

# Integrated experimental platforms to study blast injuries: a bottom-up approach

C Bo<sup>1,2</sup>, A Williams<sup>3</sup>, S Rankin<sup>1,4</sup>, W G Proud<sup>1,2</sup> and K A Brown<sup>1,5,6</sup>

<sup>1</sup> *The Royal British Legion Centre for Blast Injury Studies, Imperial College London, London, UK*

<sup>2</sup> *Institute of Shock Physics, Imperial College London, London, UK*

<sup>3</sup> *Department of Veterinary Medicine, Cambridge University, Cambridge, UK*

<sup>4</sup> *National Heart & Lung Institute, Imperial College London, London, UK*

<sup>5</sup> *Cavendish Laboratory, Cambridge University, Cambridge, UK*

<sup>6</sup> *Department of Chemistry, University of Texas, Austin, USA*

E-mail: c.bo10@imperial.ac.uk

**Abstract.** We are developing experimental models of blast injury using data from live biological samples. An integrated research strategy is followed to study material and biological properties of cells, tissues and organs, that are subjected to dynamic and static pressures, relevant to those of battlefield blast. We have developed a confined Split Hopkinson Pressure Bar (SHPB) system, which allows cells, either in suspension or as a monolayer, to be subjected to compression waves with pressures on the order of a few MPa and durations of hundreds of microseconds. The chamber design enables recovery of biological samples for cellular and molecular analysis. The SHPB platform, coupled with Quasi-Static experiments, is used to determine stress-strain curves of soft biological tissues under compression at low, medium and high strain rates. Tissue samples are examined, using histological techniques, to study macro- and microscopic changes induced by compression waves. In addition, a shock tube enables application of single or multiple air blasts with pressures on the order of kPa and a few milliseconds duration; this platform was used for initial studies on mesenchymal stem cells responses to blast pressures.

## 1. Introduction

Blast injury is a hallmark of modern military combat [1]. Treatment of the wounds in survivors represents a huge clinical challenge. In blast exposure, high pressure pulses and high strain rate deformation of hard and soft tissues result in injuries and tissue dysfunction uncommon in injuries away from the battlefield. ‘Blast lung’, an acute lung injury seen in some survivors of explosions, is an example of a traumatic injury that is primarily observed in military settings. This condition is characterized by the presence of respiratory distress, cough and hypoxia without penetrating or other blunt thoracic injuries [2]. Symptoms of blast lung may appear days after the initial blast exposure, making diagnosis particularly challenging. Another complication that can develop in traumatized limbs from war wounds is heterotopic ossification (HO), aberrant bone formation outside the skeletal tissue [3]. The development of HO has been largely attributed to systemic responses that affect the relationship between pro- and anti-inflammatory factors in the body [4,5].

Of interest is the debate regarding the parameters (pressure, duration) of the blast stimulus and their relation with the level of damage caused to the body. Richmond et al. [6] reported lethality curves for different animals as a function of overpressure and duration of the stimulus in open air experiments. From their calculations a stimulus of the duration of 400 ms with a peak pressure of 600



psi would cause 99% lethality for mammals of 70 kg weight. In a more recent study, Bass et al. [7] analysed data from more than 2550 large animal blast experiments and defined new blast injury thresholds for short duration blasts (< 30 ms) of about 1-2 MPa peak pressures. These studies give some indications on the pressure-time regime of interest in blast injuries, but they lack characterization of the pressures and strains developed inside the body at different length scales. Previous studies on the effects of pressure pulses on cells and tissues have used shock waves of tens of MPa in amplitude and duration in the order of tens of microseconds [8, 9].

This paper describes a series of “bottom up” research activities focused on understanding the effects of pressure waves ranging from tens of kPa to few MPa on cells and tissues.

## 2. Development of biocompatible chambers for a confined Split Hopkinson Pressure Bar System

We developed a system for applying pressure pulses to cell cultures using a modified SHPB system that is equipped with a biocompatible confinement chamber that permits recovery of samples for further cellular and molecular analyses [10]. We tested different chamber designs to establish the optimal configuration and experimental protocols to ensure experiment repeatability, to eliminate experimental artifacts and to maximize sample recoverability.

