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ORIGINAL ARTICLE

# Investigations of primary blast-induced traumatic brain injury

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**Abstract** The development of an advanced blast simulator (ABS) has enabled the reproducible generation of single-pulse shock waves that simulate free-field blast with high fidelity. Studies with rodents in the ABS demonstrated the necessity of head restraint during head-only exposures. When the head was not restrained, violent global head motion was induced by pressures that would not produce similar movement of a target the size and mass of a human head. This scaling artefact produced changes in brain function that were reminiscent of traumatic brain injury (TBI) due to impact-acceleration effects. Restraint of the rodent head eliminated these, but still produced subtle changes in brain biochemistry, showing that blast-induced pressure waves do cause brain deficits. Further experiments were carried out with rat brain cell aggregate cultures that enabled the conduct of studies without the gross movement encountered when using rodents. The suspension nature of this model was also exploited to minimize the boundary effects that complicate the interpretation of primary blast studies using surface cultures. Using this system, brain tissue was found not only to be sensitive to pressure changes, but also able to discriminate between the highly defined single-pulse shock waves produced by underwater blast and the complex pressure history exposures experienced by aggregates encased within a sphere and subjected to simulated air blast. The nature of blast-induced primary TBI requires a multidisciplinary research approach that addresses the fidelity of the blast insult, its accurate measurement and characterization, as well as the limitations of the biological models used.

**Keywords** Advanced blast simulator · Primary blast · Tissue culture · Traumatic brain injury (TBI) · Underwater blast

## 1 Introduction

The significant use of improvised explosive devices (IEDs) in recent conflicts has prompted the continued and increased use of highly effective body armour. Coupled with improvements in medical evacuation and treatments, this has resulted in improved survival from blast exposure, but an increased incidence of traumatic brain injury (TBI) [1,2]. A high proportion of these casualties suffer from mild TBI (mTBI), and of these, many present with symptoms only after a delay [3]. These factors have prompted renewed research interest, with a significant emphasis on studies investigating whether primary blast causes brain injury. The effects of air blast are complex, with secondary (penetrating fragments) and tertiary (impact/acceleration) blast potentially masking any deleterious effects of primary blast on the brain, while the low-level, far-field effects of the latter are likely to be subtle. This has made the diagnosis of primary blast-induced traumatic brain injury (PbTBI) challenging, with only a very few clinical cases identified in the modern era [4–6]. Exposure scenarios are difficult to recreate, and the recollection of events weeks or even months later by a veteran poses its own challenges [7].

The potential of primary blast to cause significant injury to gas-containing organs such as the ear, gastrointestinal tract, and the lungs has long been accepted. There exists a wealth of

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documentation from the Second World War describing primary blast effects on these organs in shipwrecked sailors that were in proximity to underwater explosions [8–11], as well as from submerged animal studies [12,13]. Indeed, guidelines exist today as to safe stand-off distances of divers/swimmers from underwater blast [14]. Likewise, air blast from high explosives was also recognized as capable of causing damage to gas-containing organs [8,15–17], with more recent examples of these types of injuries being identified in the casualties resulting from terrorist bomb attacks [18–21]. Nevertheless, despite these well-documented findings, there has been a long-standing debate on whether primary blast alone causes injury to solid organs such as the brain.

During World War I, large numbers of British soldiers who had been in trenches or enclosed spaces close to explosive detonations became casualties, but with no external signs of head injury. These individuals presented with symptoms reminiscent of mild traumatic brain injury, including dizziness, headache, amnesia, tinnitus, and tremor [22]. With the publication of a paper in 1915 by C.J. Myers describing this mysterious disorder [23], the term "shell shock" came into use, suggesting an underlying psychological basis. In competition with this assertion, investigations by the prominent neuropathologist Frederick Mott ascribed organic origins to shell shock, and in lectures to the Royal Society described the commotio cerebri caused by the "aerial compression" produced by explosions [24]. Despite documenting this in two human fatalities in 1917 [25], Mott eventually revised his theories to include psychological effects as also being contributory to shell shock [26]. By the end of the war, its origins were still not resolved, but both sides appeared to cautiously agree that both psychological and physical effects were contributory. In World War II, medical emphasis appeared to focus on the effects of primary blast on the lung and gastrointestinal tract (see above), rather than on the nervous system [27]. However, there were also reports of fatalities similar to those of World War I, where damage to the brain occurred in individuals that appeared otherwise unharmed [28]. These findings appeared to agree with animal studies where frank neurological damage occurred in animals due to blast exposure, but only at very high pressures that were supralethal due to lung injury [5,17,28]. Once again, however, there was discussion and debate as to the relative contributions of organic versus psychiatric causes of brain dysfunction, particularly in cases of mTBI [22,29].

In the modern era, this same debate is once again ongoing. However, there are differences. Political pressure due to the multitude of psychologically injured veterans of the Vietnam War resulted in the recognition of post-traumatic stress disorder (PTSD) by the American Psychiatric Association in 1980 [30], and it is now well accepted that combat stress can produce chronic and debilitating symptoms. In addition, and in contrast to the previous world wars, veterans are now subject to much more comprehensive medical assessments during service, as well as at discharge and afterwards. This has resulted in the identification of large numbers of mTBI casualties in the Afghanistan and Iraq conflicts. However, both the symptoms of and diagnostic criteria for mTBI in these cases overlap significantly with those of PTSD [3,7,31,32], and considerable doubt remains as to the frequency and importance of primary blast in these individuals [33,34].

The difficulties of characterizing the effects of primary blast-induced traumatic brain injury (PbTBI) also extend to experimental studies. In contrast to air-containing organs, where large differences in density may cause spalling, as well as tissue tearing, tension, and compression in response to primary blast [35], with resultant frank and obvious tissue injury, gross damage from low-level primary blast would not be expected in solid organs such as the brain. As a consequence, the subtle and poorly understood effects of primary blast make biological targets in the central nervous system difficult to identify. In addition, because open-field blast facilities are accessible to only a small subset of researchers, most laboratories have resorted to simulating blast with a wide variety of platforms and approaches. Among these are shock or blast tubes studies that use compressed gas or low explosives. However, these latter types of studies present their own unique set of difficulties and do not intrinsically simulate free-field blast [36,37]. The use of small rodents in either free-field or simulated blast studies is also problematic [37–39]. There exist anatomical differences between the brains of small rodents, such as rats or mice and humans, while the complex and nonlinear scaling factors to compare the effects of rodent blast exposures to that of humans are as yet not understood.

An alternative approach to study primary blast is to simplify the biological aspects of the problem by using isolated cell or tissue preparations that are cultured under strict conditions. By using such model systems, many of the homoeostatic mechanisms aimed at reducing the effects of stress or insult present in the whole animal are eliminated. A number of different culture types derived from multiple organs and species have been utilized to study the effects of primary blast [40–58]. Although there are limitations to this approach, notably due to the boundary effects imposed by culture vessel surfaces and walls, the use of cell and/or tissue culture models also enables the investigator to study the direct effects of shock waves on the brain/skull without the need to consider the effects of scaling that currently complicate the interpretation of rodent PbTBI studies.

