Development of an Alveolar Transbronchial Catheter for Concurrent Fiber Optics-Based Imaging and Fluid Delivery

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Nathan Knighton[1]
Department of Bioengineering,
University of Utah
36 S Wasatch Dr.
Salt Lake City UT, 84112
nate.knighton@utah.edu

Brian Cottle
Department of Bioengineering,
University of Utah
36 S Wasatch Dr.
Salt Lake City UT, 84112
brian.k.cottle@utah.edu

Veronique Dentan
Mauna Kea Technologies
9, rue d'Enghien
Paris, 75010, France
veronique@maunakeatech.com

Tom Vercauteren
University College London,
Wellcome / EPSRC Centre for Interventional and Surgical Sciences
Charles Bell House
43-45 Foley Street
London W1W 7TS, United Kingdom
t.vercauteren@ucl.ac.uk

Ahsan Akram
EPSRC Proteus Hub, MRC Centre for Inflammation Research
The University of Edinburgh,
The Queen's Medical Research Institute
47 Little France Crescent
Edinburgh EH16 4TJ, United Kingdom
ahsan.akram@ed.ac.uk

[1] Corresponding author

Annya Bruce
EPSRC Proteus Hub, MRC Centre for Inflammation Research
The University of Edinburgh,
The Queen's Medical Research Institute
47 Little France Crescent
Edinburgh EH16 4TJ, United Kingdom
Annya.Bruce@ed.ac.uk

Kevin Dhaliwal
EPSRC Proteus Hub, MRC Centre for Inflammation Research
The University of Edinburgh,
The Queen's Medical Research Institute
47 Little France Crescent
Edinburgh EH16 4TJ, United Kingdom
Kev.Dhaliwal@ed.ac.uk

Robert Hitchcock[1]
Department of Bioengineering,
University of Utah
36 S Wasatch Dr.
Salt Lake City UT, 84112
r.hitchcock@utah.edu

ABSTRACT

Optical molecular imaging is an emerging field and high resolution optical imaging of the distal lung parenchyma has been made possible with the advent of clinically approved fiber based imaging modalities. However, currently, there is no single method of allowing the simultaneous imaging and delivery of targeted molecular imaging agents. The objective of this research is to create a catheterized device capable of fulfilling this need. We describe the rationale, development, and validation in ex vivo ovine lung to near clinical readiness of a triple lumen bronchoscopy catheter that allows concurrent imaging and fluid delivery, with the aim of clinical use to deliver multiple fluorescent compounds to image alveolar pathology. Using this device, we were able to produce high-quality images of bacterial infiltrates in ex-vivo ovine lung within 60 seconds of instilling a single microdose of (<100 mcgs) imaging agent. This has many advantages for future clinical usage over the current state of the art.

INTRODUCTION

Lung disease represents a large burden of disease and healthcare resources worldwide. Lung cancer, pneumonia and chronic lower respiratory disease are three of the top ten leading causes of death in the United States [1]. Furthermore, the incidence and prevalence of these diseases is expected to increase and the incidence of other respiratory conditions, such as the pulmonary nodule, cause significant clinical uncertainty. These pathologies highlight the need, in the pulmonology field, for rapid and accurate diagnosis and treatment in order to prevent patient deterioration and close the gaps between symptoms, diagnosis and therapy.

Rapid patient deterioration and slow diagnostic methods represent a disconnect in current healthcare practices when it comes to lung disease in hospitalized settings. Utilizing imaging modalities to diagnose, classify, and stratify respiratory conditions is commonplace and often relies on using structural information through chest x-rays and computed tomography scans. However, there is an increasing need to understand these conditions with respect to their biological activity and optical molecular imaging holds significant promise in this regard.

Whilst chest x-rays and computed tomography (CT) scans provide structural information, they do not provide information regarding disease activity and this often leads to subsequent procedures for invasive sampling or biopsies. One of the most commonly used methods includes flexible bronchoscopy [2], which can allow biopsy [3] and Broncho-Alveolar Lavage (BAL). Biopsies are performed via bronchoscope and samples of lung tissue are sampled using a biopsy needle or cytology brush. BAL is a method of instilling fluid into the lungs (often >50mls) and collecting the fluid for subsequent analysis and microbial culture. BAL has variable performance in diagnosing pneumonias [4, 5] and has limited utility in cancers [6], but has limited benefit for diagnosing fibrotic scarring [7].

Advances in laboratory assays have improved the accuracy of diagnosis but accurate sampling remains a challenge. In some scenarios, patients can deteriorate rapidly in a short time span, especially critically-ill patients who are ventilated. When these patients develop inflammation or infection in the lung, the mortality rates can be as high as 70% [8]. Standard procedures such as BAL can take up to 48h to return results [9]. During this intervening time, patients may be prescribed inappropriate therapy or deteriorate rapidly while awaiting a confirmatory diagnosis. If samples are lost or contaminated, the procedure must be repeated – adding yet more time to the diagnostic process. There exists an unmet clinical need for rapid diagnosis of acute lung disease for critically-ill patients [10].

There is little doubt that rapid diagnosis and treatment of lung disease will both decrease costs and improve outcomes associated with these devastating diseases. New advances in medical technology, including molecular diagnostic probes and imaging, hold the promise to close the gap between symptoms, diagnosis and therapy. These advances may also lead to treatment options that avoid the complications of systemic therapeutics. Integrated clinical systems may allow diagnosis and localized treatment in a single procedure. We are now seeing advanced technologies in medical optics that can enable development of devices to fulfill these unmet clinical needs.

Fiber-optics based confocal microscopy is an emerging technology that utilizes a fiber-optic bundle in a probe configuration for the purpose of imaging tissues. The technique is also known as probe-based confocal laser endomicroscopy (pCLE). This imaging technology allows users to microscopically evaluate cellular and sub-cellular tissue structures within the body in real time [11]. pCLE has been used in the lungs to differentiate between healthy and unhealthy alveolar structure and for imaging cancers and chronic lower respiratory disease [12]–[14]. The ease of use and real time visualization allow pCLE to be used with molecular imaging Smartprobes in the lung for investigating

diverse pathologies such as fibrogenesis [15], inflammation, and infection. Fluorescent reporters have been used to detect neutrophil elastase – a protease implicated in the pathogenesis of inflammatory lung disease [16]**.** For potential methods of identifying infection, several teams have developed imaging markers used with pCLE to identify bacterial infections [17] both systemically and *in situ* [18], 19]. Of particular interest is a two-color methodology that has shown the potential to identify neutrophil recruitment, fungal infection, and bacterial presence in *ex vivo* human lungs[20].

pCLE interrogation coupled with bespoke optical molecular reporters has the potential to narrow the gap between symptom onset and treatment of lung disease. Given the current ambiguity with radiographic images, the ability to differentiate cancer cell phenotypes, bacteria, and fibrosis *in situ* could provide immediate diagnostic feedback to guide therapeutic intervention. A major hurdle to achieving this goal is the difficulty of delivering chemical imaging agents to the areas of interest during the imaging procedure so that pathology can be rapidly evaluated. Current bronchoscopes pass to the 3rd or 4th generation bronchial tree and have one working channel for the introduction of catheters or interventional tools. As such combining pCLE and molecular reporters currently requires sequential instrumentation of the working channel with pCLE followed by delivery catheters and then subsequent pCLE. This current methodology is time-consuming, has co-localization issues, and there is an increased risk of sterility issues. Thus to significantly improve the utility of pCLE to interrogate distal alveolar pathology, an effective way of delivering imaging agents during the imaging procedure needs to be developed.

Thus the purpose of this development project was to specify, develop, manufacture, validate to near clinical readiness and document a Triple Lumen Bronchoscopy Catheter (TLBC). The TLBC has two lumens to allow for *in situ* micro-dose delivery of potentially two different fluids (such as imaging

agents and therapeutics during a clinical procedure comprising bronchoscopy) and one lumen for passing the pCLE fiber-optics bundle into the distal human lung. Figure 1 shows the concept of the TLBC catheter during use. The TLBC is intended to be used during a bronchoscopy procedure to identify specific diseased tissues with pathology specific imaging reagents observable by pCLE. The research described in this publication focuses on the TLBC and not on the development of the imaging reagents nor on a specific clinical procedure.