### 2.1. Chamber design and SHPB compression experiments

The currently design of our biocompatible confinement chamber is shown in figure 1. The main body is composed of a polycarbonate cylinder with inner O-ring grooves. Two polycarbonate discs with an O-ring groove on the inner side are pushed against the main body by brass taps to improve sealing of the liquid inside the main body during the experiments. The chamber is mounted on a compressive SHPB system composed of four bars made from Inconel steel of 12.7 mm in diameter and 190 mm (striker) and 500 mm (input, output and momentum trap) in length. Liquid samples are inserted using syringes through 1 mm diameter counter bored holes, which are then sealed with a nylon screw. The chamber is instrumented with two foil strain gauges GFLA-3-350-70 (TechniMeasure, UK) located halfway along the chamber length and diametrically opposite in order to measure the circumferential strain. The hoop strain  $\varepsilon_\theta$  measured with a Wheatstone bridge is used to calculate the radial stress  $\sigma_r$  developed inside the chamber according to the equation

$$\sigma_r = \frac{1}{2R_i^2} E_C (R_i^2 - R_o^2) \varepsilon_\theta \quad (1)$$

where  $R_i$  and  $R_o$  are the inner and outer radii of the main cylinder, respectively, and  $E_C$  is the Young's modulus of the confinement chamber's material (2.4 GPa, as supplied by manufacturer). The longitudinal stress  $\sigma_l$  developed inside the chamber is calculated assuming stress equilibrium using classical SHPB theory iterated for the number of pressure pulses calculated from the hoop strain as

$$\sigma_l = E_B \varepsilon_T \quad (2)$$

where  $E_B$  is the bar's Young's modulus and  $\varepsilon_T$  the transmitted strain, measured with semiconductor strain gauges, Kulite type AFP-500-90, bonded to the Hopkinson bars. The average pressure developed in the chamber is defined as

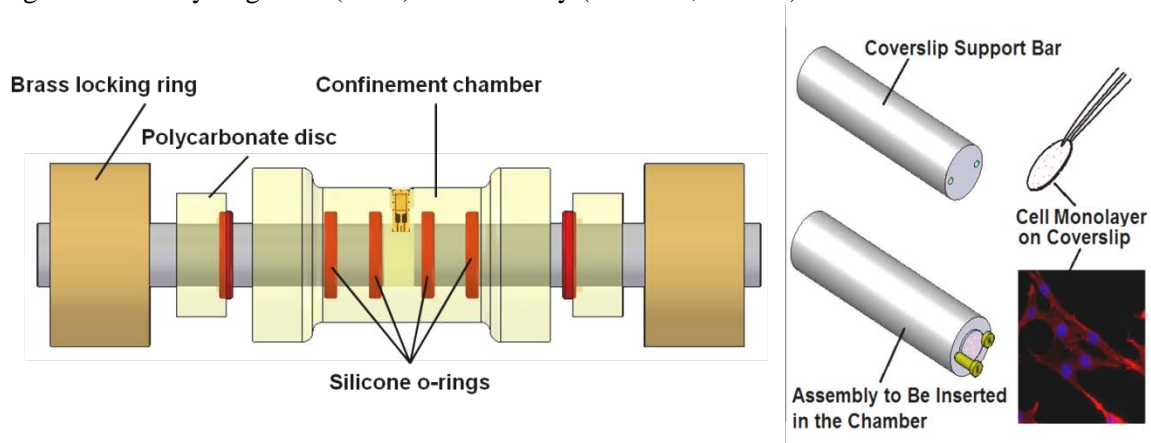
$$\sigma_C = \frac{\sigma_l + 2\sigma_r}{3} \quad (3)$$

Two pressure conditions are used in the cellular experiments. These conditions, known as Blast 1 and Blast 2, are achieved by firing the striker bar with impact velocities of 3.4 ms<sup>-1</sup> and 7.8 ms<sup>-1</sup>, respectively.