This paper describes a number of approaches and platforms that have been utilized by the authors to investigate PbTBI and the caveats that must be applied to them and overviews previously published results obtained during their use. The challenges encountered during this work high-

light the necessity of using multidisciplinary teams to tackle this research area. Significant physical science expertise is required to ensure that the blast challenges used are appropriate, measurable, and interpreted correctly, while health science expertise is essential to articulate the limitations of the biological models used and interpret the resultant changes incurred by primary blast.

## 2 Methods

### 2.1 Advanced blast simulator

An advanced blast simulator (ABS) was used in these studies and is depicted in Fig. 1. Static pressures were measured using PCB Piezotronics model # 113A28 gauges (Depew, NY) placed at 2780, 3280, 3780, 4280, and 4780 mm from the diaphragm (cellulose acetate, ULINE, Brampton, Ontario, Canada). Total pressures experienced by the test samples were measured using a custom-developed Pitot probe located at the test location 4280 mm from the diaphragm. A complete description of this probe is given in Ref. [59]. Briefly, it is 120 mm in length and 3 mm wide on its support strut fabricated via 3D printing. It is populated by Millar SPR-407 gauges (ADInstruments Inc, Colorado Springs, CO), which serve as a forward stagnation sensor, a mid-static sensor, or a dual-mode aft sensor that can be configured to measure either redundant static pressure or negative-phase stagnation conditions. Dynamic pressures were obtained by calculating the difference between the static and total pressures recorded at this location. Pressure data were recorded using a custom Labview interface and recorded on a GaGe Octopus 8389 CompuScope PCIe digitizer board at a sampling rate of 500,000 samples/s.


**Fig. 1** Advanced blast simulator (ABS). The ABS consists of a divergent driver (foreground, **a**) separated from a transition section by a frangible diaphragm. The transition section leads to the ambient-pressure test section. The ABS is 5.79 m in length with a circular test section diameter of 30.5 cm, **b** the test sample port where the clear cylindrical animal restraint device is placed, as well as the end wave eliminator (EWE) that prevents rarefaction waves from re-entering the test section. Also depicted is the positioning of the high-speed cameras that view test samples through two polycarbonate window ports

The overpressures used in these studies (15–30 psi) are relevant to low-level, far-field blast, with the corresponding dynamic pressures also being consistent with these conditions. The highest (30 psi) pressure (~7.5 ms duration) corresponds to what would be expected from a surface burst of 28.2 kg of C4 explosive 7.6 m from the target, with greater than 99% survivability as predicted from the Bowen curves [38].

### 2.2 Underwater explosive (UNDEX) pond

The UNDEX pond is purpose-designed for the study of underwater explosion effects and is located on the DRDC Suffield Research Centre Experimental Proving Ground. It has a diameter of 50 m and a maximum depth of 7 m at the centre. It is shaped as an inverted, truncated cone, and holds over 7 million litres of fresh water. The explosive charges used consisted of C4 explosives (~91% RDX) located at a depth of 1 m and were located at varying stand-off distances from the gauges and target dialysis tubing-encased brain cell aggregates, which were submerged to a depth of ~2 m. Underwater blast wave pressures were measured using PCB Piezotronics model #138A-series sensors (Depew, NY), using a sampling rate of 10 million samples/s and a signal conditioning bandwidth of 1 MHz. These gauges were deployed in a free-field configuration by suspending them at various depths from the surface. A detailed description of the explosive configurations and experimental exposure set-up used in this work has been published previously [53].

### 2.3 Animal work

In conducting this research, the authors adhered to the "Guide to the Care and Use of Experimental Animals" and "The Ethics of Animal Experimentation" published by the Canadian Council on Animal Care. For studies examining the head-only exposure effects of simulated primary

blast, adult male Sprague–Dawley rats (∼280–330 g) were acquired from Charles River Laboratories (St. Constant, Quebec, Canada) and acclimated for at least one week prior to exposure.

The methodology used in these studies for head-only exposure of rats to simulated blast has been described previously [38]. On the day of use, animals were anaesthetized with 3% isoflurane in oxygen and placed into a restraint (Fig. 1b) consisting of a clear plastic tube (3 in. internal diameter), with the neck held in a plastic collar and the head protruding. The head was secured in two different configurations. In the first (head restrained), the head was placed against mesh netting aligned vertically between two pins placed in line with the side of the head and restrained with additional netting. In the second configuration (no head restraint), only the vertical netting was utilized, with the chin of the anaesthetized animal supported using a thin strip of material placed underneath [38]. The restraint containing the animal was set into the wall of the advanced blast simulator (ABS) 4280 mm downstream from the diaphragm, so that the head protruded into the test section. Test groups consisted of sham control, and head-only, side-on exposures of single-pulse shock wave static overpressures of 15, 20, 25, and 30 psi of ∼6- to 7.5-ms positive duration. At one day post-exposure, the animals were killed and the brains isolated. Brain lysates were prepared and stored at −80 °C until analysis.

### 2.4 Brain cell aggregate culture

Detailed descriptions of the methodology used to generate rat cortical cell aggregate cultures in these studies have been described previously [53,54] and are adaptations of the methods published by Honneger [60,61], as well as Sa Santos et al. [62]. The embryos from timed pregnant Sprague–Dawley rats (Charles River Laboratories) were isolated at 17 days of gestation and the brains removed. Cells from the cerebral hemispheres were isolated and placed into 125-ml spinner flasks in culture medium supplemented to 15% FCS. The spinner flasks were placed onto stirrers in 125 ml of culture medium. Cultures were closely followed, and the rotational speeds adjusted to ensure a gradual increase in aggregate size to 350–500 μm diameter. Aggregate cultures were used for experimentation at 27–28 days in culture.

### 2.5 Aggregate exposure to underwater or simulated air blast

Prior to either UNDEX or simulated air blast exposure, the aggregates were isolated from their spinner flasks, combined, and redistributed into a predetermined number of test samples. For UNDEX exposures, the test samples were transferred into sterile dialysis tubing (Fig. 2a) and the ends tied off with thread to form a cylinder ∼10 cm long. This was transferred to the UNDEX pond and mounted in an upside-down fashion to nylon fishing line using clips. Several seconds prior to charge detonation, a solenoid was activated that released the bottom of the cylinder, allowing it to return to an upright position prior to the explosive detonation at time zero. This caused the aggregates to be in suspension at the moment of blast wave arrival. The aggregates were exposed to three blast intensities designated as low (∼43 psi), medium (∼391 psi), and high (∼2030 psi) with durations of ∼100–300 μs. After blast exposure, the aggregates were returned to routine culture conditions until harvested for analysis at 3, 7, 14, or 28 days post-blast exposure.

