Mechanical and histological characterization of trachea tissue subjected to blast-type pressures

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Abstract. Injuries to the respiratory system can be a component of polytrauma in blast-loading injuries. Tissues located at air-liquid interfaces, including such tissues in the respiratory system, are particularly vulnerable to damage by blast overpressures. There is a lack of information about the mechanical and cellular responses that contribute to the damage of this class of tissues subjected to the high strain rates associated with blast loading. Here, we describe the results of dynamic blast-like pressure loading tests at high strain rates on freshly harvested ex vivo trachea tissue specimens.

1. Introduction
Evidence from recent conflicts shows that both soldiers and civilians today are capable of surviving more extensive wounds compared to victims of explosives in previous wars. This increase in survival is likely due to improvements in injury mitigation and military trauma care that have been developed in recent years [1]. As a result, there is an imperative to elucidate the underlying biology of blast injury, as a means towards developing improved therapeutic treatments.

Primary blast injuries are defined as those resulting from exposure of the body to environmental pressure variations accompanying the blast wave [2]. Primary respiratory blast injuries are those caused by the direct interaction between blast waves and biological material. Several organ systems have been identified as being most at risk of sustaining primary blast injury, namely: tympanic membranes, the gastro-intestinal tract and the respiratory tract, [new sentence] all of these tissues have interfaces with air or liquid [3]. The most well-known type of injury of this type is blast lung, a primary blast injury that involves pulmonary contusion, oedema and the rupture of alveoli or surrounding blood vessels [4]. Survivors of an initial blast may experience a delayed trauma, such as adult respiratory distress syndrome (ARDS) [5], which can put their lives at risk unless they receive appropriate clinical intervention. Delayed, life-threatening injuries to the upper airway, including the trachea, can also occur. In these cases, symptoms consistent with the development of airway edema and blast lung are observed 6 to 12 hours after blast exposure, often requiring mechanical ventilation.
to then be applied [6]. A comprehensive understanding of the biological and material responses of these tissues to blast waves is required to improve strategies to mitigate the effects of blast and to more effectively treat the wounded.

Blast loading subjects tissues to high strain rates. Split-Hopkinson pressure bar (SHPB) systems have been used to study the dynamic material responses of biological tissues using a range of strain rates. Information from such studies can be used in simulations of the human body to understand how it responds to the high levels of mechanical stress that lead to traumatic injury [7-16]. Effective use of an SHPB platform for soft tissues can be challenging and often requires adaptation of experimental equipment and methods to enable viable data to be obtained. For example, bar materials, pulse shapes and sample preparation and mounting methods may have to be modified [7, 11-16]. Here, we present initial studies of the dynamic material properties of freshly harvested swine trachea tissue using a split-Hopkinson pressure bar (SHPB) system. These studies are an initial step towards developing new in vitro and computational models of blast injury to the respiratory system, which may lead to improved injury mitigation and therapeutic treatment strategies.

2. Methodology

2.1. Split Hopkinson Pressure Bar

Trachea tissues were obtained from six to eight week old piglets, sourced from a Specific Pathogen Free (SPF) closed herd. Piglets were sacrificed by intravenous administration of sodium pentobarbitone at a dosage of 0.8 mg per kg body mass. The trachea was excised and the trachealis muscle incised longitudinally to expose the tracheal epithelial surface. Full thickness tissue samples were taken as circular discs using an 8-mm diameter biopsy punch. The discs were placed in Phosphate Buffered Saline (PBS) solution on ice (4 °C) for the purposes of transport. Prior to testing, the discs were removed from the PBS solution, lightly dried, and relevant dimensions (diameter, thickness and weight) were measured (table 1).

Each disc was then placed between the lubricated end faces of the input and output bars of an unconfined SHPB system as shown in figure 1. High strain rates (of approximately 6000 s⁻¹) were applied using magnesium bars because of their low impedance, allowing good stress transmission through the sample, as well as high signal to noise ratio from the strain gauges. Semiconductor strain gauges were used to record the input and output signals. Triplicate samples were measured and the resulting data used to obtained average engineering stress-strain.

| Sample | Diameter ± 0.005/mm | Thickness ± 0.005/mm | Weight ± 0.0005/g |
|--------|---------------------|----------------------|-------------------|
| Sample 1 | 7.54                | 1.73                | 0.096             |
| Sample 2 | 8.09                | 1.62                | 0.107             |
| Sample 3 | 7.85                | 1.86                | 0.111             |

2.2. Histology

Compressed and uncompressed (control) tissue samples were fixed in formaldehyde and then prepared for histological analysis. Paraffin wax-embedded sections were cut at a thickness of 4 microns and stained with Haematoxylin and Eosin to evaluate changes in cell and tissue morphology. Optical data were obtained using a Nikon Eclipse E400 microscope and a JVC camera.
3. Results and Discussion

3.1. Split Hopkinson Pressure bar

The averaged engineering stress-strain curve calculated for unconstrained trachea samples, collected as triplicates from the same animal, is shown in Figure 2. The viscoelastic modulus (used for materials like tissue) was determined after establishing the point at which equilibrium is reached by 2-wave analysis. For fresh porcine trachea, the modulus obtained was $10.6 \pm 0.9$ MPa for triplicate measurements of trachea samples at strain rates of approximately 6000 s$^{-1}$. 

