Impulsive Pressurization of Neuronal Cells

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OUTLINE

- Motivation and Objectives
- A Kolsky bar technique for impulsive pressurization
  - Experimental design
  - Operation
  - In-vitro cell containment vessel
  - Verification of design parameters
- Impulsive cell pressurization experiment
  - Neuronal cell preparation and study
  - Preliminary experimental results
- Summary

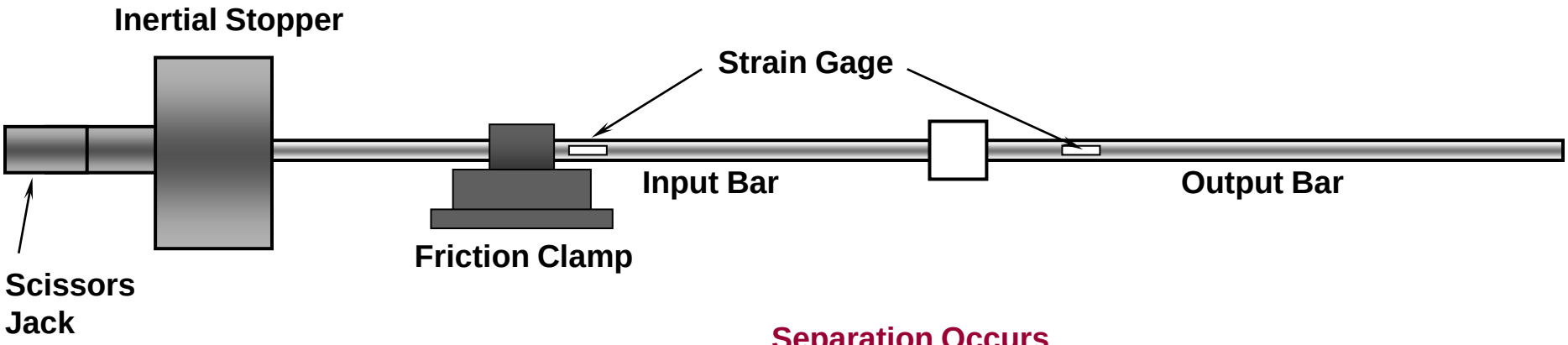
Motivation

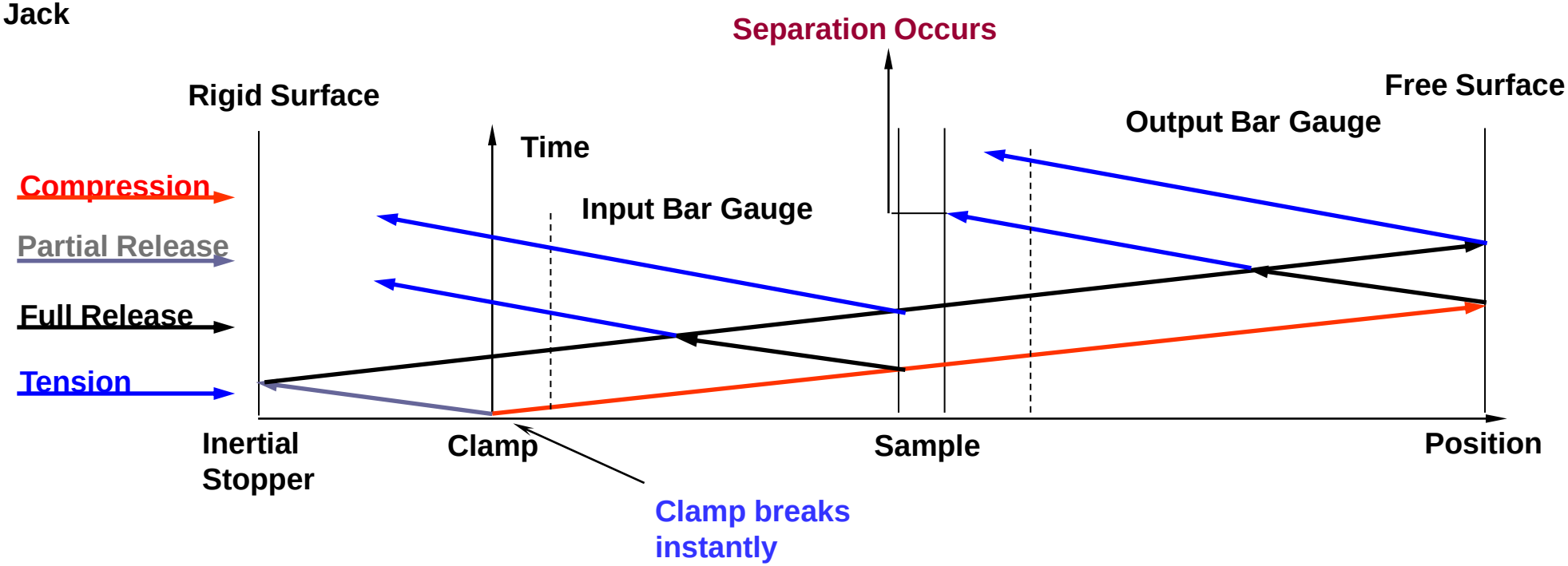
- Blast-induced traumatic brain injury (bTBI) has become one of the leading injuries of the U.S. soldiers involved in the current military operations, though currently the mechanism of the injury is unknown.
- One possible mechanism for bTBI is blast-induced overpressure, or high stress impulse in the brain.
- A new experiment needs to be developed to study the functions of the neuronal cells exposed to the pressure impulses relevant to the blast loading generated by improvised explosive devices.

OBJECTIVES

- To develop a Kolsky bar experiment that is capable of creating and measuring a stress wave with the intensity and duration of a blast wave.
- To apply this method for single stroke impulsive pressurization of in-vitro neuronal cells.