For simulated air blast exposures, the aggregates suspended in culture medium were placed into sterile 50-mm-diameter polypropylene spherical shells (∼56 ml volume; Fig. 2b) fitted with threaded collars. The shells were completely filled so as to avoid bubbles and sealed using bolts. The aggregate-filled spheres were immediately taken to the ABS facility and fitted into a mounting apparatus consisting of a centrally located sting that is supported along the centreline of the shock tube longitudinal axis by an aluminium cross/box structure. The assembly was secured into position by the friction of the rails against the ABS inner walls so that the leading edge of the sphere was in line with the pressure gauge located 4280 mm from the diaphragm (Fig. 2b). Immediately prior to driver pressurization, the sphere was turned 180° by rotating the threaded collar; this ensured that the aggregates were in suspension during shock wave exposure of the sphere. The port was rapidly sealed and the driver pressurized to predetermined pressures so that the sphere was exposed to nominal 15, 20, 25, or 30 psi overpressures. The sphere was then quickly removed from the ABS and returned to the laboratory, where the aggregates were returned to routine culture conditions until harvested for analysis, as above. Aggregate cultures were assessed for lactate dehydrogenase release, caspase-3 activity, enzyme activities (glutamine synthetase, acetylcholinesterase, choline acetyltransferase), brain structural proteins (glial fibrillary acidic protein (GFAP), neurofilament heavy chain (NFH), myelin basic protein), and cellular signalling proteins (Akt and vascular endothelial growth factor (VEGF)). In all cases, the effect of blast on test endpoints was compared to that of sham control cultures treated identically to exposed samples, but without actual exposure to blast or simulated blast.

### 2.6 Sphere internal pressure measurements

Pressure changes within the sphere were measured using Millar SPR-524 gauges (ADInstruments Inc.) immersed in the interior of a water-filled sphere and oriented such that the incident air shock wave would transmit through the sphere wall and engulf the transducer. The sphere was mounted on

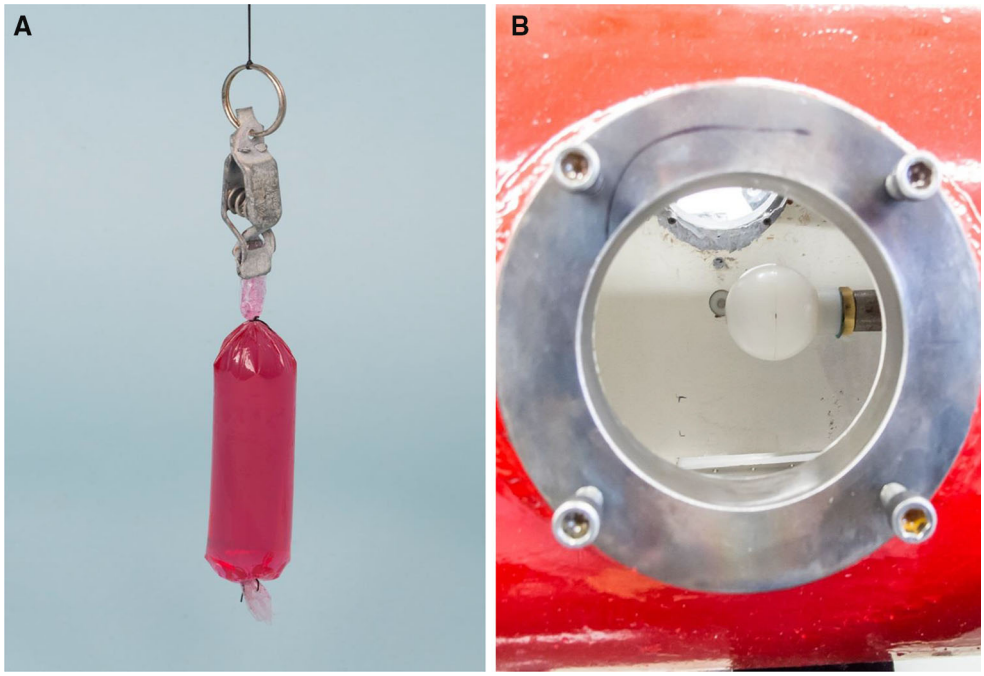


**Fig. 2** Brain cell aggregate containment during underwater explosion (UNDEX) or simulated air blast exposure. To take advantage of the suspension nature of rat brain cell aggregate culture, the aggregates were placed in dialysis tubing during UNDEX exposure (**a**) or in polypropylene spheres during simulated air blast (**b**). The dialysis tubing was transparent to the blast wave, and the cylinder was inverted just prior to charge detonation so that the aggregates are in suspension during exposure. Likewise, immediately prior to the simulated blast, the sphere located in the test section opposite the gauge at 4280 mm from the diaphragm was rotated 180°, ensuring that the aggregates are in suspension at the time of shock wave arrival

the end of the protruding sting, as described above, and the gauge placed centrally at positions sequentially located 9, 25 (centre), and 48 mm from the leading edge of the sphere facing the driver. Transducer cables extended through the bolt securing the sphere to the sting, out the back of the aluminium cross/box assembly and emerged out one of the shock tube wall pressure ports. The test arrangement using a similar Millar gauge (SPR-671, ADInstruments Inc.) is described in more detail by Lee and Rude [63]. The bandwidth of the signal conditioning amplifier (Vishay 2310B) was set to approximately 250 kHz, and the data recording system sample rate was set to 5 M samples/s to ensure that the rapid pressure changes of the underwater shocks were captured.

## 3 Results and discussion

A large number of devices and model systems have been utilized to expose animals or tissue culture samples to primary blast. These include open-field blast, bazookas and howitzers, long guns of different calibres, modified commercial nail and paint ball guns, custom-developed equipment based on compressed air or laser-ignited microexplosives, as well as traditional blast tubes and shock tubes [38,64–85]. These various approaches produce pressure traces with a broad range of shapes, pressures, durations, and static/dynamic pressure ratios. The reporting of these variables is often incomplete, with identification only of peak overpressure without further details of the pressure history, dynamic pressure components, or anomalies/artefacts such as rarefaction waves. Furthermore, detail on how the measurements were made is also often absent. As a result, while all of these systems generate pressure changes that may be capable of inflicting damage or change in biological target material, it is not clear that they simulate free-field blast. In addition, interpretation of the data obtained when exposing either animals or tissue cultures to these pressure insults must be made within the physical constraints that the exposure platform, as well as the biological model, imposes.

