 850 nm OCT images; the region between the red arrows shows the area of interest, (c) pathology magnified to 1.25 times, (d, g and h) pathology magnified to 100 times.

including apex, mediastinum, and diaphragm. Pleural thicknesses at these three points were measured and mean thicknesses were calculated. Lung tissue (including pleura, alveoli, intercostal muscle, fascia, skin) were cut into 6×6 cm$^2$ pieces, fixed in 10% neutral buffered formalin (NBF), and embedded in paraffin. Serial sections (4 μm thick) were stained with hematoxylin and eosin (H&E) and examined by microscopy.

2.7. Statistical Analysis
Data were presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). A two-tailed p-value <0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION
The gross examinations of granulation showed scattered nodular lesions in the parietal pleural surface and the nodular lesion increased in size for 4 weeks as indicated by blue arrows in Figs. 5(a), 5(e), 6(a), 6(d), 7(a), and 7(e). The distance from the basement membrane to the top of granulation using OCT examination showed gradual increase according to time (Figs. 5(b), 5(f), 6(b), 6(e), 7(b), and 7(f)). The pathologic examination showed infiltration of inflammatory cells surrounding talc particles (Fig. 6(f)). The pathologic findings were well matched with OCT images (Figs. 5(d), 5(h), 7(c), and 7(g)). The change of pleural reaction in the talc group at the 1st and 2nd weeks showed the significant thickening (average thickness: 45 ± 7.5 μm, 80 ± 10.7 μm), while the pleural reaction in sham and normal groups showed pleural change from normal to minimal thickening (average thickness: 16 ± 5.5 μm, 17 ± 4.5 μm and 12 ± 7.5 μm, 13 ± 2.5 μm. p=0.03, p=0.03 respectively). (Figs. 5, 6 and Table 1).

With regard to the early pleural reaction, Adamson et
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FIG. 6. Chest wall and moderate pleural thickening two weeks later after pleurodesis; (a and d) gross images, (b and e) 1310 nm OCT images; the region between the red arrows shows the area of interest, (c) pathology magnified to 100 times, and (f) pathology magnified to 100 times (the green arrows show typical talc particles).

TABLE 1. Measured pleural thickness by pathology and ex vivo OCT image.

| Pathology     | 1st Week     | 2nd Week     | 4th Week     |
|---------------|--------------|--------------|--------------|
| Talc          | 50 ± 7.5 µm  | 90 ± 7.5 µm  | 100 ± 10.5 µm|
| Normal saline | 15 ± 7.5 µm  | 15 ± 7.6 µm  | 14 ± 5.5 µm  |
| Normal        | 11 ± 7.5 µm  | 12 ± 3.5 µm  | 11 ± 2.5 µm  |
| Pvalue        | 0.04         | 0.02         | 0.01         |

| Ex vivo OCT image | 1st Week     | 2nd Week     | 4th Week     |
|-------------------|--------------|--------------|--------------|
| Talc              | 45 ± 7.5 µm  | 80 ± 10.7 µm | 90 ± 12.5 µm |
| Normal saline     | 16 ± 5.5 µm  | 17 ± 4.5 µm  | 15 ± 6.5 µm  |
| Normal            | 12 ± 7.5 µm  | 13 ± 2.5 µm  | 12 ± 3.5 µm  |
| Pvalue            | 0.03         | 0.03         | 0.01         |

al. reported that the peak blood level of early mesothelial proliferation occurred in the first 2-4 weeks after asbestos exposure [20]. However, there was no study of early pleural reaction after pleurodesis using imaging modality. In this study, the high-resolution capability of OCT was helpful in showing the time sequence of events in the delicately changing pleural space. OCT was able to detect pleural changes even between the 1st and 2nd weeks. There was no significant change in the thickness of pleural reaction in talc group at the 2nd and 4th weeks (average thickness: 80 ± 10.7 µm, 90 ± 12.5 µm), and the pleural reaction in sham and normal groups also showed no significant pleural change from normal to minimal thickening (average thickness: 17 ± 4.5 µm, 15 ± 6.5 µm, and 13 ± 2.5 µm, 12 ± 3.5 µm)(Figs. 6, 7 and Table 1). In the study of Kennedy et al., it was reported that pleural adhesions formed shortly after talc administration and did not increase over time after initial pleural reaction. Our results from the pleural thickness between the 2nd and
FIG. 7. Chest wall and moderate pleural thickening four weeks later after pleurodesis; (a and e) gross images, (b and f) 1310 nm OCT images; the region between the red arrows shows the area of interest, (c and g) pathology magnified to 40 times, and (d and h) 10 MHz US images and the region between the red arrows shows the area of interest.

4th weeks were well correlated with findings from previous studies [21].

In our study, the measurement of pleural reaction by pathologic examinations were well matched with the measurement by OCT images. In comparison with previous study using pathologic examination, Muta et al. [19] reported similar result in the thickness of pleural reaction measured on the 30th day after talc exposure in rats. Therefore, OCT has a potential to provide in vivo imaging of mesothelium based on thoracoscopic examination and the detection of pleural lesions in an early stage. Therefore further in vivo study using real-time OCT would be warranted in an animal model. Brenner group firstly demonstrated the in vivo thoracoscopic imaging capabilities of three-dimensional OCT systems with improved forward-scanning rigid GRIN lens rod probes for detecting pleural cancer [22]. Clinically, mesothelioma and pleural plaques occur exclusively in parietal mesothelium, not in the visceral mesothelium [23].

In the present study, US examination visualized localized nodular pleural reaction after talc pleurodesis but could not measure the thickness of pleural reaction, while OCT could show thickening of pleural layers (Fig. 7). In study of fusion image using intra-vascular ultrasound (IVUS) and OCT image for detecting atherosclerosis, multimodal fusion imaging showed a complimentary role from the view of localization of lesion with IVUS and specification of lesion with OCT [24, 25]. Therefore, OCT might have a potential complementary role in the early detection of pleural lesions when used in combination with other diagnostic approaches such as endoscopic ultrasound. Our study contributes to the knowledge of the biologic behavior of mesothelium in the carcinogenesis of pleura. Additionally, further developments in imaging technologies such as ultrahigh resolution imaging are expected to improve the diagnostic accuracy and ability to characterize pathologic events occurring after injury. In this study, two kinds of OCT (850 and 1310 nm) were used for visualizing the pleural reaction from early response at the 1st week to late responses at the 2nd and 4th weeks. The 850 nm OCT system had a higher depth resolution than the 1310 nm OCT system so that it provided more accurate thickness estimation in an early stage. The 1310 nm OCT system has a deeper imaging depth. Both systems, however, were enough to image the whole thickness of mesothelium.

IV. CONCLUSION

An animal model of pleurodesis for visualizing the pleural reaction using OCT was feasible. Compared with the normal and normal saline groups, the talc group showed significant pleural reaction. Two house-made OCT systems were utilized to image the mesothelial layer in the pleura. Increase in parietal pleural thickness might be a mechanism underlying the effectiveness of talc pleurodesis for pleural effusions including malignant effusions.

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