### 2.2 Biological samples and assays

Mesenchymal stem cells (MSCs) were selectively cultured from the periosteum and bone marrow of Balb/c mice up to passage three in a Dulbecco's Modified Eagle Medium (DMEM) containing 20% (v/v) Fetal Calf Serum (FCS), 1% (v/v) Penicillin/Streptomycin, 0.2% (v/v) Heparin and 5 ng/ml basic fibroblast growth factor (bFGF). When 80-90% confluency was reached cells were detached from the culture flask using 0.05% Trypsin/ethylene-diaminetetraacetic acid and resuspended in

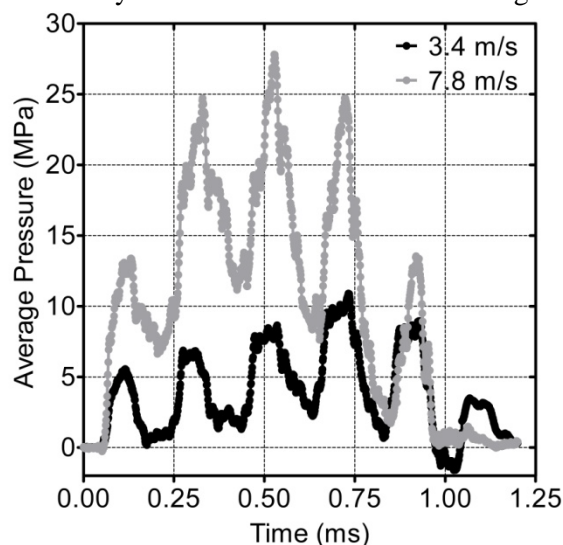
DMEM + 1% (v/v) FCS. Cell cultures at a concentration of  $10^6$  cells/ml were aliquoted as 800- $\mu$ l samples into 1.5-ml microfuge tubes. Samples (five per condition) were divided into five groups: control, sham (cells that are inserted using a syringe in the chamber and recovered without being subjected to pressure waves), Blast 1 and Blast 2 (cells that are inserted in the chamber and subjected to pressure pulses of different magnitudes) and freeze/thaw (samples that are subjected to two series of freeze and thawing procedures to obtain a total cell lysate). Three assays were performed to assess the effects of the pressure pulses on MSCs: cell counting with Trypan blue dye, cell viability using a one-step MTS assay (CellTiter96, Promega), and cell damage/lysis assessed on cell-free supernatants using a lactate dehydrogenase (LDH) release assay (ab65393, Abcam).



**Figure 1.** Experimental assembly for confined SHPB liquid experiments. Components of assembly are labelled and liquid samples are introduced into the confinement chamber through 1 mm bore holes.

### 2.3 Results

The average pressure achieved inside the chamber in a compression experiment can be calculated from the signal recorded from strain gauges mounted on the SHPB bars and the hoop strain of the chamber. Average pressure-time curves from compression experiments performed on liquid specimens varying the impact velocity of the striker bar are shown in figure 2.