### 3.1 Development of the advanced blast simulator (ABS)

In order to assess the effects of low-level primary blast on the brain, it is necessary to develop the means with which to reproducibly simulate free-field blast waves. The traditional laboratory shock tube is a straight pipe consisting of a driver section charged with high-pressure gas, separated from the test section by a frangible membrane. Rupture of the membrane results in the sudden release of high-pressure

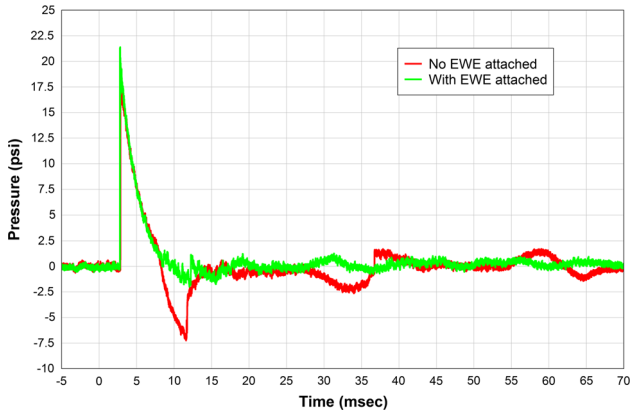


**Fig. 3** Pressure histories of 20 psi trials with and without the end wave eliminator (EWE). The divergent driver and transition sections shape the waveform so that it travels down the test section as a shock wave featuring an immediate rise in overpressure. Without the EWE (*red trace*), rarefaction waves re-enter the test section, creating an exaggerated underpressure and subsequent artefacts with time. Inclusion of the EWE (*green trace*) eliminates this artefact, resulting in a waveform that includes a sharply defined static overpressure rise time, underpressure, and secondary shock wave

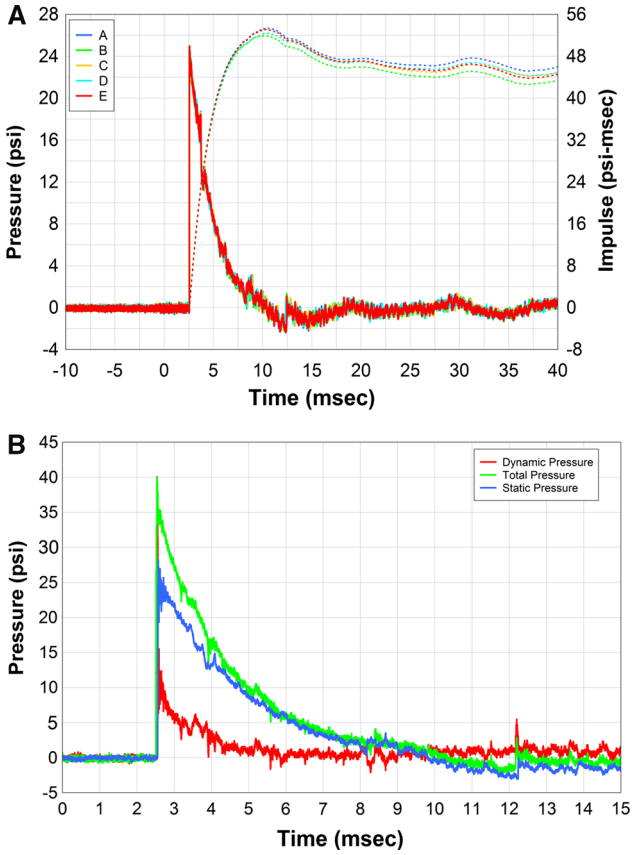


**Fig. 4** Representative waveforms created using the ABS. **a** Depicts the high reproducibility of the ABS in producing simulated blast of nominal 25 psi overpressures. Representative total, static, and dynamic pressure traces are shown in **b**. Subtraction of static from total pressure yielded the dynamic pressure

gas that drives the shock wave into the test section. The wave dynamics developed in this type of system do not intrinsically simulate free-field blast [36,37] and to address this, an advanced blast simulator (ABS) was designed from first principles to replicate the wave dynamics of explosive blast [36]. The ABS consists of a hexagonal divergent-area driver (Fig. 1a foreground) that is pressurized through a feedback system to a predetermined pressure. This is separated from the transition section by a frangible diaphragm. This section consists of a hexagonal cross section that expands into a circular cross section where it meets the test section. These geometries shape the expanding wave from the driver and smoothly re-direct the flow into the constant-area test section as a planar wave without introducing transverse waves. Helium, rather than air, is used as the driver gas to further shape the waveform, as it increases the amplitude, steepens the decay rate, and reduces the negative phase [36]. In order to prevent reflections from the open end of the test section travelling back up the tube, reflecting off the closed end of the driver and creating a chain of reverberations, an end wave eliminator (EWE; Fig. 1b) was used. The tunable EWE controls venting of the gas from the test section and can be adjusted for each driver/target pressure to eliminate rarefaction and the accompanying artefacts it causes in the pressure history. The EWE also serves to minimize noise and gas efflux into the test facility. The selection of a suitable diaphragm material was also a priority, as non-ideal diaphragm rupture is known to affect the output of shock tubes and blast simulators [86]. The desired material must be cost-effective, easy to use, and rupture instantaneously so as to prevent jetting and adversely affect the waveform. After screening a number of different plastic materials, cellulose acetate was chosen. This highly frangible material comes in a number of different thicknesses and fails reproducibly at defined driver pressures. When pressurizing the driver to predetermined and constant pressures, multi-layered diaphragm "packages" ruptured consistently to yield pressures at the target site in the test section within 1–3% of each other. These packages consisted of sheets no thicker than 5 mil since thicker sheets (10 mil) upon rupture yielded fragments that were occasionally injurious during rat exposure pilot studies.

Representative pressure histories produced by the ABS are shown in Figs. 3 and 4. Without the inclusion of the EWE, a 20 psi trial yields a pressure history that exhibits a sharply defined rise time with a ~6.5-ms duration. There are an exaggerated underpressure and additional artefacts downstream due to rarefaction waves travelling back up the test section and then reflecting off the closed end of the driver (Fig. 3, red trace). In addition, although not shown in this figure, superimposed over this underpressure is a greatly exaggerated dynamic pressure travelling in the opposite direction. The addition of the EWE eliminated these artefacts, and the green

trace in Fig. 3 shows a shock wave with a sharply defined primary overpressure, underpressure, and secondary wave closely simulating a free-field blast wave. The reproducibility of the ABS in simulating blast is illustrated in Fig. 4a, where the static overpressures and impulses from five 25 psi trials superimpose each other. A very important, but seldom measured endpoint in PbTBI studies is dynamic pressure, which can vary markedly depending on where the test samples are located inside the test section, or if end-jet testing is being carried out. End-jet testing, in particular, produces significantly increased dynamic pressures as the gas expands upon exiting the tube, conditions that do not simulate free-field blast detonations [37,87]. A mini-Pitot probe was developed to facilitate the measurement of this important endpoint [59]. Figure 4b shows the total and static pressures from a 25 psi test shot, as well as the derived dynamic pressure. The static overpressure closely follows the Friedlander waveform, with an instantaneous rise time followed by an exponential decay. The positive-phase duration time is approximately 7 ms, followed by a negative phase and a secondary shock wave. The total pressure trace shows an immediate reflective peak, followed by an exponential-like decay. The dynamic pressure is derived by subtracting the static from the total pressure.

