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Development of a chamber to investigate high-intensity compression waves upon live cell cultures

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Abstract. Understanding how high-intensity compression waves damage human tissue is critical to developing improved therapeutic interventions for treating traumatic injuries arising from explosive devices. Assessment of the cellular and molecular basis of damage that is physiologically relevant and requires the study of biomaterials using appropriate techniques which subject samples to compression waves at magnitudes relevant to traumatic injury. Here, we present the design and initial calibration of a polycarbonate chamber to be used in a split Hopkinson pressure bar system to study live cell cultures.

1 Introduction

There is a critical need to develop improved therapeutic treatments for traumatic injuries arising from high intensity compression waves. Current conflicts in Afghanistan and Iraq have been characterized by the use of improvised explosive devices (IEDs) that result in a range of fatal and non-fatal injuries [1], including blast lung, damage to

the ear and bowel injuries, but in particular limb injuries.

The interaction of the blast wave with the human body represents an important mechanism in traumatic limb amputation [2,3] and there is clinical evidence that a primary blast wave can have an adverse effect on coagulation [4] and tissue healing. Late complications of injuries caused by explosions include a high incidence of infection, nerve injury and formation of abnormal bone (heterotopic ossification) [5]. Preliminary studies on the aetiology of this

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latter pathology, consequent to wound traumatic injuries, reveal that severe trauma leads to the release of osteogenic factors (implicated in the formation of bone), but the cellular and molecular processes related to the growth of the ectopic bone remain unclear [6].

Understanding how blast waves damage human tissue is critical to preventing and treating many of the disabling consequences of explosions. However, little is known about the effect of blast waves on cellular and molecular damage in biological tissue. The difficulties in handling live biological samples and the lack of appropriate equipment have limited the research on the effects of extreme mechanical impacts on live human tissues.

Several studies have been conducted to understand the mechanisms whereby mechanical stimuli applied on cells give rise to biological responses and pathology. Different systems have been developed to subject *in vitro* cell cultures to shear or/and compression stresses [7–9]. However, all these devices have the limit of applying very low shear and compression stresses to the cells. A common outcome of these studies is that the severity of cell damage is correlated to the magnitude of stress and the rate of application, but the mechanisms by which deformation generates cell damage are still unknown.

The objective of this work is to develop an experimental system for subjecting live cells and tissues to high intensity compression waves. A confinement chamber has been designed that allows the compression of *in vitro*-grown cell cultures to pressures relevant to blast injuries using a split Hopkinson pressure bar (SHPB) system. Ini-

tial assessments show that the chamber is resilient to the stresses applied and is appropriately designed to enable containment of liquids and transmission of high compression waves suitable for use with cell cultures.

2 Materials and experimental procedures

2.1 Experimental set up and data acquisition

The classical SHPB setup has been modified with the insertion of a confinement chamber to test biological samples in a liquid environment. Fig. 1 shows the arrangement used for the experiments in the Cavendish Laboratory. The system is composed by input, output and trap momentum bars made of Inconel steel, of 12.7 mm diameter and 50 cm length. Each bar is held in place by adjustable supports which allow the alignment of the system. The striker bar, 12.7 mm in diameter and 180 mm in length made of Inconel steel is fired out of a helium gas gun 1.22 m long. A double valve pressure system allows the control of the firing pressure, hence of the impact velocity. The strain of the bars is recorded using semi-conductor gauges (Kulite type AFP 500-90) with a sampling rate of 10 MHz. The elastic displacements measured in the bars are used to determine the dynamic response of the specimen.

2.2 Data analysis

In a classical (unconfined) SHPB arrangement the signals from the gages mounted on the input and output bars are used to determine the stress-strain behaviour of the

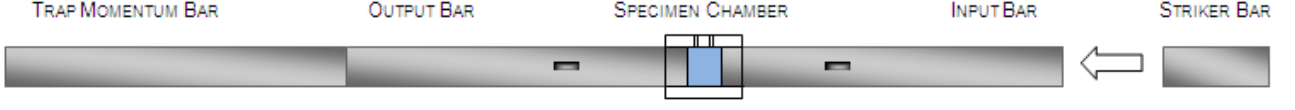


Fig. 1. Modified Split Hopkinson Pressure Bar set-up. The chamber, shown in blue, is mounted for the insertion of liquid samples.