A New Design of Kolsky Compression Bar





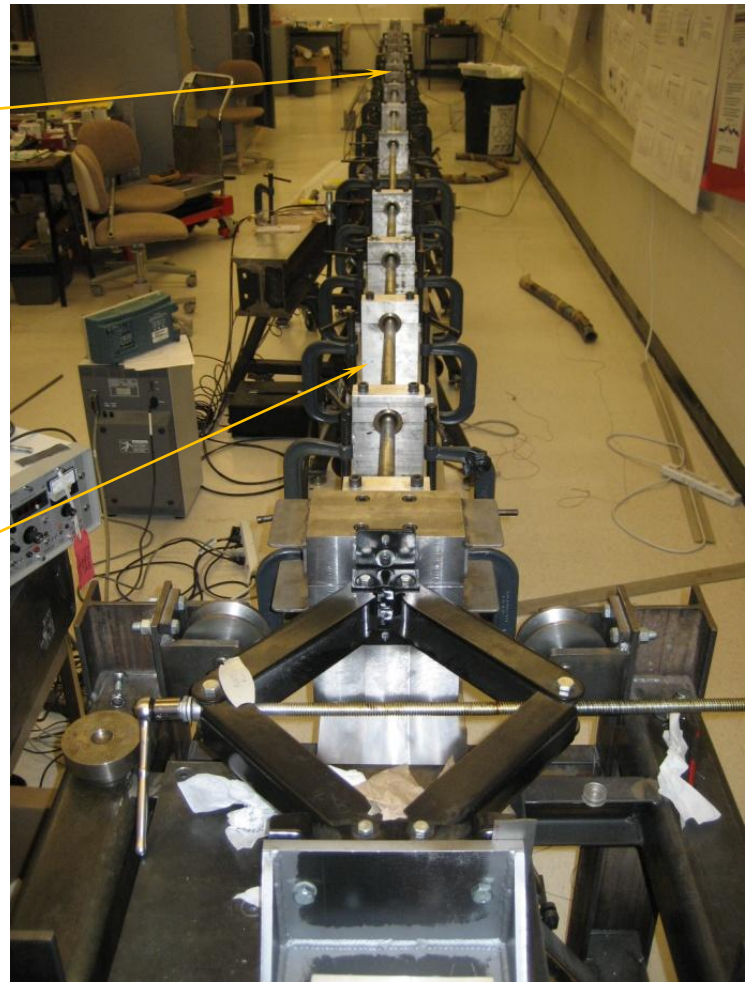
The Experimental Setup

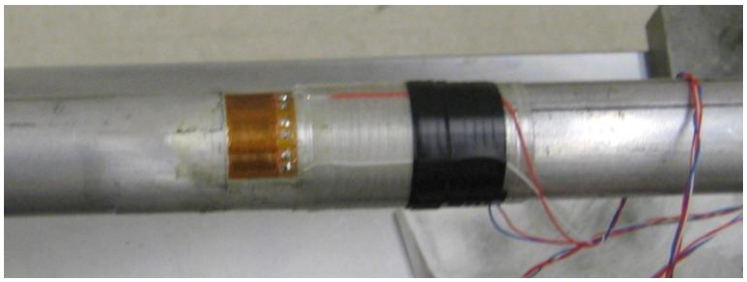
Friction Clamp

Output Bar

Piezo-Buzzer Trigger

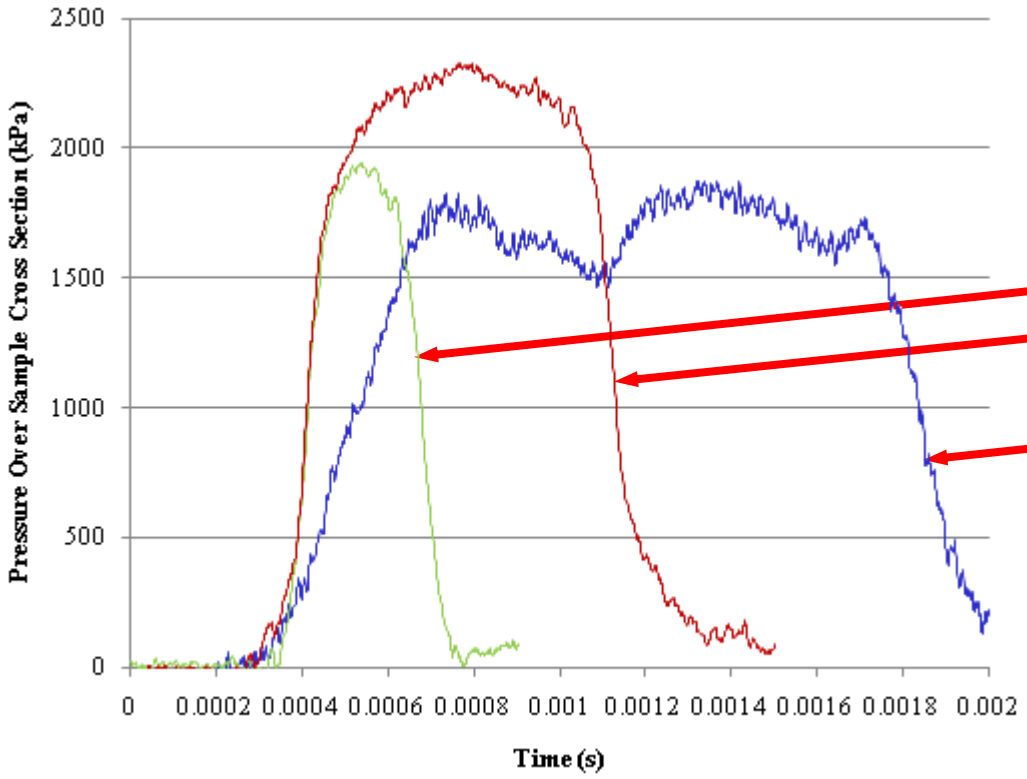
Input Bar





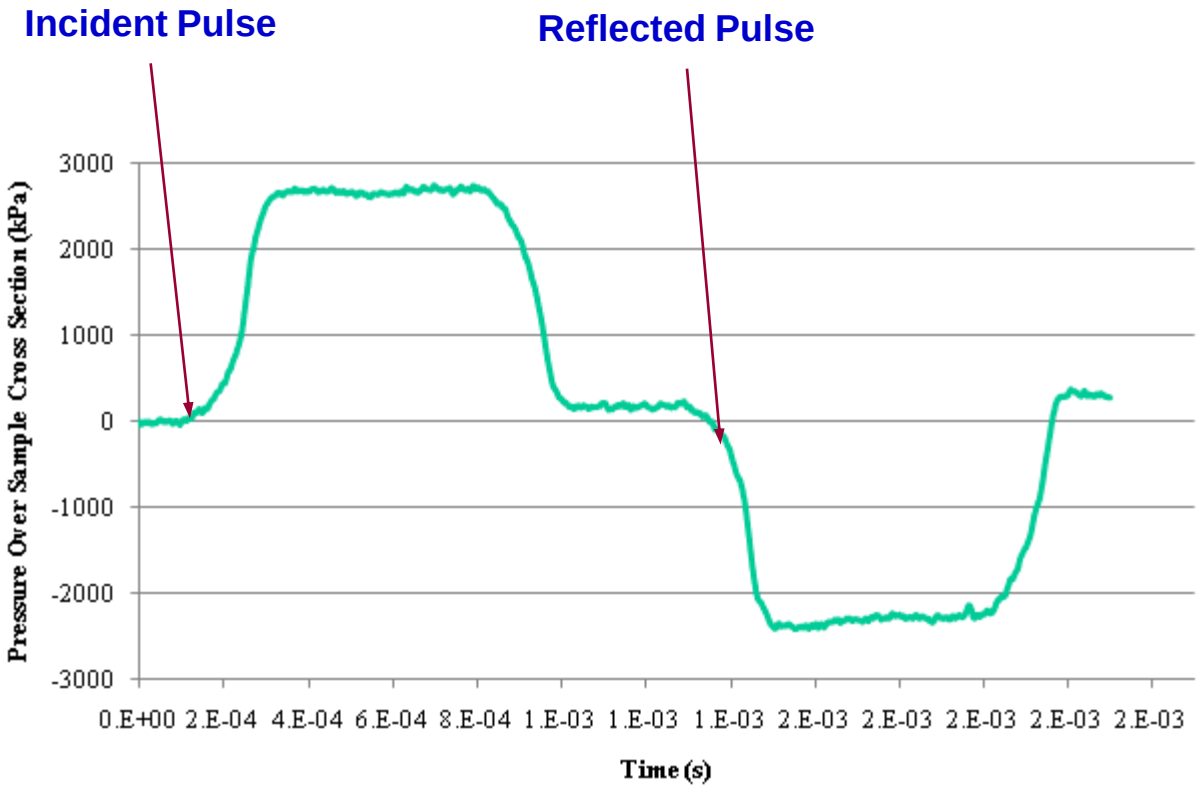
Compound Strain Gauge

Variable Pulse Duration



- The clamp's position on the bar alters the duration of the stress pulse.
- This allows the time to be varied widely from .3ms to over 1.5ms.

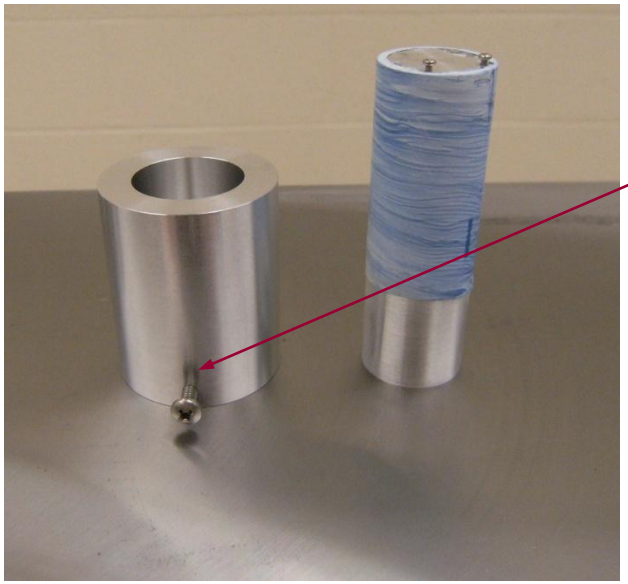
Typical Pulse Profile

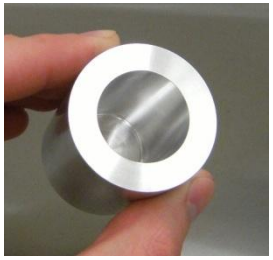


0.65 ms long pulse

- The size and shape of both the incident and reflected pulses are similar.