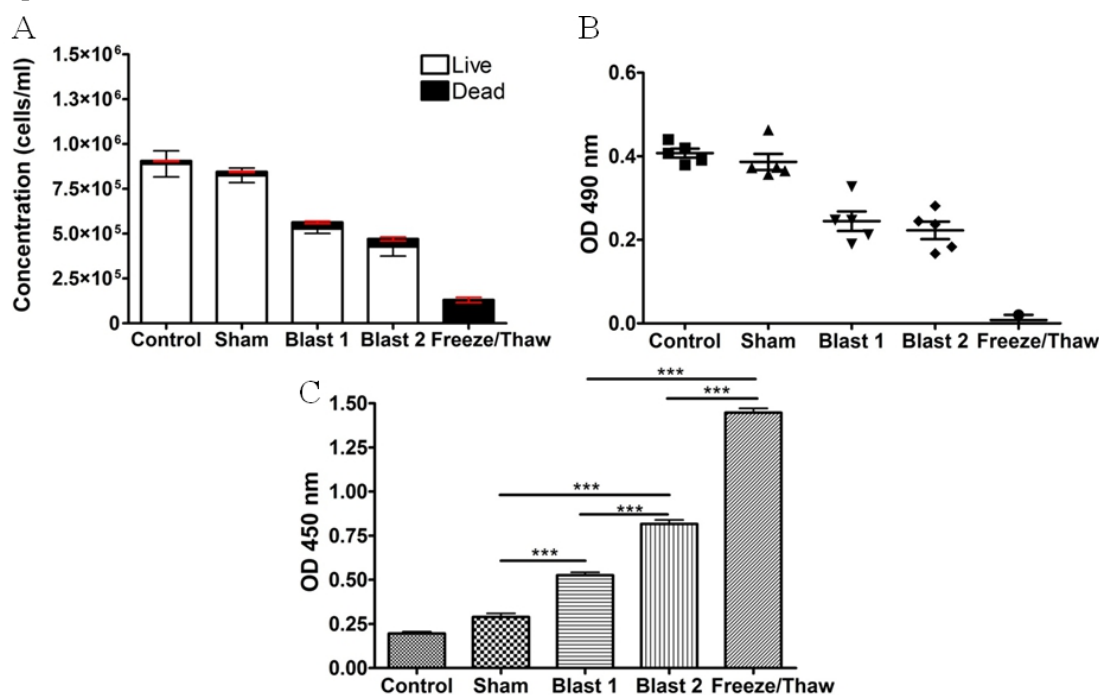
**Figure 2.** Pressure traces calculated from the strain gauges signal using iterative SHPB theory and hoop strain measurements for compression experiments of liquid samples in the confinement chamber. Average data ( $n=3$ ) for two striker bar's impact velocities are shown in black (Blast 1 - impact velocity = 3.4 m/s) and grey (Blast 2 - impact velocity = 7.8 m/s).

Increasing the impact velocity resulted in an increase of the values of peak pressure and pressure impulse, as shown in table 1.

**Table 1.** Average values of peak pressure and pressurization impulse (n=3) for SHPB compression of liquid specimens.

	Peak Pressure (MPa)	Impulse (MPa ms)
Blast 1	$9.5 \pm 0.5$	$4.6 \pm 0.3$
Blast 2	$27.0 \pm 1.0$	$12.0 \pm 0.8$

Survival of MSCs post compression was assessed using Trypan blue dye to count cells and by measuring the cell respiration levels using the MTS assay. Results suggest that cell survival decreases as a function of the intensity of the pressure pulse (figures 3(a) and 3(b)). Cell viability was investigated by measuring the release of LDH into the supernatant of the recovered samples. LDH levels in the samples increased as a function of the intensity of the compression waves applied. These data suggest that cells have lysed due to the subjected mechanical stimulus in the SHPB experiments (figure 3(c)). Although a correlation between the peak pressure (and the pressurization impulse) and the level of damage, measured as cell viability, was observed, other mechanical parameters (i.e. strain rate) and mechanical phenomena (i.e. cavitation) could have contributed to the lysis of cells in suspension, as previously reported in extracorporeal shock wave treatment and laser shock experiments [8, 9].



**Figure 3.** Comparisons of MSC survival at two different dynamic pressures, Blast 1 and Blast 2 (described in text). See text also for descriptions of controls (CTL), sham and freeze/thaw samples. A, Cell concentrations of MSCs determined using the Trypan blue. B, Cell respiration measured with a MTS colorimetric assay. C, LDH levels in the supernatants of samples recovered post compression. (\*\*\*) =  $p < 0.001$ , one-way analysis of variance (ANOVA) statistical test).