The development of the ABS has enabled the reproducible generation of single-pulse shock waves that simulate free-field blast, including sharply defined static and dynamic overpressure rise times, underpressures, and secondary shock waves.

### 3.2 Animal studies

The soldier of today wears body armour that, if worn properly, is highly protective. However, the face remains substantially unprotected, with helmets specifically designed to prevent penetrating fragments and concussive forces, but not the pressure changes accompanying a blast wave. For this reason, head-only exposure models are most likely appropriate to better understand the mechanisms of PbTBI. Using the ABS technology, a rat head-only exposure model system was developed where the animals were exposed to overpressures of 15–30 psi of ~6.1- to 7.5-ms duration [38]. The presence of the rat head in the flow did not change the pressure histories (described above), with the exception of a perturbation in the decay phase of the peak at ~0.8 ms, which did not affect the impulse. Dynamic pressures were calculated separately and ranged from $4.8 \pm 0.2$ psi at 15 psi to $21.7 \pm 0.7$ psi ($n = 6$) at 30 psi [38]. It is important to note that these exposures represent low-level blast, and few or no signs of distress were noted in the animals as they recovered from the anaesthetic in a similar fashion compared to sham control animals.

Initial experiments did not restrain the heads of the anaesthetized animals, with only chin support and a line of netting contrecoup to the shock wave direction. High-speed video showed that exposure of this head configuration to simulated blast produced violent head movement and flexure. Although this did not result in the head impacting the sides of the test section, a very significant rotational whiplash action occurred [38]. The head response caused by the dynamic pressures is dependent on the impulse relative to the mass and surface area taking the load, and this type of global head movement would not be expected with a target the size and mass of a human head under similar loading conditions. Subsequent experiments reduced the global head movement by securing the head against the vertical netting. Analysis of a number of different biomarkers of brain damage revealed the importance of head movement in these experiments. Most interesting were changes in glial fibrillary acidic protein (GFAP), a structural protein that has been touted as a highly specific serum biomarker for TBI in humans [88,89] and that has also been reported to be elevated in test animals exposed to primary blast using a variety of model systems [80,81,83,84,90–92] with varying head configurations. As depicted in Fig. 5, the elevation of this protein in either the cortex or hippocampus was dependent on whether the head experienced whiplash during exposure to primary blast; in the unrestrained head configuration, GFAP elevations up to four times that of sham control animals were observed. In contrast, when head movement was minimized, no increase in this brain protein occurred. This head movement is a scaling artefact, and one must therefore conclude that in these studies GFAP is not a biomarker of primary blast, but is instead indicative of brain injury due to acceleration/deceleration effects. This is in contrast to the widely-held assertion that GFAP elevation is a common response to brain mechanical insult, including primary blast [83,84].

The results of these studies [38] clearly showed that when head movement was minimized, GFAP was not indicative of primary blast exposure. However, they also showed that pressure-dependent differences were found in the neuronal structural protein neurofilament H (NFH) [38]. More recent work using this animal model system in this laboratory (unpublished studies) has also shown that the cellular survival protein Akt, an important component of the phosphatidylinositol 3-kinase signalling pathway, is also activated in a pressure-dependent fashion. Clearly, primary blast causes subtle changes in the brains of exposed animals in this model system. Although head movement was greatly reduced during simulated primary blast exposures where the head was restrained, motion was not entirely eliminated and the restraint used in these studies represented a compromise. Use of a more rigid support may produce brain injury artefacts due to compression of the flexible rat skull that do not represent the stresses imparted to the human head from exposure to free-field blast. We are currently investigating the design of head restraints that allow adjustment of head accelera-

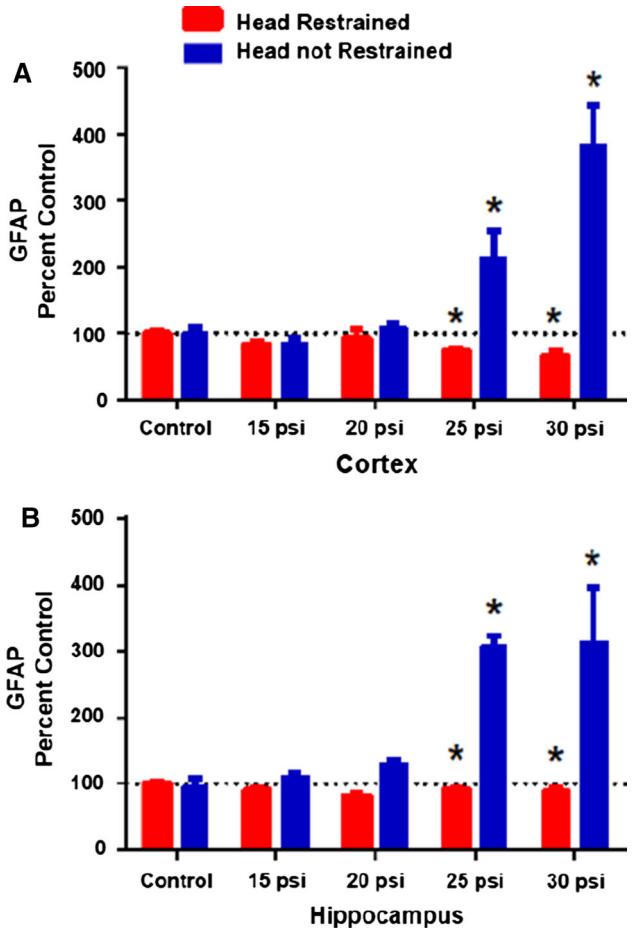


**Fig. 5** Effect of head restraint on brain glial fibrillary acidic protein (GFAP) expression after head-only simulated primary blast. Rats were exposed in head-only fashion to simulated primary blast with their heads either restrained (*red bars*), or not restrained (*blue bars*). At one day post-exposure, the levels of GFAP were determined in the cortical (**a**) or hippocampal (**b**) regions of the brain. In both brain regions, GFAP was significantly elevated after primary blast exposure only when the heads were not restrained. Asterisks denote statistical differences from sham control values ($p < 0.05$, Sidak's multiple comparisons test, $n = 5$). Figure derives from previously published data (Ref. [38])

tion in order to satisfy the scaling laws for blast-induced motion and enable better translation of results from animal testing to the human case. It seems clear that although the exposure conditions used in these studies are relevant to low-level free-field blast in humans, their use in most small rodent studies is probably inappropriate due to artefacts of exaggerated head motion and flexure from the blast. Scaled exposures comprised of similar overpressures, but reduced durations (<1.0 ms) are more likely to produce information of relevance to the human being exposed to low-level primary blast. These studies illustrate the scaling problems encountered when using small rodents in blast experiments and that caution must be used when interpreting the data derived from such model systems.