- The lack of vertical drift shows that the heavy mass closely approximates a ideal rigid wall.

In-Vitro Cell Containment Vessel

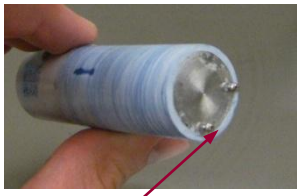




- Water vent allows large air bubbles to exit when the piston is pushed in .
- Leakage due to sealing are lower. since there is only one area to leak from
- Small o-ring on screw completely seals the chamber.

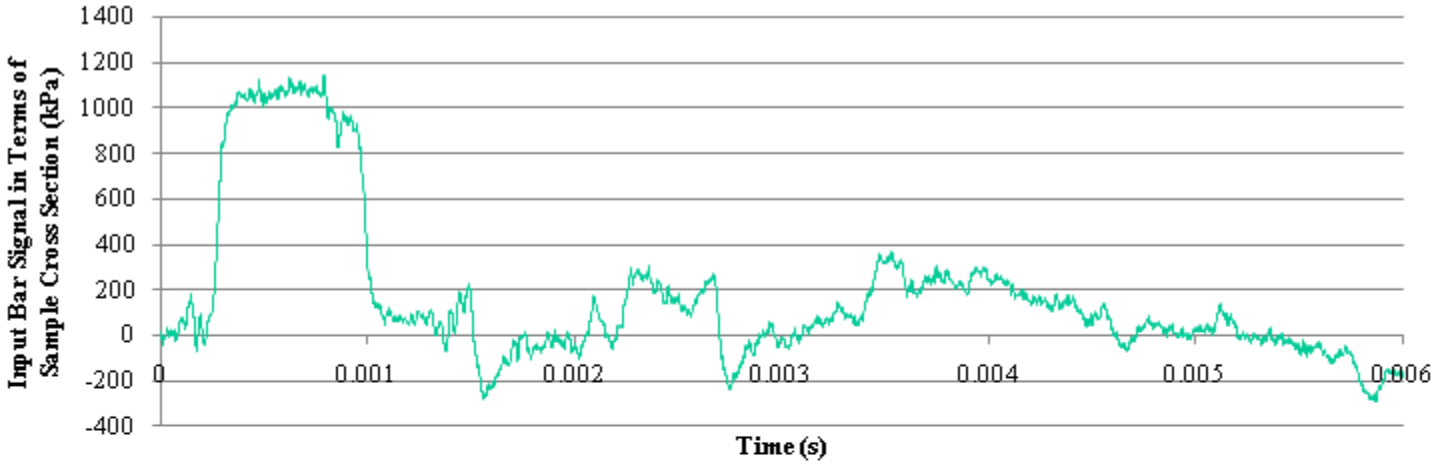
- The hollow piston matches impedance with the output bar.
- Sample is a glass slide and is secured with two stainless steel screws.
- Teflon eliminates surface gauging and friction, seals the chamber, and is biologically neutral.
- A thin layer of Teflon has little effect on the pressure.