### 3. Compression of soft tissue: from low to high strain rates

To develop biofidelic models of blast injuries it is necessary to define the mechanical properties and structural changes of wounded tissues at high strains rates. Examples in literature of dynamic mechanical characterisation of biological tissues have been reported with high strain rates in the order

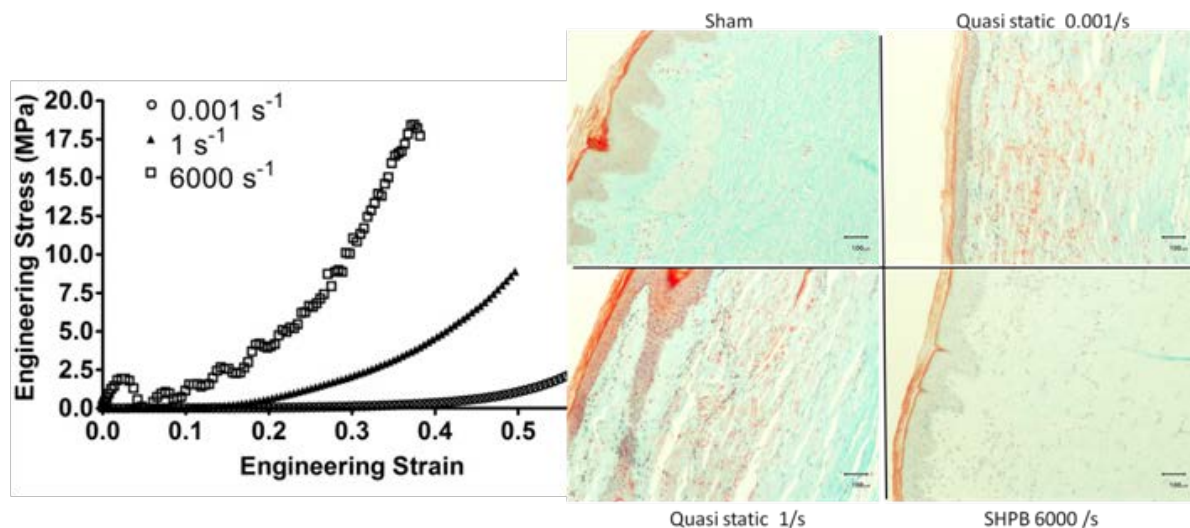
of  $10^3 \text{ s}^{-1}$  [11, 12]. In our research the tissues under study are those associated with musculoskeletal trauma to the extremities, and respiratory tissues associated with blast lung.

### 3.1 Compression of porcine skin samples at different strain rates

Skin samples were obtained from the rump and thigh of a weaned pig (6–8 weeks old), sourced from a Specific Pathogen Free (SPF) closed herd. The pig was sacrificed, euthanized by intravenous administration of sodium pentobarbitone ( $0.8 \text{ mg/kg i.v.}$ ). A rectangular sheet was harvested from each anatomical area of interest and the adipose layer was removed from each with a scalpel. Cylindrical specimens, about 8 mm in diameter, were obtained using a biopsy punch. These specimens were stored in phosphate buffered saline solution at  $4^\circ \text{C}$  until mechanical tests performed, up to 5 hours post mortem. Prior to testing, each sample was positioned between two microscope slides and the thickness was measured with a calliper. Compression experiments were performed with an Instron 5566 using  $0.001 \text{ s}^{-1}$  and  $1.0 \text{ s}^{-1}$  strain rates. High strain rates ( $6000\text{--}9000 \text{ s}^{-1}$ ) were applied to samples placed in a Split Hopkinson Pressure Bar. Magnesium bars were used to reduce the impedance mismatch between the output bar and the skin samples thereby maximising signal transmission, and semiconductor strain gauges were used to record the input and output signals.

### 3.2 Histological analysis of compressed samples

After compression tissue samples were recovered and fixed in formaldehyde for 24 hours. Samples were sectioned along the cylinder axis and cellular structures were analyzed using light microscopy with the following histological stains: Haematoxylin and Eosin to detect changes in general morphological features, Masson's trichrome to investigate the collagenous components of skin, and Miller's stain to identify elastin fibres within the samples.



**Figure 4.** Studies of compression of porcine skin harvested from the rump area under different loading regimes. Left, average stress-strain curves for skin specimens obtained from three different loading experiments. Right, Masson's trichrome staining of sham and compressed samples at different strain rates (Magnification 10X).