tested sample. According to Gray III [10] specific conditions need to be satisfied to validate a SHPB test: the stress waves must propagate in the bars uniaxially, the surfaces of the bars in contact with the specimen must remain flat and parallel at all time and the specimen has to reach dynamic stress equilibrium in a reasonably short time interval (compared to the entire length of the experiment) [11]. If these hypotheses are verified, the strain rate, strain and stress histories in the specimen can be calculated from the input and output bar gages' signals as:

$$\dot{\varepsilon}_s(t) = \frac{C_0}{L_s} [\varepsilon_i(t) - \varepsilon_r(t) - \varepsilon_t(t)] \quad (1)$$

$$\varepsilon_s(t) = \frac{C_0}{L_s} \int_0^t [\varepsilon_i(t) - \varepsilon_r(t) - \varepsilon_t(t)] dt \quad (2)$$

$$\sigma_s(t) = \frac{A_0}{2A_s} E_0 [\varepsilon_i(t) + \varepsilon_r(t) + \varepsilon_t(t)] \quad (3)$$

where $\varepsilon_i(t)$, $\varepsilon_r(t)$ and $\varepsilon_t(t)$ are the measured strains on the bars correspondent to incident, reflected and transmitted pulses respectively, C_0 is the sound speed in the bars, A_0 the bar cross section area and A_s and L_s are the instantaneous area and length of the sample [12]. When the sample reaches dynamic stress equilibrium and if it deforms uniformly it follows that:

$$\varepsilon_i(t) + \varepsilon_r(t) = \varepsilon_t(t) \quad (4)$$

and thus the set of Eq. (1)-(3) can be simplified as:

$$\dot{\varepsilon}_s(t) = -\frac{C_0}{L_s} \varepsilon_r(t) \quad (5)$$

$$\varepsilon_s(t) = -\frac{C_0}{L_s} \int_0^t \varepsilon_r(t) dt \quad (6)$$

$$\sigma_s(t) = \frac{A_0}{2A_s} E_0 \varepsilon_t(t) \quad (7)$$

In a fully confined SHPB test the sample is not allowed to deform in the radial direction during compression. This results in the development of a uniaxial strain state along the central axis rather than a uniaxial stress state [13].

The stress condition are two dimensional: an axial stress σ_a , in the main direction of motion, and a transverse stress σ_t , in the radial direction. The axial stress can be easily calculated from Eq. 7, considering that the area ratio A_0/A_s is equal to one, as the specimen has the same diameter as the bars and is not allowed to expand radially. The transverse stress cannot be directly calculated from the signal recorded by the strain gauges on the input and output bars. Bragov et al. have analyzed the biaxial stress state of the specimen in a system where a jacketed sample can radially deform by a very small ($\ll 1\%$) amount, so allowing hoop strain and by extension radial stress to be measured. In those studies [15] a relation between the

axial and transverse components has been proposed

$$\sigma_t = \frac{\nu}{1 - \nu} \sigma_a \quad (8)$$

where ν is the Poisson ratio of the specimen. Moreover, according to Saraf. et al [13], the maximum shear stress in the sample, defined as:

$$\sigma_{sh} = \frac{(\sigma_t - \sigma_a)}{2} \quad (9)$$

is a measure of the effect of viscous dissipation in the specimen.

Biological samples intended for use in this chamber are live cells dispersed in cell culture that can be described as a Newtonian fluid with low viscous content. We assume that, during the compression process, equilibrium in the thin liquid layer is reached in a short time compared to the entire duration of the experiment and thus shear stress quickly drops to zero. When this condition is fulfilled $\sigma_a = \sigma_t$ and a hydrostatic pressure state is developed in the sample. Considering that the biological sample is mainly composed by water which is an incompressible media, it is possible to calculate a hydrostatic pressure for experiments at different impact velocities, this corresponding to the peak axial stress as calculated from one-wave theory (Eq. 7).