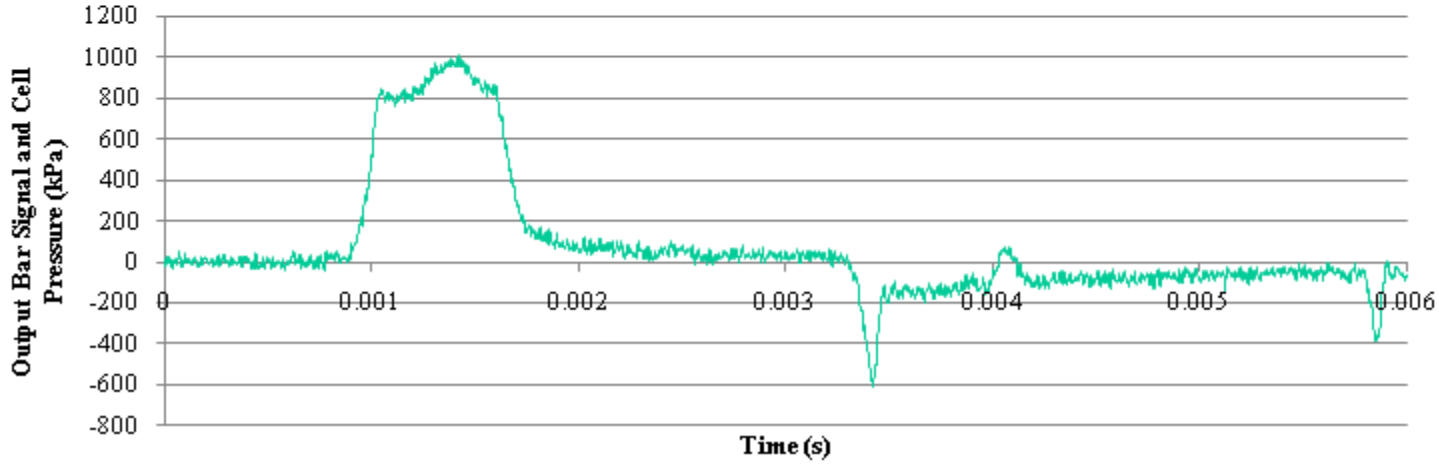
Piston



Sample securing screws

Typical Measurements In-Vitro Cell Containment Vessel



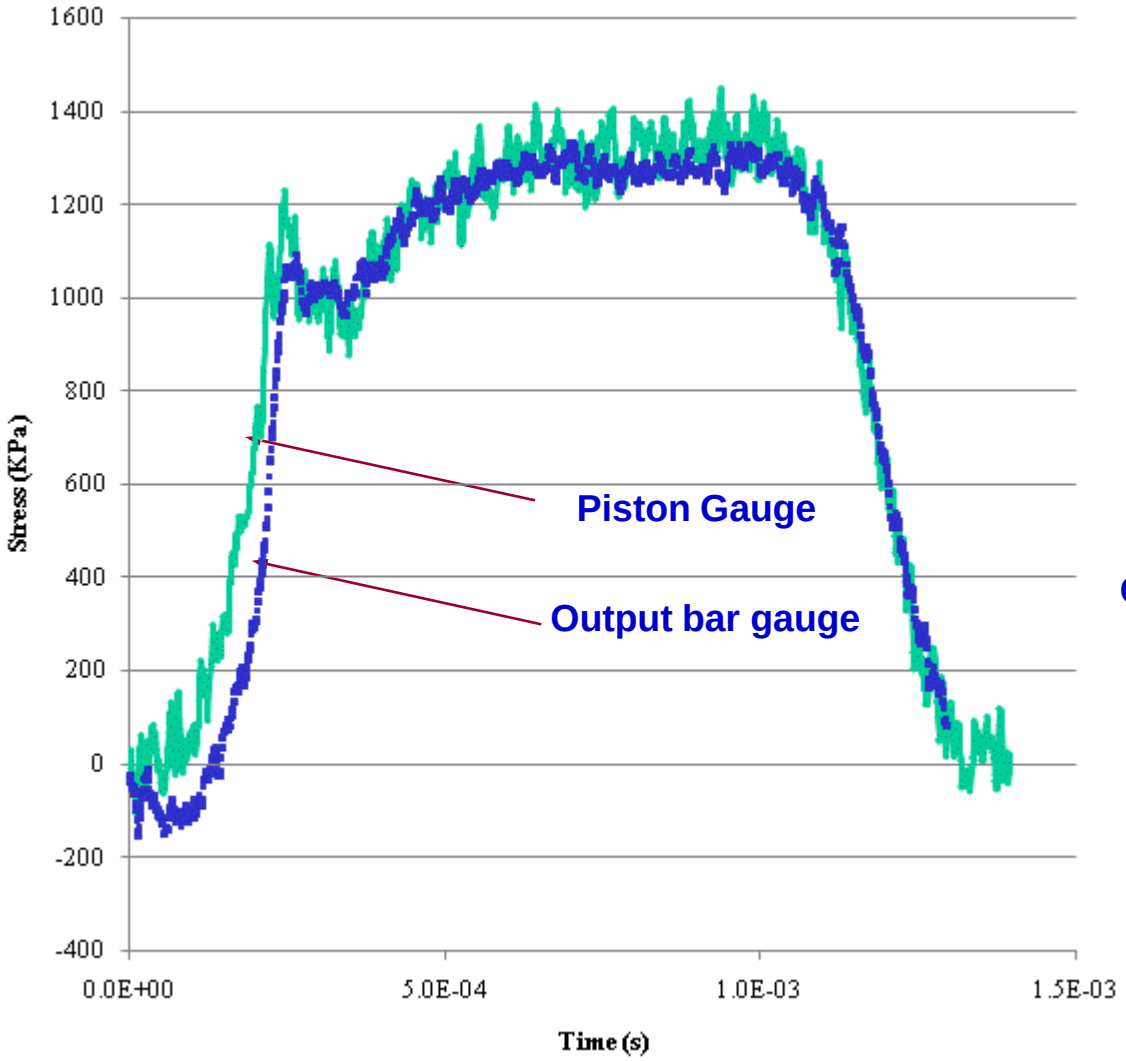


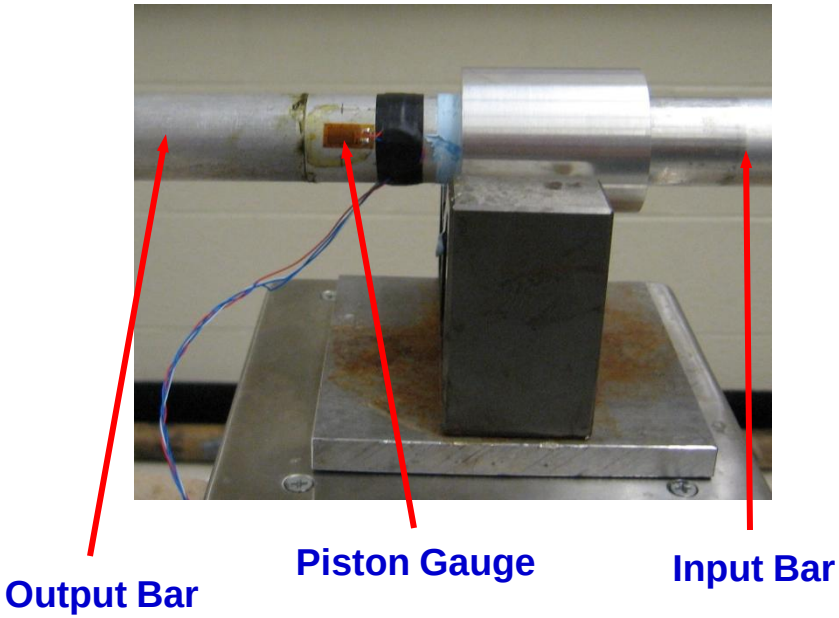
- The output wave is similar to the input wave in both intensity and duration.
- Sample reloading after the initial pulse is difficult to determine.
- Theoretically the output wave should be proportional to the pressure history of the sample.

Cell Pressure Verification