### 3.3 Results

The experimental results show that the mechanical response of skin in compression is strongly dependent on the strain rate of loading and on the anatomical location from which the samples were collected. Specimens collected from the rump showed a stiffer response compared to samples harvested from the thigh (figure 4).

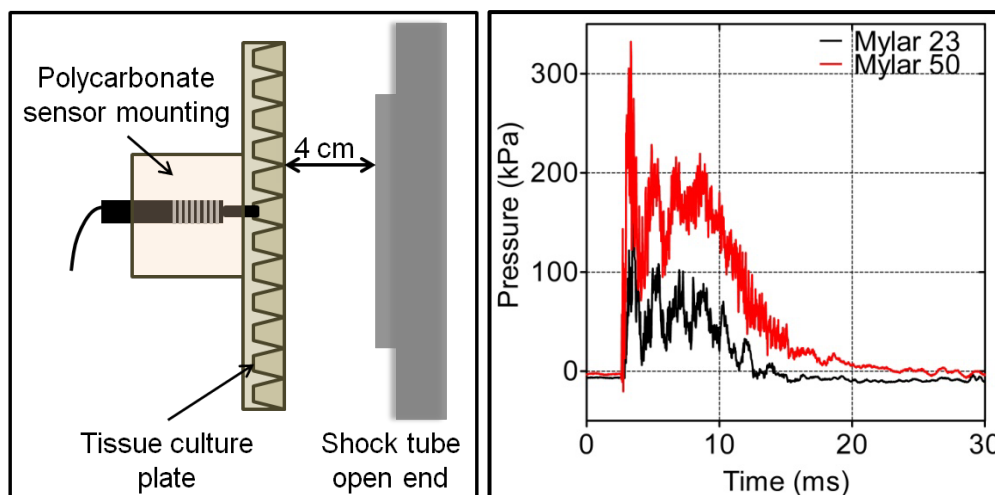


Staining with Masson's trichrome revealed structural changes in skin subjected to compression at low and medium strain rates using the Instron 5566 machine. Histological analysis of skin samples demonstrated infiltration of dye in the samples subjected to compression compared to the sham (figure 4). The level of dye infiltration decreases as the applied strain rate of loading increases. The increased presence of dye in slowly compressed samples indicates disruption of tissue organization, most likely due to damage of collagen structures.

#### 4. Replicating mild blast injuries using a shock tube

In future work, the intention is to analyze the effects of shock pulses on cells at the order of hundreds of kPa and milliseconds duration, which correspond to mild blast injuries [13]. To this end, a shock tube for *in vitro* overpressure experiments has been developed. The device consists of three stainless steel 1.22 m long tube sections. These tubes are connected by gaskets and flanges, within which pressure transducers and vents are embedded. The driver and driven sections are separated by a diaphragm. The driver section is pressurized from a standard gas cylinder of dry air to a maximum pressure of 18.2 bars. The wave evolution along the tube and at the open end is monitored using ultra high frequency piezoelectric pressure sensors (Dytran Instruments - 2300V1). For a full characterization of the system please see the accompanying paper, Nguyen *et al.* [14].

The *in vitro* set up consists of a tissue culture plate secured on a custom-made stainless steel support mounted at the end of the shock tube in the open configuration (figure 5 (left)). Each well of the tissue culture plate is filled with liquid medium and the entire plate is sealed with a non-permeable tape (Corning). Initial experiments have focused on the characterisation of the pressure developed within the liquid filled-wells. A custom made polycarbonate sensor mounting is attached with epoxy resin to the rear surface of the tissue culture plate, in which a hole is drilled to allow insertion of the pressure sensor (Dytran Instruments - 2300C4). As shown in figure 5 (right), varying the diaphragm thickness corresponds to waves of increasing peak pressure generated inside the wells of the tissue culture plate. Preliminary results from overpressure experiments on a MSCs monolayer suggest that the viability of cells exposed to the shock wave is reduced depending on the orientation of the plate with respect to the open end of the shock tube.