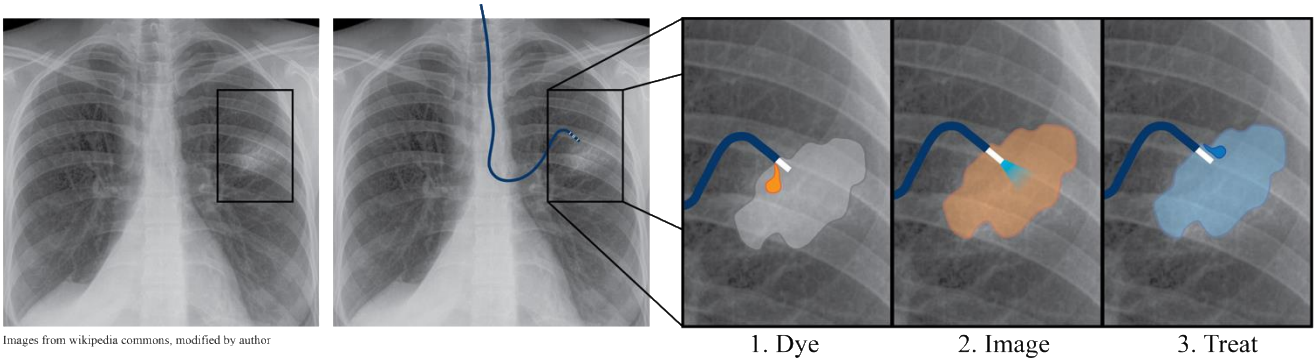

Figure 1 - Device usage diagram. The TLBC is inserted via flexible bronchoscope and navigated to the region of interest. Imaging agent is then added, and the subject tissue analyzed. In the event the tissue shows pathology requiring treatment, therapeutic liquids can then be applied. This is all accomplished without having to remove/reinsert catheter (Original image from Wikipedia commons, modified by author).

METHODS AND MATERIALS

Creating safe, functional devices acceptable for clinical studies is an important aspect of translating research ideas into realistic medical solutions. Input parameters and requirements should be applied to designs within the context of appropriate regulatory frameworks to ensure that good manufacturing practice results in outputs that are safe, effective, and verifiable for use in clinical studies.

Design control

TLBC catheters were built and documented following the regulatory design control pathway mandated in Title 21 Part 820 of the US Code of Federal Regulations (CFR).

Catheter Design Inputs

A set of design input requirements was developed for the TLBC. Theses inputs were derived from bronchoscope compatibility requirements as well as clinician input. The major compatibility considerations being that the TLBC should be compatible with bronchoscopes with working channels >2mm diameter and the TLBC should be radiopaque. Additionally, the TLBC needed to be compatible with existing pCLE imaging fiber-optics probes and rigid enough to enable transbronchial passage. Clinician inputs required a bolus of 200 µl of liquid agent be delivered over 5 seconds and that it not be too difficult to depress the plunger of a 1ml syringe while delivering these liquids. Preliminary experimentation defined this force value as not more than 30-35N force. Poiseuille's equation was used to determine a minimum appropriate diameter. This value was bracketed and served as the input requirement for the final lumen size.

Risk analysis was also performed throughout the project, according to the consensus standard ISO 14971[21]. The continued analysis served to inform applicable input requirements. The addition of clearly identifiable depth markings, sterility, and minimizing tissue damage during transbronchial passage as requirements came directly from these analyses and clinician input.

The TLBC was designed to be a single-use disposable device in line with all current catheters in clinical use in bronchoscopy. Materials were chosen to meet both the performance requirements as well as cost targets. Furthermore, the sterilization methodology of ethylene oxide was chosen and as such, the materials and design for both the device and packaging were chosen with this requirement in mind.

The TLBC devices were intended to support an ongoing clinical trial and were not intended for broader distribution or uses that were not considered investigational. The catheters were therefore

not conditioned at temperature or accelerated aging prior to verification and validation testing. All verification and validation tests however were performed after sterilization. The potential health hazards of this device fall broadly into four categories: toxicity, sterility, function and breakage. Each of these hazard classes has been addressed in the device verification and validation: Toxicity – ISO 10993 toxicity studies have been successfully completed. There is no reason to believe this lack of toxicity would change over time with the materials that are used in the catheter assembly or packaging. Sterility – A complete sterilization validation has been successfully completed on these packaged devices. In addition, the contract packaging company (ProTech, Inc) has documented evidence that the package used for the EDDCs has been validated for a three year shelf life for a similarly packaged product. Function – Each of the catheters has been checked for lumen leakage, lumen patency and lumen integrity. All catheters that were sent for sterilization and subsequent clinical use and V&V testing passed all of these functionality tests. The component materials, adhesives, surface treatments and processes are generally accepted as stable and not susceptible to degradation over a period of several years at standard shipping and storage temperatures. In addition, if functionality were lost there would be no risk to the patient because no unwanted materials or treatments would be introduced into the body. Due to the experimental nature of the treatments and close clinical supervision, the loss of function would be detected by the clinician. Breakage – Successful verification testing has confirmed that all components and adhesive joint meet acceptance criteria. In addition, if breakage were to occur, all adhesive joints are external to the patient and no components could enter the catheter, bronchoscope or patient airway. Furthermore, the clinician would know immediately that breakage had occurred and would replace the catheter or end the procedure at their discretion. In summary, the lack of accelerated aging prior to verification testing does not impose any health hazard

to the patient and the project team recommended that these catheters be used for clinical use in their current configuration. The shelf life of the sterile barrier (3 years) was adopted from previous results with a similar catheter and the identical packaging.

Catheter Design Outputs

The TLBC was designed to guide a pCLE fiber-optics probe to the tissue site of interest within the distal lung and to simultaneously deliver small amounts of liquid for imaging and evaluation of pathological processes. The TLBC was required to be introduced into the lung via a flexible bronchoscope having a working channel with a minimum diameter of 2.0 mm. To achieve this requirement, the catheter was designed with a multi-lumen cross section. The cross section for the TLBC consists of a circular outside diameter of 1.84mm surrounding three separate lumens. The largest lumen (1.14 mm diameter) enables introduction of the pCLE imaging fiber-optics probe through the catheter and to the site of interest. This lumen is offset slightly from the central axis of the catheter. Two 0.25mm diameter fluid delivery lumens are located opposite of the larger lumen in a cross-sectional arrangement as seen in Figure 2A. The overall design of the TLBC is a 100cm long catheter that joins a triple hub (Figure 2B) where two of the extension tubes are for fluid delivery and the central tube is for introducing the pCLE imaging fiber-optics probe.