- Does the piston strain pass through the interface without interference?
- Does the cell chamber reload?
- Is the cell chamber pressure related to the bar strain gauge measurements?
- What effect do bubbles have on the transmitted pulse?

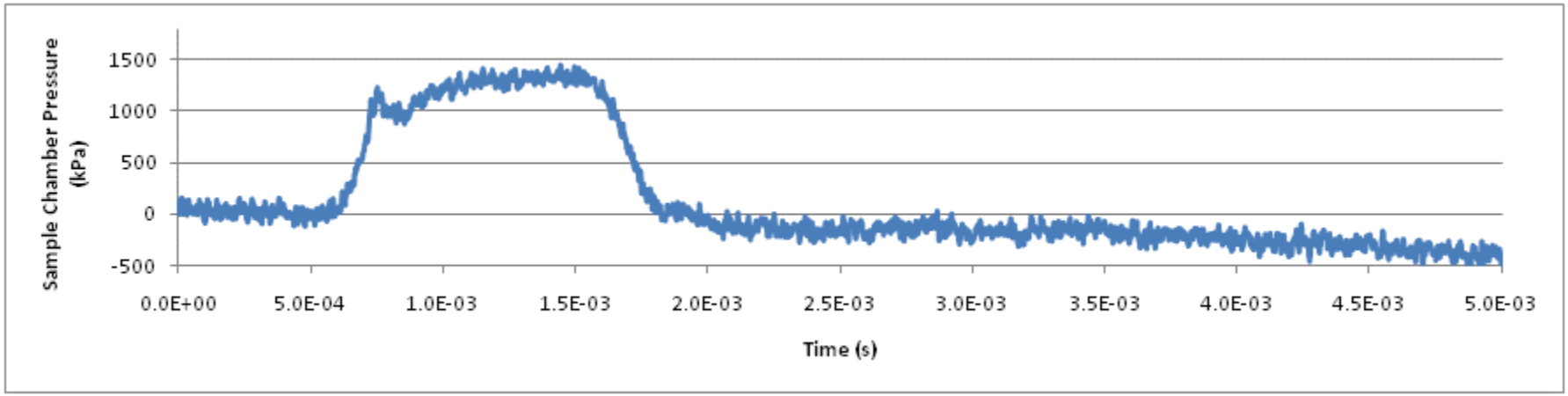
Comparison of Piston and Bar Gauges





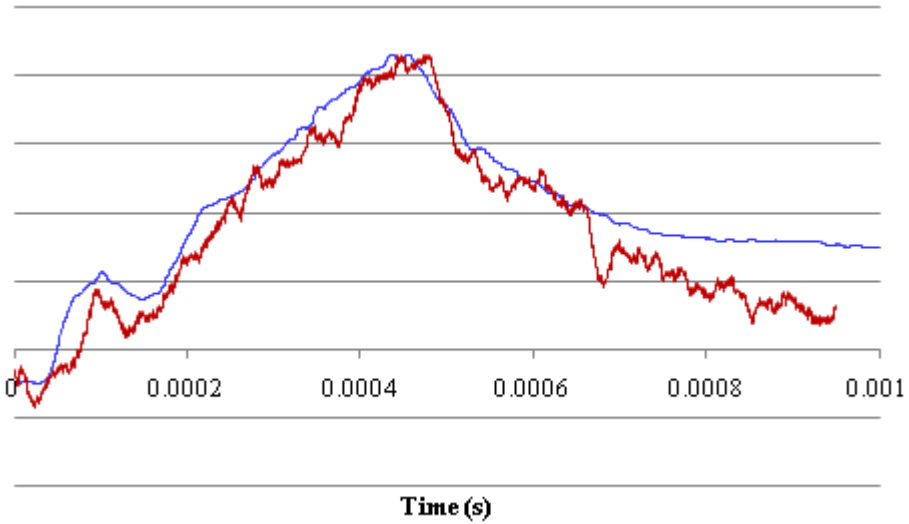
- The shapes nearly match up.
- Small difference between the two profiles may be due to dispersion.