3 Results and discussion

3.1 Chamber design

The first attempt to characterize fluids subjected to dynamic loading using SHPB technique was reported by

Kenner [16]. This study focused upon how waves are propagated through a fluid column held in a rigid tube with a view that this methodology has the potential to be used to investigate the mechanics of head injuries. Kenner defined the requirements necessary to consider the fluid containing tube rigid and the conditions to apply one-dimensional analysis to determine the nature of the pulses transmitted to the fluid. Other attempts to investigate the dynamic behaviour of fluids have been conducted by Åhrström et al. [17] who used a high-alloy confinement system to test oil specimens. This system, differing from the previous one mainly because the liquid column was reduced to few centimetres in length, was developed to study the behaviour of elastohydrodynamic lubricants. In both examples rigid confinement was applied to minimize the shear within the liquid specimens and identify the pressure state reached inside the tested material. A different approach has been proposed to study the dynamic behaviour of fluids at rate of shear up to $10^4/s$. Ogawa [18] developed a through bar method in which the input bar extends through the output bar, a coaxial cylinder, with the sample sandwiched between the two. He formulated the expressions for the shear stresses on the input and the output side of the fluid, but no validation of the shear modulus obtained using this method is presented by the author. Subsequently Lim et al. [19] developed a passive confinement method for SHPB technique to study the shear response of viscous fluids. In their system the fluid is held in place between the classical input and output SHPBs using a soft, flexible band which allows radial expansion of the specimen during the

tests. Their work particularly focused upon the definition of characteristic times for axial wave propagation, flow and pressure development.

Different examples of confined SHPB tests have been found in literature mainly related to the studies of soil materials. Song et al. [20] compared the effects on the stress-state levels reached in the specimen due to different materials used as confinement tubes and the dynamic response of the sand as a function of the initial density. Stress components in a soil specimen and in the confined jackets have been defined by Bragov et al. [15]. In those experiments different pressure bars and jackets materials were used to perform tests at different load magnitudes. In addition, strain gauges were mounted on the jackets to measure the radial stress in the sample. This method has proved to be useful for the determination of parameters necessary for the development of a model of the sample.

There appear to be no reports in the literature of using modified SHPB systems to study the effects of compression waves upon live biological sample. Several different animal or human tissues among which bone, skin, muscle and brain matter have been investigated using SHPB technique, but all these experiments focused mainly on the determination of mechanical properties of the samples [21–24]. Saraf et al. applied confinement cells to the traditional SHPB system to determine the bulk response and the shear stress of soft human tissues under dynamic loading [13]. However, in these studies the samples were not maintained in a live state so no assessment could be

made of compression wave-induced biological changes.



Fig. 2. Confinement chamber: the main body is a hollow cylinder with a 50.00 mm outer diameter and 12.7 mm inner diameter, 88.00 mm in length. Two mirror holes, 15.00 mm in diameter and 35.00 mm in length, have been drilled from the lateral surfaces of the cylinder. These holes allow the insertion of an O-ring on each side which is kept in place by a metal ring compressed by the lateral cups that are screwed to the chamber. Each piece can be easily cleaned and mounted back on the system.

The approach presented in this study focuses on the possibility to test and analyse live biological samples, i.e. cell cultures, in order to assess the cellular and molecular basis of short-time overpressure damage. A chamber has been designed to contain biological samples in liquid environment during the SHPB tests and to allow the recovery of uncontaminated samples after impact. As shown in Fig. 2, the chamber is composed by three parts: a main body, where the sample is placed when the chamber is mounted on the bars, and two screw caps, which fit in the main body to keep it in place and secure the sealing using O-rings. This configuration assures the sealing of the liquid between the input and output bar, while al-

allowing a smooth movement of the bars without applying pre-stresses to the system. The inside of the chamber is connected to the outer circular surface using two 1 mm diameter counter bore holes used to insert the liquid via syringes. To prevent spillages during the experiments the small insertion holes are sealed using rubber discs and metal screws.

Most of the chamber's components are made of polycarbonate. This material has been chosen because it offers a combination of strength, rigidity and toughness, being able to maintain the liquid confined during impact. In addition, it is transparent which allows the inspection of undesired air bubbles inside the chamber prior impact and the use of high speed photography. Good heat resistance, low water absorption and biocompatibility are also among the important properties of this material. Finally, polycarbonate can be sterilized using all major methods and can be easily cleaned with disinfectants.