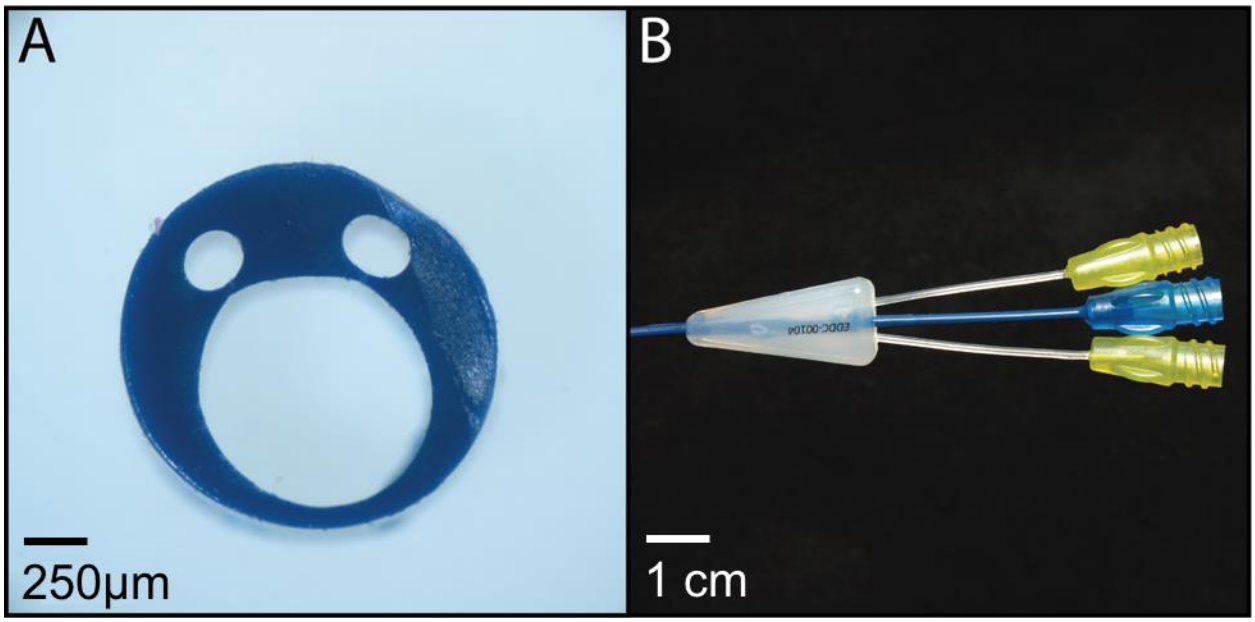

Figure 2 - (A) Catheter cross-section showing arrangement of lumens, (B) Catheter hub showing extension tubes and luer connectors for fluid (outer - yellow) and pCLE introduction (middle - blue).

The tubing for the main catheter shaft was custom extruded (Vention Medical) from 7233 SA01 MED Pebax™ (polyether block amide - Arkema). The Pebax™ was compounded with 20% barium sulfate. The barium sulfate radioopacificant was added to ensure the entire catheter shaft was visible under x-ray imaging. The catheter tubing was cut into 100 cm lengths for main shaft tubing and 6 cm segments for pCLE extension tubing. The fluid delivery extension tubes of 1.27mm outer diameter and 0.33mm inner diameter were extruded (Microspec, Inc.) from clear 6333 SA01 MED Pebax™ (Arkema) and cut into 6 cm lengths. One end of each extension tube was mechanically abraded using a fine wire brush, wiped with 70% isopropyl alcohol, and then plasma etched using ionized argon over the entire length. This was done to enhance the adhesion of stock female luer fittings (PVC, Qosina) to the abraded ends of the extension tubes. Fittings were attached to the extension tubes using an acrylated urethane UV-cure adhesive (3311, Loctite). Luer fittings were colored according to their functional purpose with blue for the pCLE lumen and yellow for fluid delivery lumens. Luer fittings were sourced

from a supplier who certified that the fittings complied with applicable standards in ISO 593 & 594[22]. A PTFE-coated, 0.2 mm diameter, 304 stainless steel wire mandrel was then inserted through the inner diameter of each extension tube-luer subassembly prior to injection molding the catheter hub.

The proximal end of the catheter tubing was then scallop cut using a scalpel through the centers of the small fluid lumens without exposing the large pCLE lumen. Exposed ends of the wire mandrels were then inserted into the corresponding channels of the main catheter shaft tubing. This assembly was placed in an aluminum mold with the extension tubing aligned such that the pCLE extension was bracketed by the two fluid extension tubes. A benchtop injection molding machine (Model 150A, LNS Technologies) was used to mold the central hub of the catheter. The catheter hub was molded from Pebax™ resin (7233 SA01 MED - Arkema). Mold flash was cleaned from the catheter and hub and the mandrels extracted. TLBC catheters were individually tested for leakage and blockage before marking and labeling.

Catheters which passed leakage and blockage testing were plasma-etched using ionized argon along the distal-most 10 cm of the main shaft. Depth markings were applied using pad-printing ink (Tampastar® TPR170, Marabu). A 2mm wide mark was applied using a customized rotating fixture. A single mark was placed at distances of 25mm and 75mm inward from the distal tip of the catheter with a double mark applied at 50mm inward from the distal tip of the catheter. The Ink was then cured with a flow of 70˚C air for 3 minutes followed by an inspection step to ensure markings met specification.

The catheters were individually labeled with unique serial numbers. This was accomplished by applying preprinted decals onto the catheter hub and then coating the decal with a clear polymer. The catheter hubs were plasma etched with ionized argon gas and printed decals (82370, Micro Mark)

were applied to the hubs. The decals were then conformably coated with UV-cure adhesive (3311) in a nitrogen chamber. Final units intended for clinical use (Figure 3) were cleaned with 70% isopropyl alcohol and sealed in clear plastic bags in preparation for shipment to a third-party company for packaging and sterilization.