**Figure 5.** Cell-culture shock experiments. Left, schematic of the *in vitro* shock tube setup used for the measurement of the pressure waves generated inside the tissue culture plate. Right, pressure waves developed inside the central well of the tissue culture plate in correspondence of shock tube overpressures generated using Mylar diaphragms of 23  $\mu\text{m}$  (black) and 50  $\mu\text{m}$  (red) thicknesses respectively.

## 5. Conclusions

Several experimental platforms have been developed to study blast injury at the cellular and tissue level in CBIS. The SHPB and biocompatible chamber can subject cell cultures of clinically relevant samples to pressure waves of the order of few MPa and microseconds duration. Experiments on MSCs suggest that the level of damage in samples subjected to pressure pulses is correlated with the peak pressure generated in the confinement chamber. The SHPB was also used to study the mechanical properties of porcine skin tissue samples. The mechanical response of skin samples (indicative of hardening) in dynamic compression increases as a function of the strain rate of loading. Histological analyses suggest that damage to the collagen network decreases as the applied strain rate increases. Finally, in a preliminary extension of this work, a shock tube has been developed and characterised to study mild blast injuries using a cell culture model.

Further characterization of the platforms and their ability to reproduce blast injury effects is ongoing. High-speed photography and heterodyne velocimetry are providing improved visual and physical characterisation of the experimental conditions. Adapting and integrating a variety of approaches with experimental setups to study dynamic and static responses of biological samples allows us to sample a pressure-time space that can correspond to actual combat blasts. We are able to characterize the physical parameters of the applied pressure pulses (peak pressure, stimulus duration, impulse pattern) and characterize the damage in our samples with observations drawn from clinical description of human blast injuries. Data obtained from these types of studies are essential for developing realistic experimental and theoretical models of blast injury. Such models have the potential to provide novel insights about the mechanisms of damage involved in these types of injuries and yield viable experimental platforms for both fundamental and applied studies in this field.

## Acknowledgements

The Institute of Shock Physics acknowledges the support of the Atomic Weapon Establishment, Aldermaston, UK and Imperial College London. The Centre for Blast Injury Studies acknowledges The Royal British Legion for its support, as well as Imperial College London. The technical support of the Cavendish Laboratory, Cambridge, and the Veterinary School, Cambridge, along with technicians in Imperial College London was invaluable to this multi-faceted research.

## References

- [1] Ramasamy A, et al. 2011 *Phil. Trans. R. Soc. B* **366** 160-70
- [2] Ramasamy A, et al. 2011 *J. R. Soc. Interface* **8** 689-98
- [3] Smith J 2011 *Phil. Trans. R. Soc. B* **366** 291-4
- [4] Potter B K et al. 2010 *J. Bone Joint Surg. Am.* **92** 74-89
- [5] Davis T A, et al. 2011 *J. Bone Joint Surg. Am.* **93** 1122-31
- [6] Richmond D R and White C S 1962 *The Symposium on Effectiveness Analysis Techniques for Non-Nuclear Warheads Against Surface Targets* Dahlgren
- [7] Bass C R, Rafaels K A and Salzar R S 2008 *J. Trauma* **65** 604-15
- [8] Delius, M 1994 *Shock Waves* **4** 55-72
- [9] Kodama, T, Hamblin M R and Doukas and A G 2000 *Biophys. J.* **79** 1107-22
- [10] Bo C, et al. 2011 *Eur. Phys. J. Appl. Phys.* **55** 31201 1-5
- [11] Saraf H, et al. 2007 *J. Biomech* **40** 1960-67
- [12] Zhou B, Xu F, Chen C Q and Lu T J 2010 *Phil. Trans. R. Soc. A* **368** 679-690
- [13] Long J B, et al. 2009 *J. Neurotrauma* **26**(6) 827-40
- [14] Nguyen T-T N, Wilgroth J M and Proud W G 2013 *J. Phys.: Conference Series* submitted