3.2 Preliminary tests: chamber resiliency and confinement

Initial tests have been conducted to assess the resiliency of the chamber to different firing pressures. Polycarbonate discs, lubricated with petroleum jelly as suggested by Trautman et al. [14], were used as testing samples at strain rates in the range of 3000/s to 4500/s. The chamber showed no damage during the tests and the effects introduced by the chamber on the signal measured by the strain gauges mainly translates in the attenuation of the Pochhammer-Chree oscillations typical of a SHPB

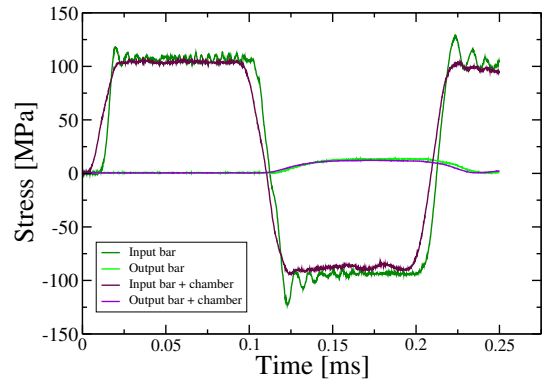


Fig. 3. Polycarbonate discs 2 mm in length and 4 mm in diameter have been tested in the classical (free standing) and in the chamber SHPB system configuration. A comparison of the stress waves on the bars in the two different configurations shows that no remarkable differences are introduced by the presence of the chamber on the strain gauges signals.

test and minor prolongations of the incident and reflected waves rising time, Fig.3. No significant influences could be observed between the tests of polycarbonate specimens with or without the specimen chamber.

This chamber has been designed with the key intention that it will be used for containing live cell cultures that will be subjected to compression waves in a SHPB system. Preliminary tests have been conducted using the chamber filled with 1.5 ml of water. In order to obtain a distinct signal from the strain gauge on the output bar, it is necessary that no air bubbles are present in the chamber. No leakage has been observed demonstrating that the chamber has an effective seal to contain liquid samples under the experimental conditions reported here. In Fig.4 the true stress vs time curves are presented for the initial experiments calculated using 1-wave, 2-wave and 3-wave analyses [11] respectively are presented. There is an ap-

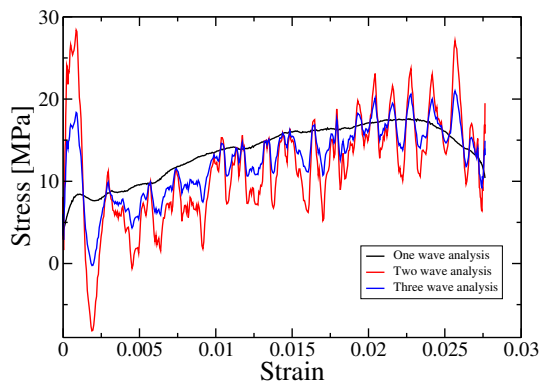


Fig. 4. Stress vs strain profile of a water sample (1.5ml) subjected to a compression wave.

preciable ringing on the 2 and 3-wave analysis while the 1-wave is quite smooth. It will be important to ensure that the sample thickness and loading conditions are varied according to sample wave speed. In this case the maximum strain achieved using 1.5 ml of water, corresponding to a large sample thickness of 12 mm, is approximately 0.03 which suggests that the assumptions of incompressibility of water and small shear stresses are appropriate in this experimental design.

4 Conclusions and future research

Improving the prospects for treatment of injuries arising from high pressure compression waves is a issue of increasing importance given the nature of the casualties arising in military conflicts where explosive devices cause a range of blast injuries, particularly to lower limbs [1]. However, there is a clear lack of knowledge about the molecular basis of the damage induced in human tissues that have sustained traumatic blast-related injuries. The work presented here aims to address this through the development

of an experimental setup in the form of a highly resilient and biocompatible chamber. This chamber enable the application of high pressure compression waves to model cell cultures that can then be subsequently analyzed for biomarkers of damage and repair.

In the work presented here we describe the construction and initial assessment of a polycarbonate sample chamber that can be used in a SHPB system. Preliminary tests on the chamber show that it is capable of sustaining up to 18 MPa of pressure generated in water and that it is able to seal the liquid specimen while still allowing the bars to move freely. Key features of the chamber design include a 1.5 ml sample volume that can accommodate, without leakage, sufficient amounts of material for molecular analyses following compression wave stimuli. The construction of this chamber now provides an appropriate platform for pursuing molecular analyses of live cell cultures, such as bone and nerve, that have the potential to reveal details about cellular changes arising in human tissue affected by blast injury.

Acknowledgments

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