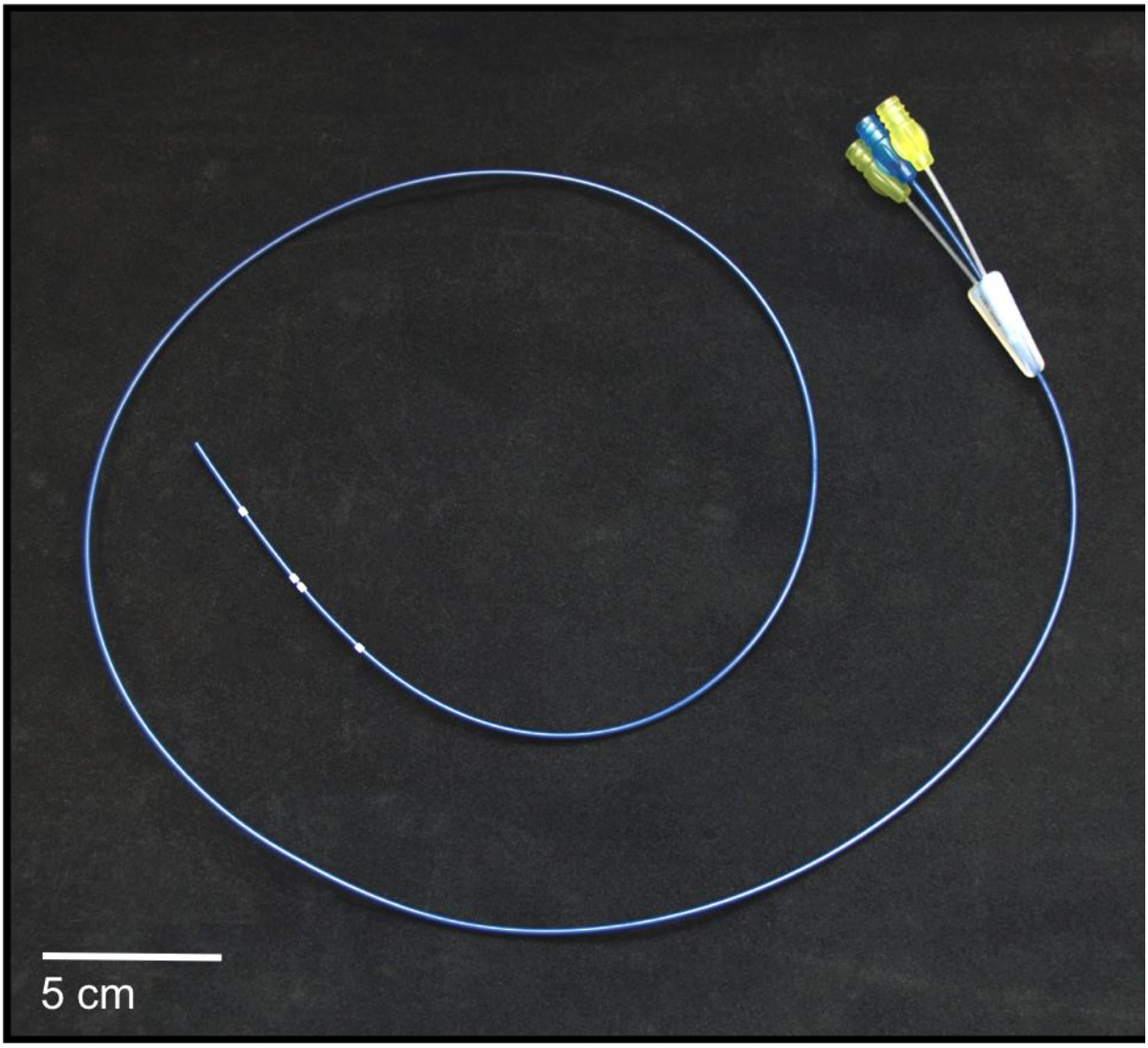

Figure 3 - Completed catheter prior to packaging and sterilization

Packaging & sterilization

Each TLBC was individually tested for functionality prior to being sent off to a third party for packaging, labeling, and sterilization (Pro-Tech Design and Manufacturing, Santa Fe Springs, California, USA). Sterilization was accomplished through ethylene oxide (EO) gas processing (Steris Isomedix, Temecula, California, USA). Once received at the third-party packager, serialized catheters were removed from the plastic shipping bags and placed within individual gas-permeable pouches. Pouches were labeled with: serial numbers, manufacturing information, manufacturing dates, and expiration dates. The pouches were placed in similarly labeled shelf boxes alongside an instruction for use document (IFU) in preparation for ethylene oxide sterilization. Shelf boxes were gathered together and placed in shipper boxes before sterilization.

Device validation

Biocompatibility of the Triple Lumen Bronchoscopy Catheter was validated by performing tests on the final product according to the requirements of ISO 10993-1 [23] and the FDA Blue Book Memorandum G95-1 [24]. The tests performed were selected using Table A.1 of ISO 10993-1 and Tables 1 and 2 of the FDA Blue book Memorandum G95-1, and after an evaluation of the use of the TLBC. The type of body contact and the contact duration were evaluated to determine the biological tests to perform.

The TLBC is intended to be used during bronchoscopy procedures, and the duration of body contact of the catheter is less than 30 minutes per bronchoscopy procedure, with contact repeated at different locations on the mucosa or the tissue during one procedure. Patient contact duration for the selection of biocompatibility tests to be performed was determined to be type A (less than 24h cumulated) according to ISO 10993-1 clause 5.3. The TLBC is a “Surface device” according to the

definitions provided in Clause 5.2.1 of ISO 10993-1 [23] and can be put into contact with the mucosal membrane. For the visualization of the lungs, the TLBC might sometimes be in contact with damaged surfaces and must therefore be categorized as “Surface device in contact with breached or compromised surfaces.” Eleven sample TLBC devices were used to evaluate biocompatibility (Nelson Laboratories Inc., Salt Lake City, Utah, USA). The tests performed were: cytotoxicity, sensitization, intracutaneous reactivity and acute systemic toxicity.

The catheter assembly and packaging was designed for sterilization using ethylene oxide (ETO). Ten test catheters were inoculated at four sites with a population of at least 1.0×10^6 CFU/site of *Bacillus atrophaeus*. Catheters were inoculated at the proximal ends of each extension tube and the hub joint region for a single fluid extension tube using thin wire. Once inoculated, each test catheter was assembled in its original packaging configuration, consisting of an individual gas-permeable pouch inside a shelf box. The inoculated wires were prepared by applying 14 µL of spore suspension (SPS Medical) to a 0.003" stainless steel wire and allowing the suspension to dry at ambient temperature for 30 minutes. Population verification (labeled for 1.8×10^8 CFU/ml - verified as 1.3×10^8) was performed on the inoculated wires.

Process Challenge Devices (PCDs) consisting of a length of Tygon tubing containing a Biological Indicator strip (NAMSA) were used to challenge the catheter sterilization process. The ends of the tubing were closed with a barbed coupler to create a closed inner space. Biological indicator (BI) strips were labeled for a *B. atrophaeus* population of 2.3×10^6 CFU/BI. The PCD was packaged in a Tyvek backed sterilization pouch, creating two layers of breathable barrier. The test products and PCDs were sterilized (Model 3017, STERIS®) with the set points found in Table 1.

Table 1 - Sterilization validation process set points

Conditioning Phase Set Points:	Exposure Phase Set Points:
Temperature: 48.9°C	Temperature: 48.9°C
Initial Vacuum: 1.1 psia	Sterilant Set Point: 7.4 psia
Vacuum Ramp Rate: 9.9 psia/minute	EO concentration: 601 *mg/L*
Relative Humidity: 61%	Gas Dwell Time: 80 minutes
Humidity Set Point: 2.1psia	
Steam Dwell Time: 120 minutes	

Immediately following cycle completion, the BIs contained in the test products and PCDs were tested for sterility by aseptically immersing them into containers of soybean casein digest broth. The containers were then incubated at 30-35°C for a minimum of seven days and scored for growth of the indicator organism, *B. atrophaeus.* Validation of the ethylene oxide sterilization process was completed in accordance with US FDA good manufacturing process (GMP) regulations 21 CFR Parts, 210, 211, and 820 (Nelson Laboratories, Salt Lake City, Utah, USA).

Benchtop verification testing

The TLBC is designed for use in bronchoscopy procedures. During the bronchoscopy procedure the TLBC is used in conjunction with a pCLE imaging fiber-optics probe (Miniaturized AlveoFlex™, Mauna Kea Technologies, Paris, France) to perform optical imaging. The Miniaturized AlveoFlex™ is a 3m long pCLE imaging fiber-optics probe with an external diameter < 1mm. When connected at its proximal end to the Cellvizio® Laser Confocal Imaging system (Mauna Kea Technologies, Paris, France), microscopic structure of fluorescent tissues can be visualized at a depth of observation ranging from 0 to 50 µm with a lateral resolution of 3.5 µm. A number of verification tests were conducted to ensure that the device met the design specifications and user requirements. Samples of the finished device and subassemblies were tested during and after the manufacturing process and as part of the

sterilization validation activities to ensure that sterilization did not alter the device specifications. The following section describes these tests.

Visibility

The correct placement of the TLBC catheter requires manipulation of the bronchoscope and catheter under fluoroscopic visualization. The TLBC I has been designed using a radiopaque polymer blend to allow for radiographic visualization. Benchtop radiographic and simulated use testing were used to verify that the design requirements for visibility were met. For radiographic testing, the combined catheter/pCLE system was x-ray imaged using a chest-representative algorithm. This test was repeated for the TLBC without the pCLE probe or flexible bronchoscope.

The second method of visualization used the integrated camera of the flexible bronchoscope to identify the depth markings placed along the distal end of the TLBC. The flexible bronchoscope was first inserted into a length of opaque tubing and the TLBC was advanced through the bronchoscope until the distal end was in the field of view. The TLBC was then advanced further until all depth markings were clearly visible. The catheter was removed from the bronchoscope and the test concluded.

Lumen patency

Flow testing was used to check for lumen patency. A pCLE imaging probe was inserted into the central imaging lumen of each catheter and advanced until the probe exited the distal end of the catheter to ensure that the lumen was un-occluded. The imaging probe was then removed. A 100ml syringe was then placed in a syringe pump (Model# 55-2222, Harvard Apparatus,) and an inline pressure transducer (PRESS-S-000, Pendotech) was attached between the syringe and one of the TLBC

fluid lumens. The pressure transducer was connected to a data acquisition system (PC mounted National Instruments NI USB-6211) as well as a 5V DC power source. The syringe pump was set to flow air through the catheter fluid lumen at a rate of 15 ml/min. Pressure readings were sampled at 100 Hz and pressure / time curves generated for each catheter (SignalView2009, National Instruments). Upon completion of the test, the syringe and syringe pump were reset, and the test was repeated for the other lumen of the catheter. 35 catheters in four sample lots were tested.