Figure 1. Schematic showing the SHPB system used to test the tissue samples. The tissue (red disc) is held between the lubricated end faces of the input and output bars of the SHPB. A hollow polycarbonate jacket is used to ensure biological containment of the sample throughout the test, but the sample is unconfined. Strain gauges present on the bars provide dynamic data, which is used to calculate the stress and strain experienced by the tissue sample.

Figure 2. Average engineering stress-strain for trachea at strain rates of approximately 6000 s$^{-1}$. The data are an average across three samples taken from the same animal. A viscoelastic modulus of $10.6 \pm 0.9$ MPa was calculated as the gradient of the stress-strain curve once the sample had reached equilibrium, as determined by 2-wave analysis (figure 3).
Figure 3. Average 2-wave analysis data for three trachea samples. Equilibrium is indicated by the black arrow.

3.2. Histopathology
Figure 4 shows a comparison of representative light micrographs of a control sample and a compressed sample of trachea tissue. The control was chemically fixed at the point of extraction and therefore not subjected to compression using the SHPB. The control shows normal histological architecture including the presence of cilia, the outermost layer of cells present at the air-tissue interface of the trachea. In comparison, compressed trachea shows a loss of cilia and an obvious compression of the underlying connective tissue layers, although the cartilage appears intact.

Figure 4. Comparison of uncompressed trachea (Control) with trachea subjected to a strain rate of approximately 6000 s⁻¹ (High Strain Rate) using the SHPB system described above. A representative light photomicrograph of a control sample is shown in the upper left quadrant and a representative light photomicrograph of a compressed sample is shown in the lower right quadrant. Embedded schematics are included to identify the cellular structures visible in these images, from cilia located at the air-interface to cartilage. A scale bar indicating 100 µm is also shown.
4. Discussion
The trachea is a viscoelastic, multi-layered, fiber-oriented composite of soft tissues [17]. A fibrous, collagen-rich hyaline cartilage is the main component, and is primarily responsible for its structural stability. The extensive meshwork of fibers in the soft connective tissues of the trachea helps to explain the non-linear and anisotropic material behaviours of the trachea [18]. The mechanical properties of trachea and its subcomponent tissues have been studied in both compression and tension from a number of mammalian species and in human cadaveric tissues, yielding moduli of the order of 10 kPa to 100 MPa [19-23]. This wide variation is due in part to the unfolding of collagen fibrils under relatively low force, followed by a re-orientation resulting in strain-stiffening of the tissues at increasingly higher forces [24]. Variations in modulus values have also been attributed to species-specific differences in the tissues and to sample orientation [23]. Less attention, however, has been paid to the biological viability of the tissues, and most reports use materials maintained or sourced under conditions that would cause tissues to be stressed (altering their normal pathology) and/or degraded.

As a step towards creating experimental models of blast injury based on live trachea tissue, we have begun to characterize the material properties of fresh trachea tissue samples using the SHPB at moderately high strain rates (of approximately 6000 s⁻¹). We are comparing the cellular architecture of control and compressed tissue using optical imaging. The modulus of 10.6 ± 0.9 MPa obtained in these studies falls within the range of measurements of trachea tissues cited above and is near the lower limits (10-30 MPa) of modulus values (bending and elastic) for collagen [25, 26]. Optical micrographs indicate that control samples show normal cellular architecture, identical to that observed in live trachea [27]. In adult humans, the outermost epithelium contains at least eight different cell types [28], but is dominated by ciliated cells, as seen in figure 4. In comparison, the representative optical image of the SHPB compressed samples is not intended to represent the true pathology of a blast injury as this tissue is compressed by a direct impact rather than by a blast wave. However, ablation of the cilia layer of cells and compression of the underlying connective tissue provide an indication of the relative resilience of layered structures in the trachea likely to be affected by blast loading conditions. In addition, the relatively normal appearance of the cartilaginous layer suggests stiffening effects may occur in this layer of tissue, most likely arising from structural changes to collagen fibrils.

5. Conclusions
We have presented data describing mechanical properties and alterations of cellular structures of trachea tissue subjected to moderately high strain rates using an SHPB system. The data obtained are highly reproducible and provide information about the material properties of trachea in a biologically viable form, supported by accompanying histological data. Future studies will use high-resolution tissue-imaging techniques to provide more detailed data about potential structural alterations to collagen in the cartilaginous layers of the trachea. Similarly excised tissue samples will also be used in the development of an ex vivo trachea tissue model for creating blast-like pathologies using complementary platforms, such as a shock tube, for studying blast injury.

Acknowledgements
This work was supported by the Royal British Legion Centre for Blast Injury Studies at Imperial College London. The Institute of Shock Physics acknowledges the support of the Atomic Weapons Establishment, Aldermaston, UK and Imperial College London. The invaluable technical support of the Cavendish Laboratory workshop and the Cambridge Veterinary Histopathology Laboratory is gratefully acknowledged by the authors. We also thank D. Thompson for proofreading.
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