### 3.3 Brain cell aggregate studies

The use of animals to assess blast injury in humans presents a number of problems that are difficult to overcome. Studies using large animals with physiologies closely related to that of humans (i.e. swine or non-human primates) are difficult, cost prohibitive, and the blast facilities necessary to carry them out are rare. Small animals, notably rats and mice, present their own set of challenges that include dissimilarities in brain physiology and morphology compared to humans, as well as very significant scaling issues that are as of yet not understood. Cell and tissue culture models represent experimental platforms that have the potential to simplify the study of blast and shock on a number of different levels. The systemic effects that the intact organism uses to maintain homoeostasis are absent, enabling cell-/tissue-specific responses to a given stimulus or insult. In contrast to the intact animal, the experimental treatment actually experienced by the cell/tissue culture—be it chemical, biological, or physical in nature—is also frequently much easier to quantify. In the case of blast and shock, tissue culture systems also represent an opportunity to understand the injury biomechanics of pressure change directly on brain tissue, without consideration of the scaling issues and resultant artefacts of dynamic pressure that should be considered in rodent studies.

A rat brain cell aggregate culture model has been developed that has a number of features that render it useful in the study of primary blast-induced brain injury. Cells from embryonic rat brains are placed into spinner flasks and gently stirred under close rotational control. Over the course of 3–4 weeks, the cells arrange themselves into spheres (300–500 μm) of defined architecture that exhibit many of the organotypic structures and functions of the intact brain [60–62]. Figure 6 shows examples of aggregates that have been in culture for ~4 weeks. Panels A and B are electron micrographs that highlight the complex multi-cellular make-up of the aggregates at 950× and 11,400× magnification, respectively. Panel C depicts a confocal micrograph of an aggregate at 25 days in culture and shows the characteristic and highly defined cellular architecture obtained by this time point. The glial cells (green) have formed a protective layer around the perimeter of the aggregate, surrounding an extensive latticework of neuronal cells (red), as well as glia and other cell types (not stained) in the interior. The use of these cultures offers several advantages in the study of shock and blast effects on the brain. They are composed of the major cell types of the brain that have differentiated function, which suggests that they will respond to pressure changes in a fashion more similar to that of the whole brain, compared to single cell type cultures. In addition, they can be maintained in culture for periods of weeks to months, ensuring that any persistent or delayed effects of blast are detected; a potentially important characteristic if indeed the delayed symptoms of

mTBI observed in many returning veterans [3] are due to primary blast exposure. Lastly, in contrast to the great majority of culture models where the cells are adhered to plastic or glass surfaces and maintained in culture vessels of varying geometries, brain cell aggregates are suspension cultures. Transfer of the aggregates into vessels with defined boundaries and physical properties enables the definition of experimental shock or blast parameters, as well as measurement of the actual pressure changes experienced by the brain tissue.

It is not currently known what properties of a pressure change (i.e. rise time, peak amplitude, decay rate, duration, complex) are of importance in producing brain injury, nor is it known with any certainty what biological effects that primary blast causes to induce mTBI. The rat brain aggregate system provides the flexibility with which to potentially answer some of these questions and a research effort to understand the effects of primary blast on brain tissue was undertaken using this model. The initial efforts focused on defining the endpoints, as well as ascertaining the sensitivity of the model system. If no pressure-dependent changes in aggregate function were found, or the changes were uniform to different types of pressure change, for example shock wave versus compression wave, then the model system would not likely be useful. Two separate studies were carried out; one intentionally simplified to isolate the effect of UNDEX-induced principal stress without shear on the aggregates [53], and one where the complexity of the simulated blast insult was much increased by encasing the aggregates within a spherical shell [54], resulting in a complex pressure history due to flexure of the sphere walls and the reflections within.

The work assessing the effects of a simplified shock wave insult took advantage of the UNDEX pond facility situated on DRDC Suffield Research Centre's Experimental Proving Grounds. The large size of the UNDEX pond enabled the placement of the target vessel containing the aggregates far from reflected waves, thus allowing for exposures (~43, 391, 2030 psi) to nearly planar, free-field underwater blast waves. The highly hydrolysed dialysis tubing cylinders used to encase the aggregates (Fig. 2a) were transparent to the shock front, with representative pressure histories from gauges placed either inside or outside of the dialysis tubing showing virtually identical traces (Fig. 7a). Thus, the aggregates were directly exposed to measurable and well-characterized underwater blast waves, which exhibited realistic sharp rises and exponential decays free of reflecting boundaries. This shock exposure produces only principal stress, and all but eliminates the shear forces and global accelerations that are also thought to be important in blast injury. In contrast, the second set of trials involved simulated air blast of aggregates encased within a spherical shell (Fig. 2b). In these experiments, the spheres containing the aggregates were exposed to single-pulse shock waves of 15–30 psi generated by the ABS. Figure 7b shows a representative 25 psi trace, show-

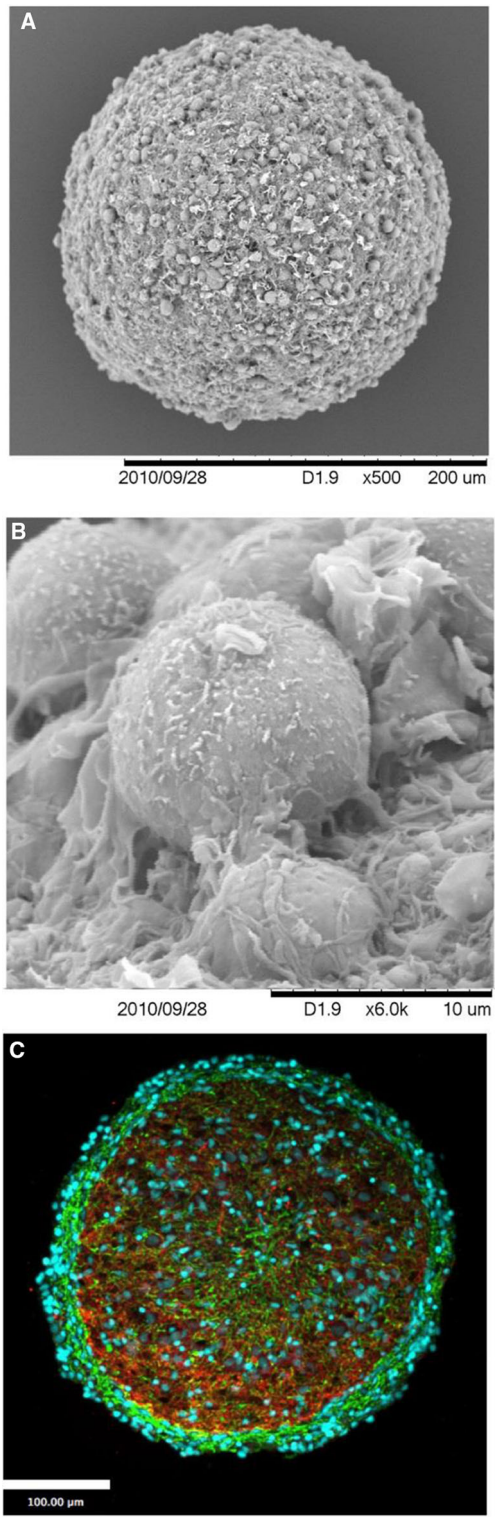


**Fig. 6** Micrographs of rat brain cell aggregates. **a**, **b** Electron micrographs of a brain cell aggregate at 28 days after initiation at increasing magnifications (**a** ×950; **b** ×11,400), **c** a confocal micrograph of a brain cell aggregate at 25 days after initiation. The cells have arranged themselves so that a protective layer of glial cells (*green*-stained glial fibrillary acidic factor) is on the outside, surrounding an extensive network of neuronal cells on the inside (*red*-stained NeuN), as well as other cell types not stained for