Leakage testing

To test for leakage a pressure decay test was performed. In this test a pressure transducer (PRESS-S-000, Pendotech) was attached to one of the TLBC fluid lumens and the remaining two luer fittings were sealed with non-vented luer caps. The distal end of the catheter was sealed with a plug that prevented cross-talk between the lumens. An angioplasty inflation device (BasixCompak™, Merit Medical) was used to pressurize the sealed lumen by attaching to the extension tube / pressure transducer assembly. The same data acquisition system and sample rate were used for the pressure decay and flow testing. The inflation device was used to generate an initial pressure of 18 psi, and the pressure was recorded for three minutes. After two minutes, the luer caps that were used to seal the non-pressurized lumens were removed. Removing the luer caps from the parallel lumens and recording the pressure change allowed for evaluating luminal failures resulting from cross-talk between the lumens. The test was then repeated for the other fluid lumen. The methodology was validated via submersion testing in water. The data were processed using custom Matlab script (MATLAB r2014, Mathworks). High frequency noise was removed using a moving average filter with a window size of 100 samples. Data were normalized to zero psi at time t=0, and pressure curves were plotted using as change in pressure over time.

Tensile testing

Sixty catheter subassemblies were prepared for testing joint strength. The subassemblies were packaged and sterilized prior to testing. Ten representative samples for each of the molded and adhesively affixed joints were prepared. Samples were sterilized using the same process described above. Testing was performed on a tensile testing machine (Model# 3342, Instron). Samples were tested to failure, and failure was described as either; A) failure of the interface between the tubing / molded hub or tubing / adhered luer fitting, or B) material yield of the extension tubing. Samples passed if the tensile strength of the joint exceeded 20N. The rationale for choosing 20N is based on the yield strength of the Pebax used in the extension tubes [25]. The calculated tensile yield force for these tubes is 27N. The calculated breakage force for these tubes is 59N. Using a 20N acceptance value for these components and interfaces provides a safety factor of approximately 1.4x at yield and approximately 3x at failure.

Proof of concept testing

In an *ex vivo* ovine infection model, lungs were removed from ewes, and ventilated *ex vivo* [26] This lung tissue was used to evaluate device insertion and transbronchial passage into the lung utilizing a bronchoscope. The TLBC catheter was inserted into segment into which bacteria had been preinstalled, while imaging, and a bacterial specific fluorescent “Smartprobe” agent was instilled. This approach differs from our previous work in that the fluorescent probing agent was delivered *in situ* via the TLBC rather than surface application to excised tissue segments [19, 20] utilizing a separate catheter, requiring removal of the imaging fiber [26]. The Ewe lung has been shown to be an acceptable model for human lungs [27]. These ovine lungs are similar in size to human lungs and

therefore can be used for TLBC testing using the same bronchoscope and ventilator as would be used in humans.

A key design requirement was the need for the catheter to pass through the bronchial wall into the lung parenchyma to reach sites of interest. In clinical usage, tactile feedback at the point of transbronchial pass allows the user to be confident of alveolar passage in combination with real-time imaging. Damage to healthy tissue was to be minimized by the design of the catheter tip. A variety of tip designs were explored including both square and tapered ends. A series of transbronchial passage tests were performed on excised lung tissue under direct visualization to assess the potential damage to pleural tissue. Three sizes of catheter outer diameter were evaluated: 1.7mm, 1.84mm, 2.36mm with increasing parenchymal damage with increasing diameter. Two catheter tip designs were explored including a square end and tapered end. This tissue was then dissected to observe the extent of damage to the parenchyma caused by transbronchial passage of the TLBC. The investigators then scored the results relative to each other.

RESULTS

Sterilization Validation

The TLBCs used in the validation demonstrated less resistance to EO sterilization than the PCDs. Therefore, the PCDs were determined to be appropriate indicators for the sterilization of the TLBC catheters. Screening for EO residuals further indicated that >99% of EO gas remnants had been cleared from the devices in accordance with the standards set in ISO 10993-7:2008 [28] for limited dwell time devices. Subsequent catheters used for additional verification and validation testing were sterilized according to the protocol developed during sterilization validation.

Benchtop testing

Benchtop testing of TLBC units was used to verify that design outputs met design inputs. Visibility testing of TLBC units verified that the TLBC catheter is visible under x-ray imaging (Figure 4A). Simulated use testing to analyze the visibility of the depth markings using the bronchoscope's integrated camera was able to clearly identify all the depth markings. A representative image is included as Figure 4B.

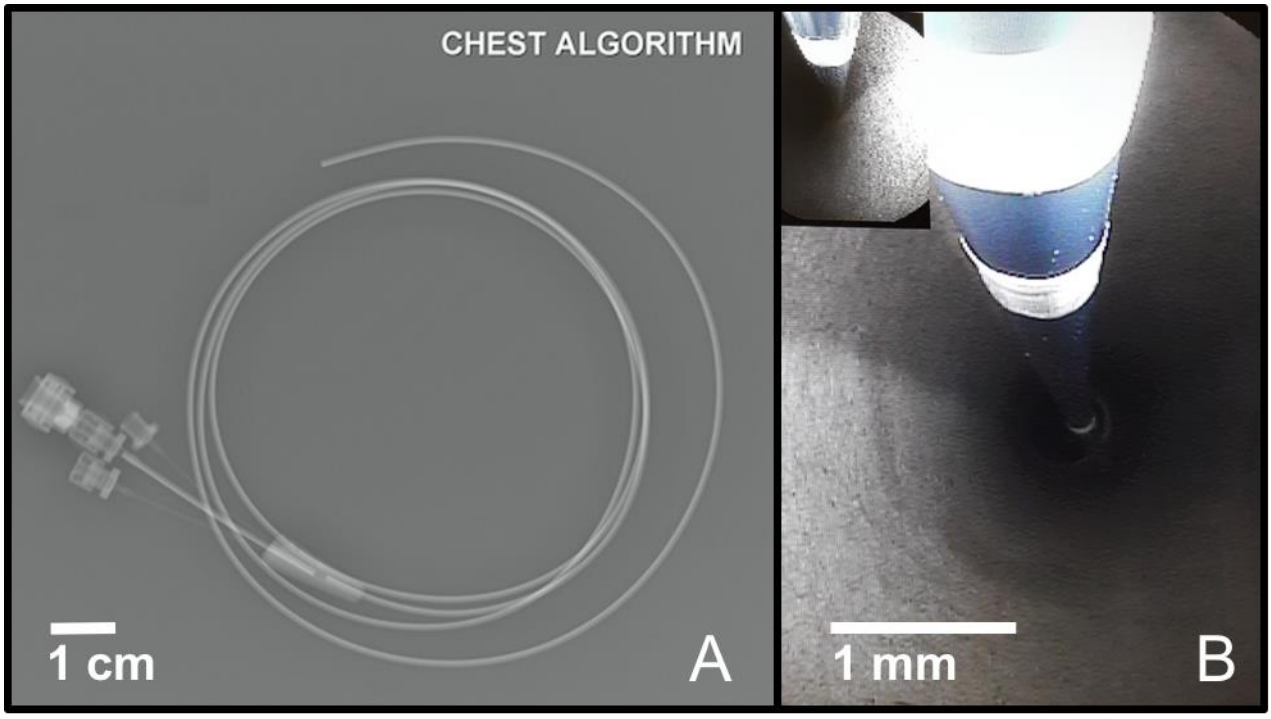

Figure 4 - (A) TLBC catheter under x-ray imaging in a chest algorithm. (B) The white depth marking bands are clearly visible using the integrated camera on the flexible bronchoscope.

The Miniaturized AlveoFlex™ pCLE imaging fiber-optics probe was able to pass easily along the length of all manufactured catheters as demonstrated in Figure 5A. Additional bending tests of the TLBC within a flexible bronchoscope confirmed that the probe remained freely moveable within the imaging lumen of the TLBC for the complete range of bronchoscope motion angles.