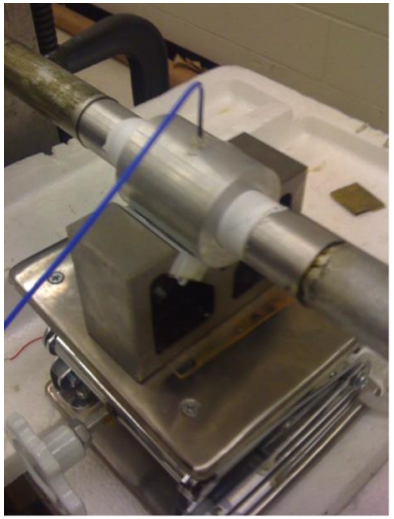
Verification Against Major Reloading



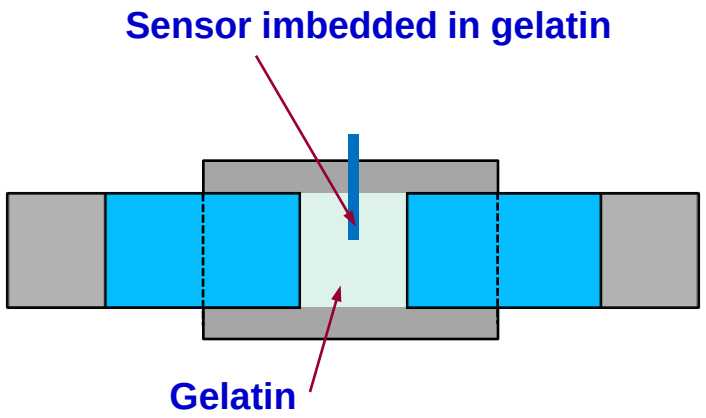
- This data is from the same experiment as the previous slide.
- The strain history is quiet after the initial wave which shows no reloading.

Actual Pressure vs. Bar Pressure

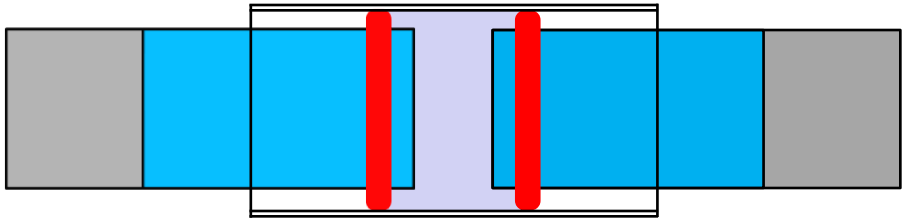


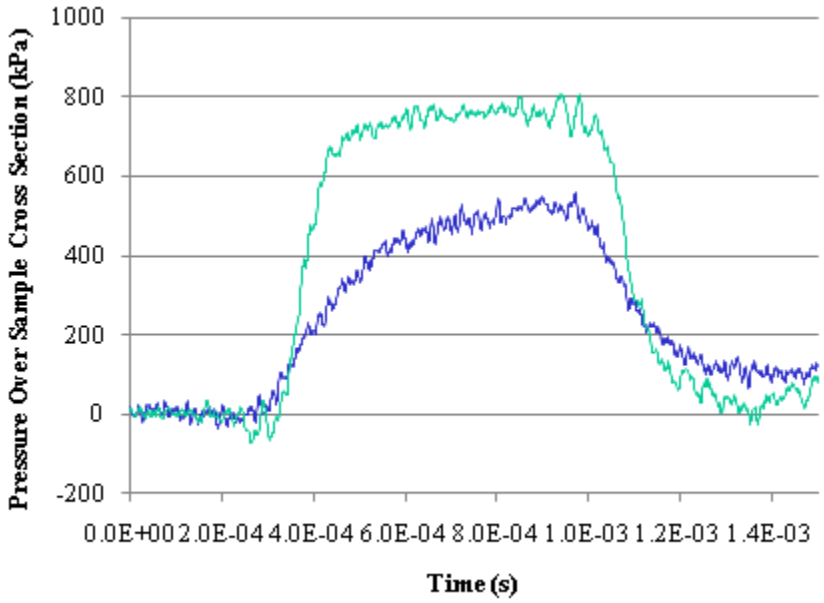


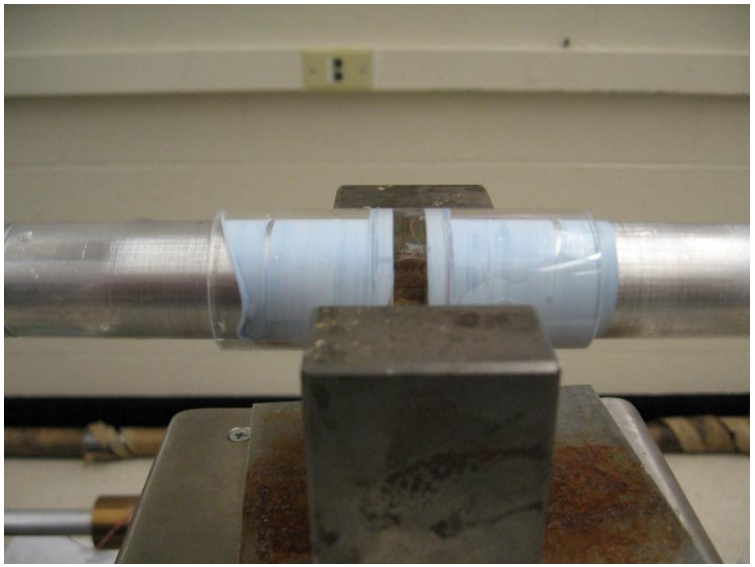
- A test was conducted on a small pressure sensor imbedded in gelatin in a small casing.
- It shows that the pressure inside the cell, and the response of the output bar are nearly identical.
- Thus the transmitted pulse obtained from the output bar should be proportional to the chamber pressure.