ing an immediate rise time followed by a decay phase with minor perturbations at ~0.8 and ~1.2 ms due to reflections off of the sphere. Although the simulated blast exposure of the sphere was single pulse in nature, the actual pressure changes experienced by the aggregates inside were spatially dependent and much increased in complexity, as illustrated by the pressure histories recorded at forward, centre, and back locations of the sphere (Fig. 7c). The forward gauge first detected the transmission of the reflected pressure off of the leading edge of the sphere, which was significantly increased compared to the 25 psi incident overpressure on the sphere. This then decayed towards ambient values in an oscillatory fashion as the reflections arrived from the back of the sphere. This trend was more pronounced at the rear of the sphere due to the closer proximity of the gauge (2 mm) from the rear reflecting boundaries, while the centre gauge recorded similar pressure trends, but in an attenuated fashion due to the superimposition of the reflecting waves.

The aggregates were placed back into routine culture conditions after blast exposure and sampled at 3, 7, 14, and 28 days post-exposure. It is not clear how primary blast affects the brain and a variety of endpoints previously utilized as indicators of brain damage or change were therefore assessed, including assays of cell death (lactate dehydrogenase, caspase-3), brain structural proteins (GFAP, NFH, myelin basic protein), cell type specific enzymatic assays (glutamine synthetase, acetylcholinesterase, choline acetyltransferase) and cellular signalling proteins (Akt and VEGF). The subtle nature of the primary blast effects on the brain was well illustrated by the largely negative findings using these endpoints. No cell death was detected, and with the exception of Akt and VEGF, no changes were noted in any of the other endpoints, with either type of pressure insult at any time point [53,54]. These findings, that primary blast does not cause overt cellular damage or inflammatory responses in brain cell aggregates, are not overly surprising if it is representative of PbTBI. While the role of primary blast in the mild TBI experienced by individuals exposed to blast may not be clear, it seems likely that any such effects would be subtle and not due to rapid structural protein alterations or cytotoxicity.

Much more significant findings were found when the cellular survival protein Akt and VEGF (Fig. 8) were examined [53,54]. Exposure to either type of pressure insult resulted in a pressure-dependent phosphorylation of Akt that was maximal by 3 days post-exposure and then declined towards baseline values thereafter, with the decrease being more rapid in UNDEX-exposed aggregates. In contrast, both types of pressure challenge resulted in a decline in VEGF levels. However, these effects were dramatically different in their time dependence. Simple UNDEX-induced overpressure caused a rapid inhibition of aggregate VEGF levels by 3 days that then recovered towards baseline values afterwards

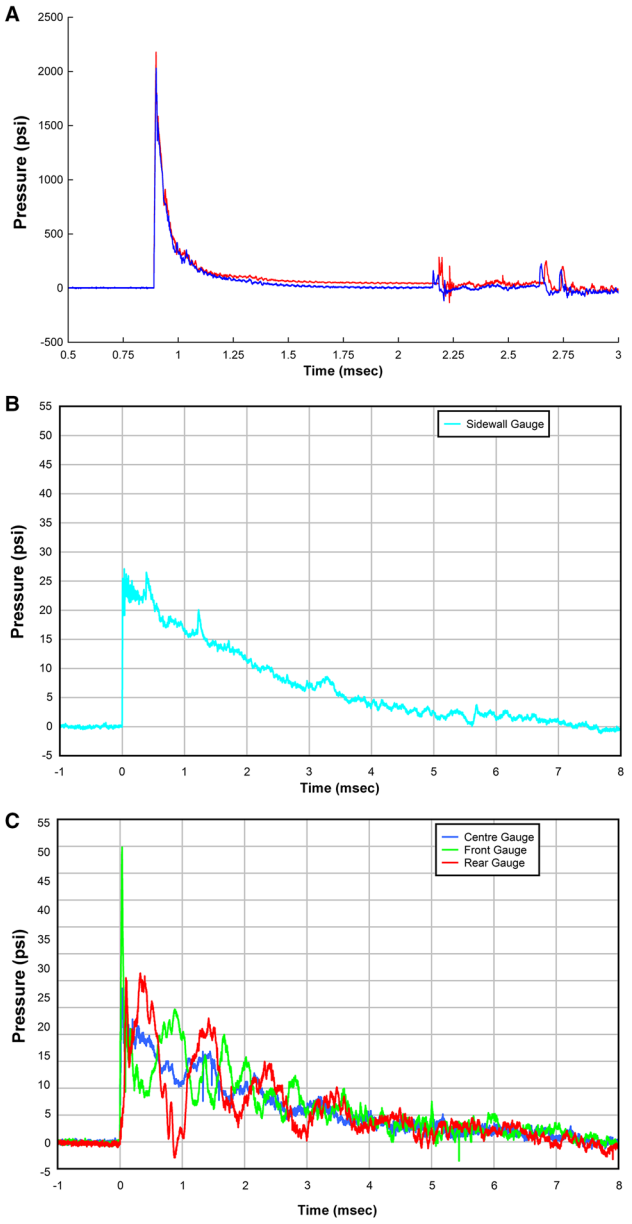


**Fig. 7** Representative pressure histories of underwater and simulated air blast exposures of brain cell aggregates. **a** The pressure history of an underwater blast (~2030 psi) was recorded by gauges placed 10 cm to either side of the dialysis tubing (*red trace*), or inside the dialysis tubing used to contain the brain cell aggregates (*blue trace*). In both cases, the traces are similar, indicating that minimal boundary effects were imposed by the dialysis tubing on the shock wave exposures, **b** representative static pressure history from a 25 psi trial using the ABS, showing an immediate rise time and an exponential decay, **c** the actual pressure changes experienced by aggregates inside the sphere exposed to a 25 psi shock wave are complex in nature and spatially dependent. The waveforms inside the sphere were recorded by gauges located at the centre (*blue trace*), front (*green trace*), or rear edge (*red trace*)

(Fig. 8b). In comparison, the effects of the complex pressure changes experienced by the aggregates in the sphere were much delayed, with little change in VEGF levels until 14 days