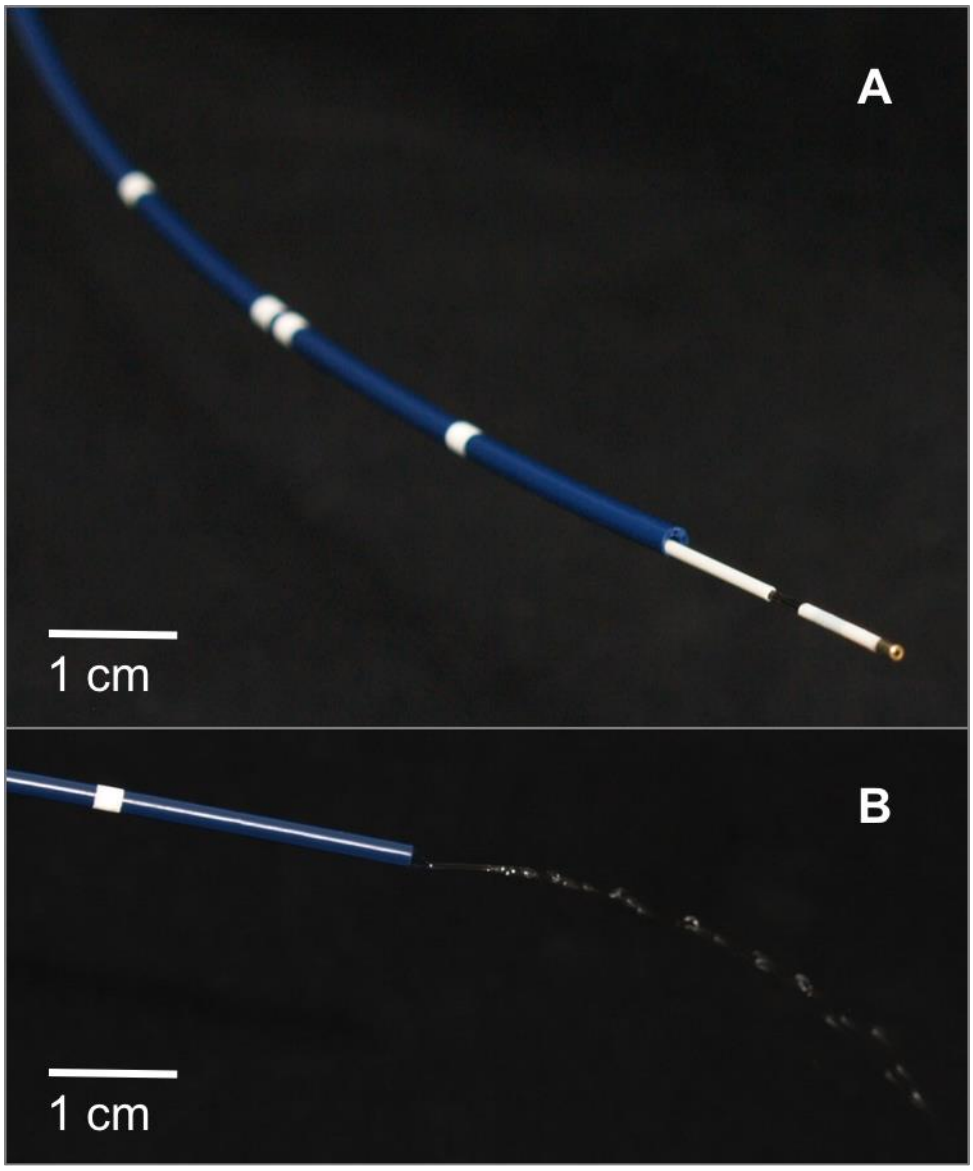

Figure 5 - (A) Image of the distal end of TLBC with the AlveoFlex™ pCLE probe extended outside of the imaging lumen. Note that the probe shown here is a slightly damaged test sample used for fit-tests only. (B) Image of distal end of the TLBC illustrating the liquid flow through the 250µm fluid lumens.

Pressurized flow testing indicated that no lumens were occluded. All pressure curves showed that flow through the lumens reached steady-state flow. Subsequent submersion bubble testing indicated that all of the catheters with a final pressure reading less than 2.5 psi had leaks, while those in the range 2.5 psi-3.0 psi were mixed. Catheters with pressures above 3.0 showed no leaks. (Figure 6).

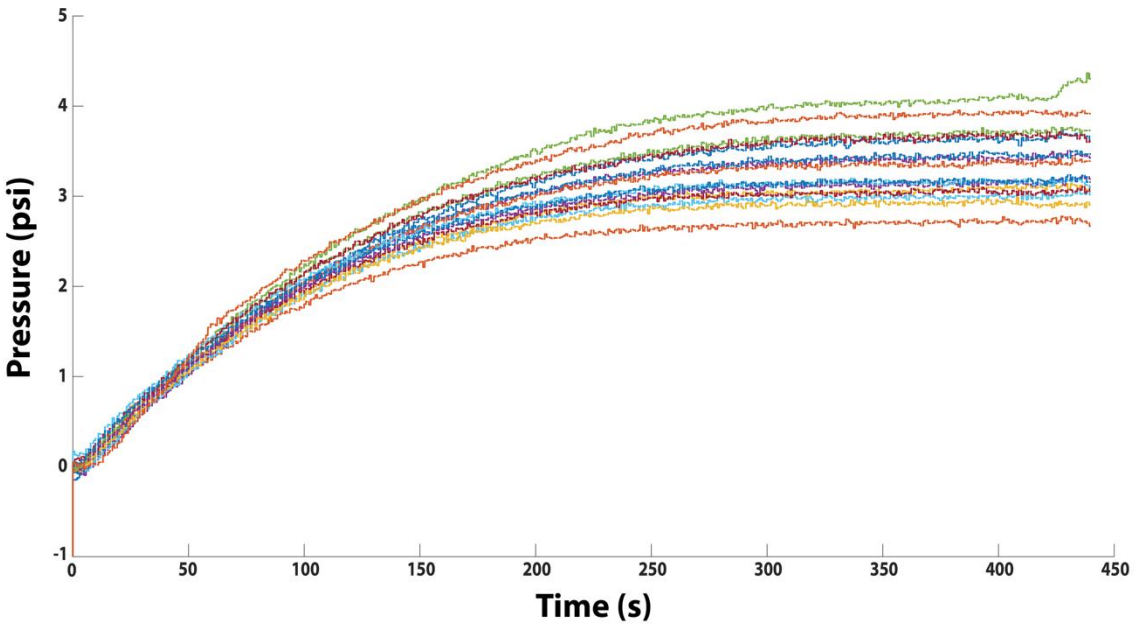

Figure 6 - Flow Test Results. Submersion bubble testing indicated that all catheters with final pressure of ≤2.5 psi were prone to leakage, and catheters with final pressures in the range of 2.5 psi - 3.0 psi were suspect. No leakage was found in catheters where the pressure was ≥3.0 psi.

Decays in pressure were found to indicate lumens having moderate to severe leaks at the luer fittings. A pressure curve that exhibited a time-delayed decay in pressure indicated crosstalk within the hub of the catheter. These results followed a predictable behavior associated with crosstalk within the catheter (Figure 7). Submersion testing appeared to validate the failure types identified with the pressure decay test. However, 7 lumens tested by submersion showed leaks that the pressure decay test did not indicate. The catheters were pressure tested a second time and these leaks were identified as new leaks. These new leaks are posited to have been caused by handling following the initial pressure decay testing.