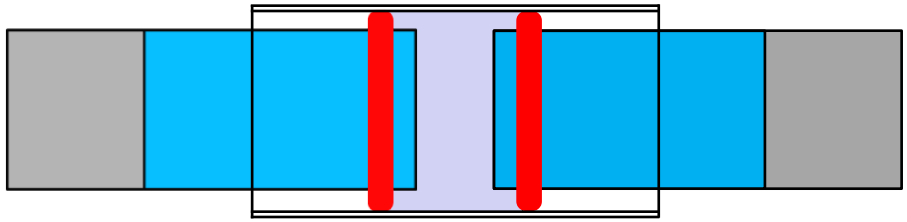
Clear Verification Chamber

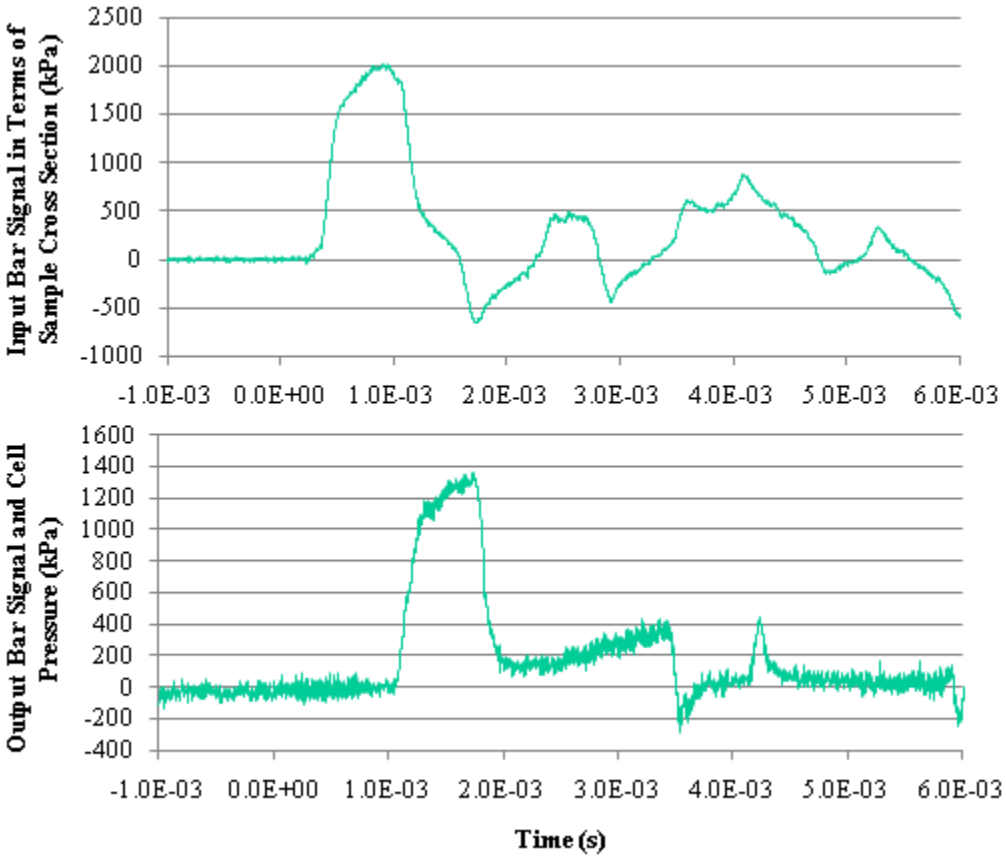


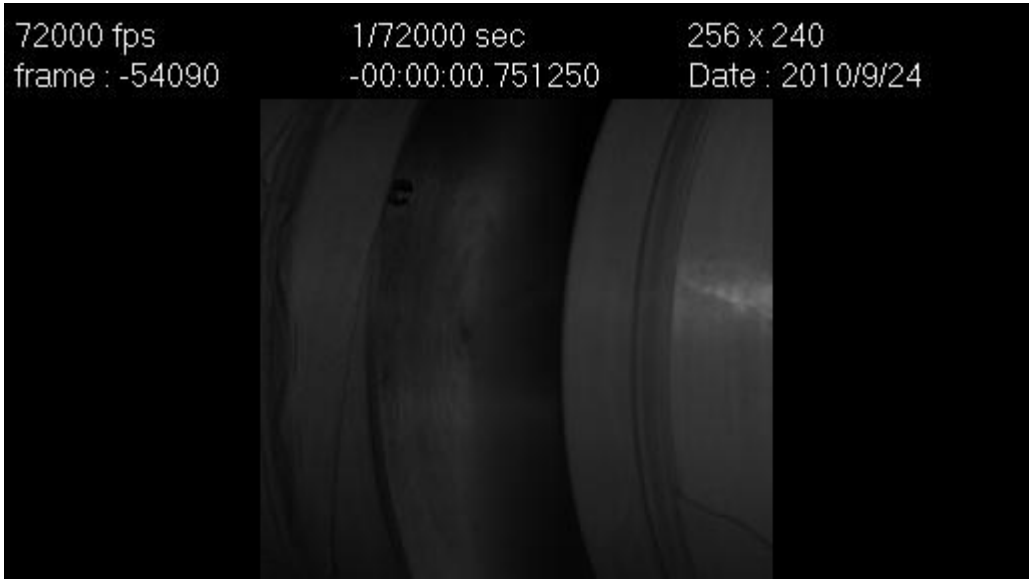




Bubble Size Affecting Transmitted Pulse

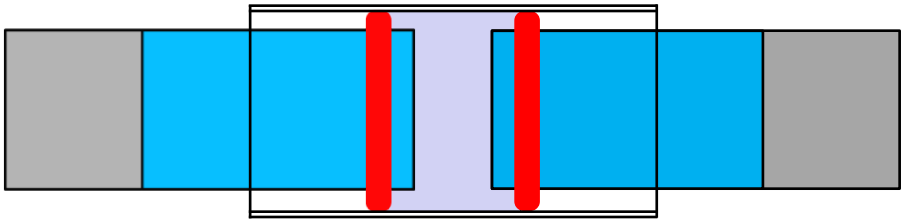