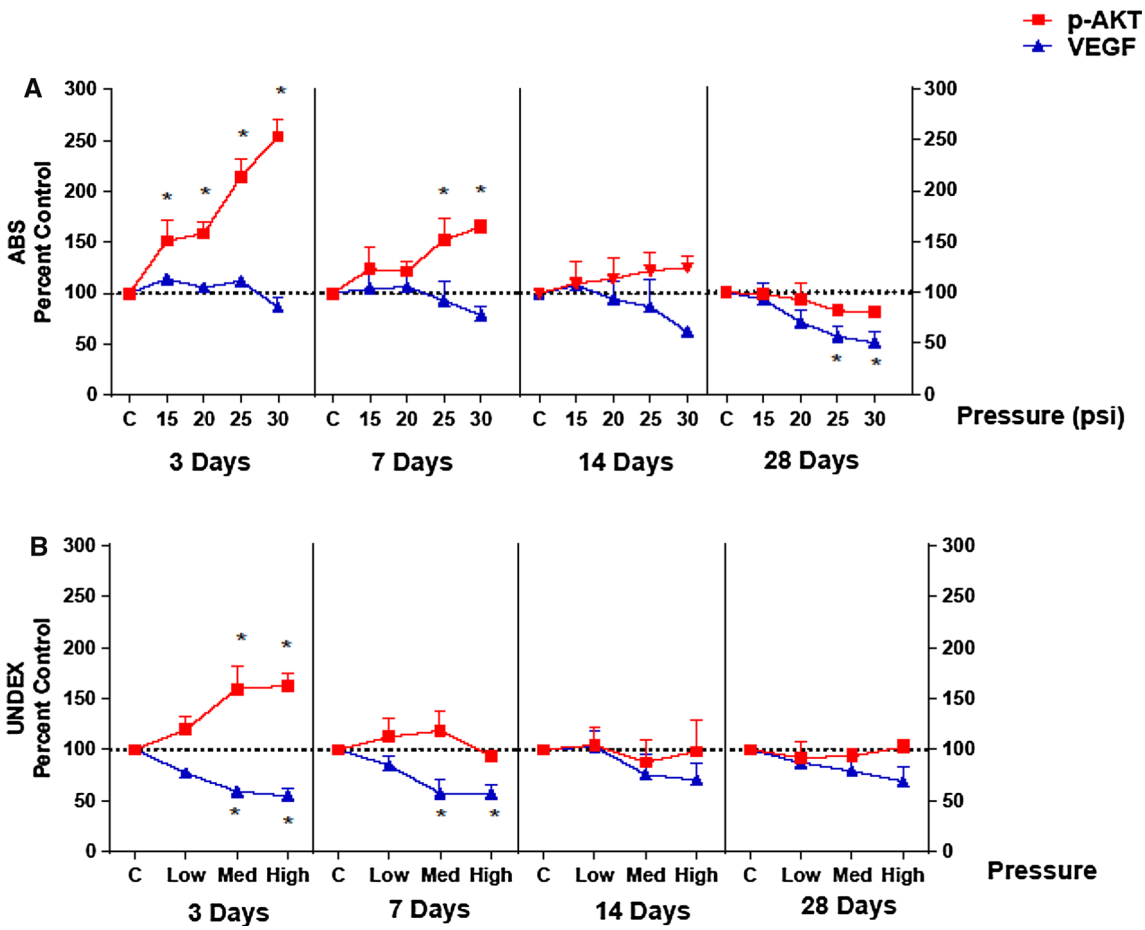


**Fig. 8** Effect of simulated primary blast on brain cell aggregate Akt and VEGF levels. Brain cell aggregates were exposed to either simulated air blast (~15, 20, 25, 30 psi) when encased in a sphere (**a**), or to underwater blast (low: ~43, medium: ~391, or high: ~2030 psi) when encased in dialysis tubing (**b**). Sham control samples were treated identically to exposed samples, but without exposure to blast or simulated blast. The cellular survival protein Akt (*red*) and vascular endothelial growth factor (VEGF; *blue*) levels were assessed at 3, 7, 14, or 28 days. In both cases, Akt was significantly elevated by 3 days and then declined towards baseline levels. In contrast, simulated air blast caused VEGF levels to decline only after a delay of 2–4 weeks, compared to the immediate decrease induced by underwater blast. Data were analysed using two-way ANOVA and Dunnett's test. Values represent the mean ± SEM from at least three separate experiments. *Asterisks* denote statistical differences from sham control values ($p < 0.05$). Figure derives from previously published data (Refs. [53,54])

that then became markedly pressure dependent and maximal by 28 days (Fig. 8a). The identification of Akt and VEGF as potential targets of primary blast is of importance. Both proteins have been implicated in a host of neurodegenerative diseases, including TBI [93–100], and pharmacological modulation of their pathways has been the subject of much interest.

The above studies show that the effects of primary blast on these cultures are subtle, with many commonly assayed endpoints of TBI not being affected and no cell death being induced, results that are in line with what appear to be the emerging consensus that primary blast induces very subtle changes in cellular function, with little or no cell death [42,43,55,57,58]. Primary blast was found to induce similar pressure-dependent changes in Akt phosphorylation with either type of exposure. However, VEGF levels were temporally dependent on the type of pressure change, with simple overpressure exposure inducing immediate and transient VEGF inhibition, compared to the significantly delayed and pressure-dependent VEGF induced by the complex pressure changes experienced in the sphere. This suggests that the rat brain cell aggregate culture model may represent a useful tool with which to elucidate what properties of a shock wave are important in causing primary blast-induced brain damage, a potentially valuable aid in the development of personal protection. In addition, these molecules have been identified in a number of neurodegenerative diseases and present potential targets for therapeutic intervention.

## 4 Conclusions

The results presented in this paper are examples from the authors' laboratories and recently published studies. They

illustrate the complexities of primary blast-induced TBI, and the multidisciplinary approach necessary to carry out such studies. An ABS system has been described that reproducibly simulates primary blast, including sharply defined static and dynamic pressure rise times, underpressures, and secondary peaks. The measurement of dynamic pressure was necessary to fully characterize the simulated blast exposures within the ABS and assist in understanding the exaggerated global head movement observed in small rodents during head-only exposures to primary blast. This head movement was an artefact of the scaling issues concerning dynamic pressure impulse, and studies showed that elevation of a commonly thought biomarker of primary blast-induced TBI (GFAP) was due to this exaggerated head movement. A brain cell aggregate culture model was developed that allows the rigid control of experimental shock or blast parameters and enables basic studies aimed at elucidating the shock wave properties that are important in causing brain damage.

Future research will continue efforts to improve the head-only animal exposure model system through the development of suitable head restraints and primary blast simulations that utilize much shorter durations. In parallel, work will continue with the brain cell aggregate model in a comprehensive research effort to understand the effects of primary blast on the biology of the brain, and to facilitate the development of personal protection and therapeutic drug treatments.

#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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