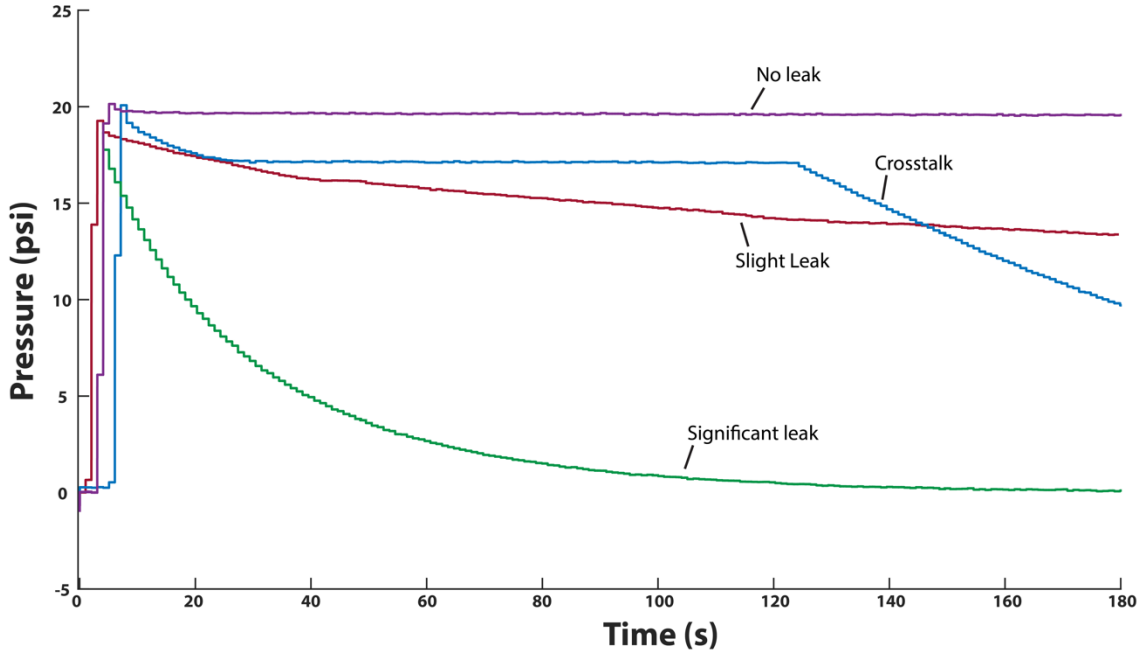

Figure 7 - Pressure Decay Testing results. Catheters with no leakage held constant pressure for the duration of the test (top). Lumen cross-talk leakage was identified by a sudden drop in pressure when the cap of an adjacent lumen was removed (second from top). Steady loss of pressure was associated with leakage through one of the joint regions of the assembly (Bottom two).

The most common mode of tensile test failure was tensile failure of the extruded tubing. This occurred in 56 out of 60 samples or 93(%) percent of cases tested (Table 2). The remaining 7(%) percent failed due to luer joint adhesion failure prior to any detectable tubing material failure. All tests reached the threshold value of 20N. Tensile failure values were an average of 25.030N for fluid tubing, and 46.911N for optics/catheter tubing.

Table 2 - Tabulated Tensile Testing Pass/Fail Results

Configuration Tested	Extrusion Failure (No. / %)	Joint Failure (No. / %)
Optical luer / Optics ext. tube subassembly	10 / 100%	0 / 0%
Fluid luer / Fluid ext. tube subassembly	6 / 60%	4 / 40%[a]
Optics Extension Tubing / Hub Joint	10 / 100%	0 / 0%
Fluid Extension Tubing 1 / Hub Joint	10 / 100%	0 / 0%
Fluid Extension Tubing 2 / Hub Joint	10 / 100%	0 / 0%
Hub / Catheter Extrusion joint	10 / 100%	0 / 0%

[a]In these failures, the failure values are consistent with the values for material failure. This could indicate that the change in cross-sectional area of the stretched tubing has caused the tubing to become detached from the relatively stiffer adhesive used to bond the luers to the tubing, rather than a true failure of the adhesive bond with undamaged (unstretched) tubing material.

Usage in *ex vivo* lung

Anatomically distinct bronchopulmonary segments of the *ex vivo* lung were instilled with bacteria, and subsequently microdoses (<100 mcgs) of a bacterial imaging agent were instilled and imaged using the TLBC. Following instillation, this imaging agent enabled the specific detection of bacteria in the distal airway and alveoli within sixty seconds (Figure 8).

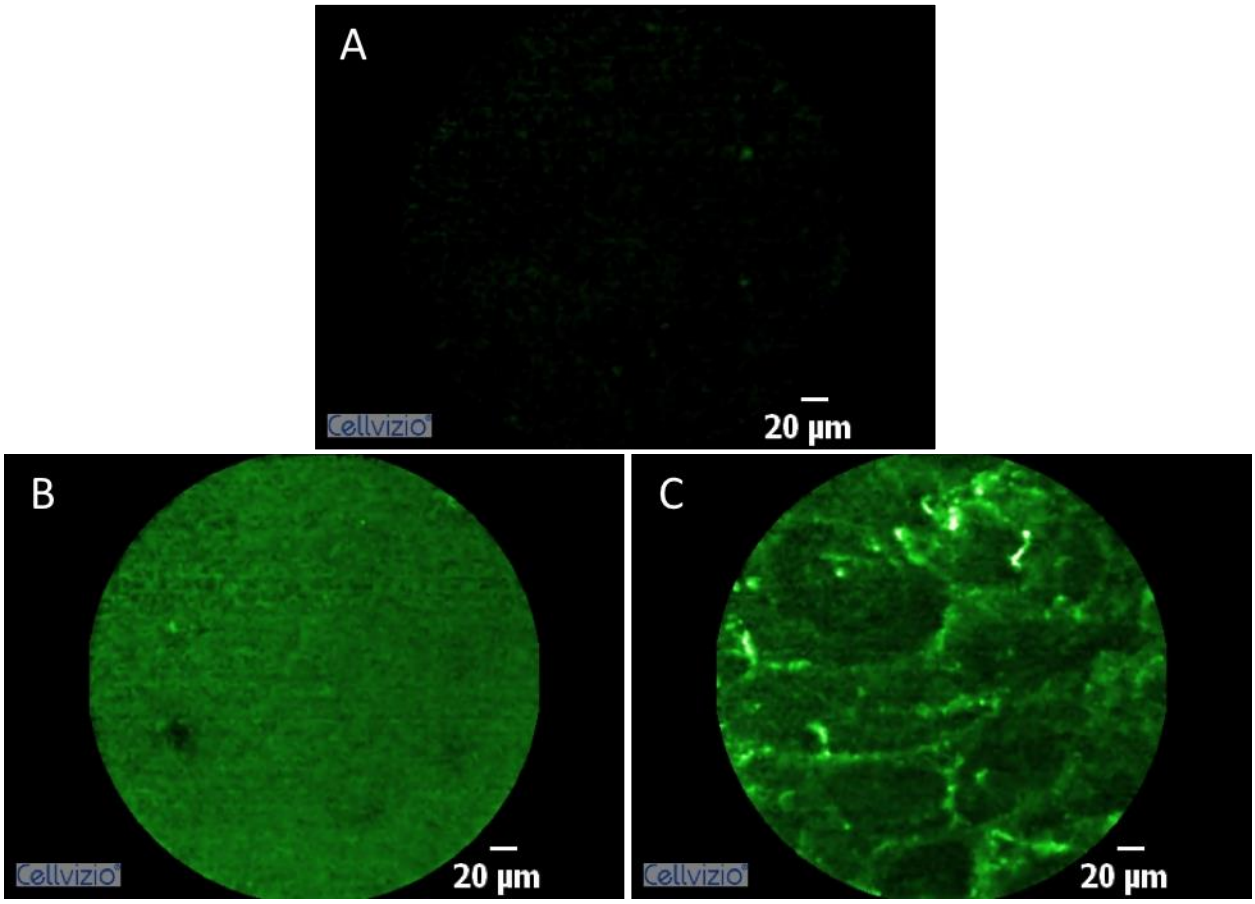

Figure 8 - (A) Miniaturized AlveoFlex with TLBC in distal ovine lung without Fluorescent probe. (B) Image immediately following fluorescent probe administration (C) Image following fluorescent probe dissipation. Bright areas show areas of bacterial infiltrates.

Testing in *ex vivo* lung tissue showed that there was a lack of tactile feedback, which could potentially result in damage to pleura or other organs. We discovered that advancing the pCLE imaging probe slightly beyond the end of the TLBC and initiating transbronchial passage with the probe in place was as effective as the other tip designs. Therefore, a final design shape was agreed on as a square end with the smallest practical size to house the imaging fiber and deliver fluids. Dissected tissue showed damage. The damage was assessed by investigators as shown in Table 3. We found that a touhy-borst type locking device placed proximally was required to stop the pCLE fiber from slipping back during transbronchial passage.

Table 3 - Evaluation of tissue damage and difficulty of transbronchial passage, low (+) to high (+++++)

	Ease of passage (+ to +++++)	**Absence of damage to tissue (+ to +++++)**
Standard AlveoFlex™ (reference)	**+++**	**+++**
Miniaturized AlveoFlex™ alone	+++++	+
Miniaturized AlveoFlex™ in TLBC	+++	+++/++++
Standard AlveoFlex™ in TLBC	+++++	+++++

DISCUSSION

One of the challenges for medical researchers is translating research discoveries into clinically impactful solutions. Reasons for this difficulty are many, and can include lack of infrastructure, experience with the design and regulatory processes, or struggling to find clinical needs to fit an already developed technology [29]. In an academic setting these challenges are amplified because universities have typically not institutionalized the commercialization of medical technology. Many institutions have Institutional Review Boards (IRBs) that review clinical trial protocols however these IRBs are placing new medical device technology under increasing scrutiny. In addition, new regulatory guidance from the FDA [30] is adding additional translational hurdles such as sterilization validation for academic medical device developers.

This project was guided by a quality systems approach [31] in order to facilitate regulatory approval for human clinical use. Using this approach, the team drafted a project plan and a design input document. Specifications were then developed alongside a comprehensive risk management plan both of which guided the final TLBC design. Manufacturing was conducted in a specific area of the laboratory where materials, processing instructions, equipment, work in process and finished goods could be controlled. Testing on the finished devices illustrated failure modes that could be investigated

for root cause. The failures that arose during the testing motivated process improvements to increase manufacturing yield and overall device quality.

The quality systems and design control process [31] serves as common framework for developing new medical technologies. The design control process guided our development of a unique catheter-based approach to image distal lung pathology using the TLBC. The TLBC and pCLE imaging fiber-optics probe when used with fluorescent reporter molecules represents advantages over the current state of the art. First, is the speed in which an optical molecular biopsy is able to deliver a diagnosis compared to currently established biopsy approach. Secondly, *in situ* characterization to guide therapeutic intervention of diverse lung diseases paves the way for eliminating the time-to-treat gap evidenced in modern medicine. Using the device-based approach that we've described here enables simultaneous minimally invasive optical imaging and molecular profiling in a single device.

Currently, no alternate device allows the simultaneous pCLE imaging and delivery of fluid/compounds into a pulmonary segment. Current methodologies require i) bronchoscopic navigation to an affected region, ii) baseline imaging with a pCLE bundle, iii) removal of imaging bundle and insertion of a catheter which allows fluid/compound delivery and iv) delivery of fluid/compound and v) removal of catheter and re-insertion of the pCLE bundle to acquire images. This approach results in requiring larger volumes of fluid/compound delivery to ensure adequate dispersion, as well as the risk of being unable to return to the same pulmonary sub-segment for imaging and fluid administration. Therefore, the TLBC is an improvement in current techniques because it allows for simultaneous imaging and fluid/compound delivery directly into the field of view, therefore utilizing less compound, providing confidence the segment imaged post-delivery remains the same, and reduced procedure time.

Optical biopsy with pCLE has already been shown to be a novel means of rapidly identifying various pulmonary indications [20]. The TLBC builds upon the groundwork of pCLE imaging fiber-optics probes by introducing a catheterized sheath that also enables delivery of aqueous formulated products in small volumes with a high degree of spatial precision. With this configuration the unit as a whole remains with the pCLE imaging fiber-optics probe and does not need to be removed during the procedure in order to deliver agents. This approach has the potential to significantly reduce overall procedure time for optical molecular endomicroscopic guided procedures in the lung. The real-time lung images allow on or off-site interaction with pathology experts for rapid diagnosis. A fast diagnosis would allow for diagnostic actions or in some clinical indications, localized therapy which could be immediately delivered via the TLBC. This potential to diagnose and guide therapy collapses the time-to-treat gap to minutes rather than the current standard of hours to days.

The emergence of localized delivery of optical molecular imaging agents coupled with pCLE are enabling new methods for analyzing lung pathologies. These technologies could be significantly augmented by catheters such as the TLBC to deliver fluorescent reporters with a high degree of spatial and temporal resolution to the distal alveolar space. Indeed, some of the fluorescent Smartprobes are enzyme sensors, and accurate measurement of dynamic fluorescent amplification improves data collection. Thus a specialized catheter was developed to bring these technologies together. The TLBC was designed specifically for this application - to allow simultaneous imaging and delivery of targeted molecular imaging agents. This new catheter places the imaging probe and imaging agents in the same tissue location and importantly enables in situ in vivo visualization of the dynamic fluorescent pathophysiological processes in the distal lung and opens new avenues of better, faster, safer, less

expensive, and more targeted analysis of pulmonary diseases. This, in-turn, has the future potential to deliver improved patient outcomes and reduced burden of health care costs.

ACKNOWLEDGMENT

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NOMENCLATURE

pCLE	Probe-based Confocal Laser Endomicroscopy
TLBC	Triple Lumen Bronchoscopy Catheter

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Figure Captions List

Fig. 1 Device usage diagram. The TLBC is inserted via flexible bronchoscope and navigated to the region of interest. Imaging agent is then added, and the subject tissue analyzed. In the event the tissue shows pathology requiring treatment, therapeutic liquids can then be applied. This is all accomplished without having to remove/reinsert catheter (Original image from Wikipedia commons, modified by author).

Fig. 2 (A) Catheter cross-section showing arrangement of lumens, (B) Catheter hub showing extension tubes and luer connectors for fluid (yellow) and pCLE introduction (blue).

Fig. 3 Completed catheter prior to packaging and sterilization

Fig. 4 (A) TLBC catheter under x-ray imaging in a chest algorithm. (B) The white depth marking bands are clearly visible using the integrated camera on the flexible bronchoscope.

Fig. 5 (A) Image of the distal end of TLBC with the AlveoFlex™ pCLE probe extended outside of the imaging lumen. Note that the probe shown here is a slightly damaged test sample used for fit-tests only. (B) Image of distal end of the TLBC illustrating the liquid flow through the 250μm fluid lumens.

Fig. 6 Flow Test Results. Submersion bubble testing indicated that all catheters with final pressure of ≤2.5 psi were prone to leakage, and catheters with

final pressures in the range of 2.5 psi - 3.0 psi were suspect. No leakage was found in catheters where the pressure was ≥3.0 psi.

Fig. 7 Pressure Decay Testing results. Catheters with no leakage held constant pressure for the duration of the test (top). Lumen cross-talk leakage was identified by a sudden drop in pressure when the cap of an adjacent lumen was removed (second from top). Steady loss of pressure was associated with leakage through one of the joint regions of the assembly (Bottom two).

Fig. 8 (A) Miniaturized AlveoFlex with TLBC in distal ovine lung without Fluorescent probe. (B) Image immediately following fluorescent probe administration (C) Image following fluorescent probe dissipation. Bright areas show areas of bacterial infiltrates.

Table Caption List

Table 1 Sterilization validation process set points

Table 2 Tabulated Tensile Testing Pass/Fail Results

Table 3 Evaluation of tissue damage and difficulty of transbronchial passage, low (+) to high (+++++)