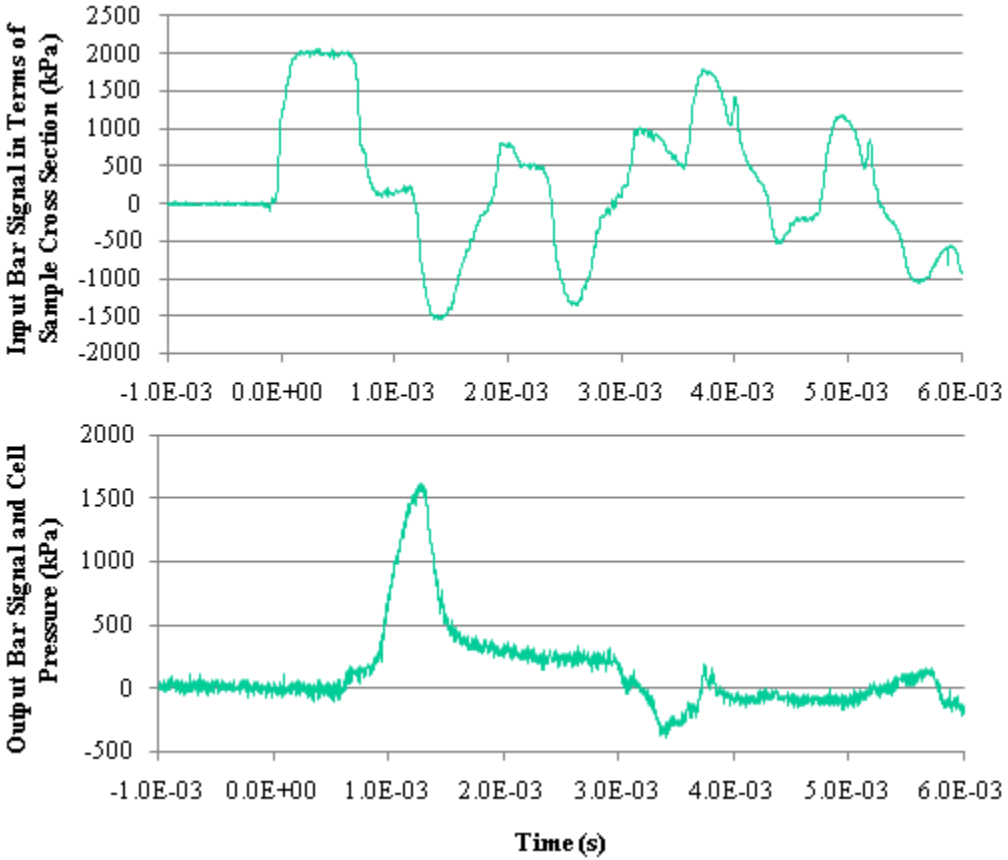


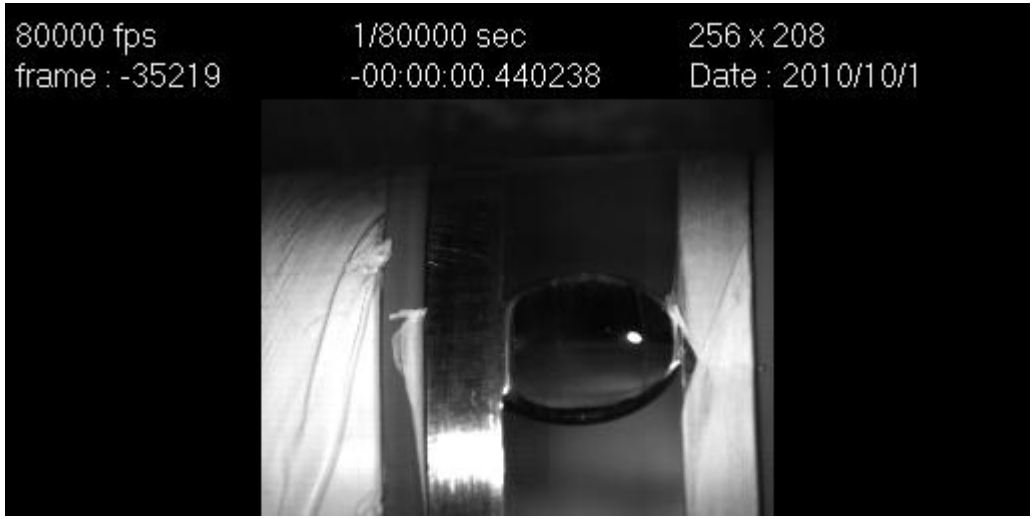


Video by Jonathan Hein

Bubble Size Affecting Transmitted Pulse



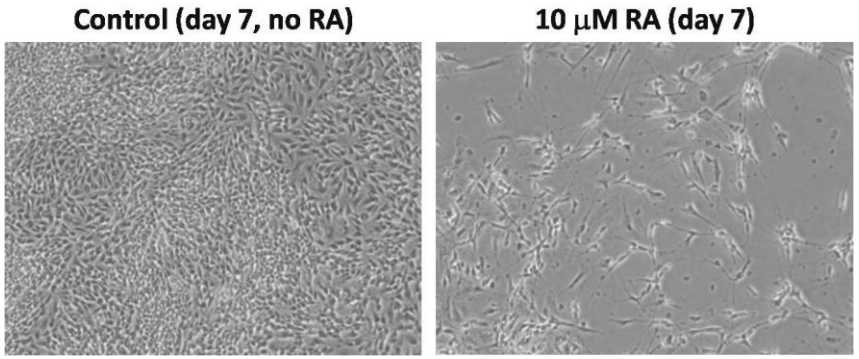


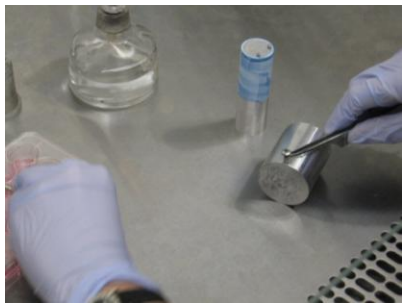
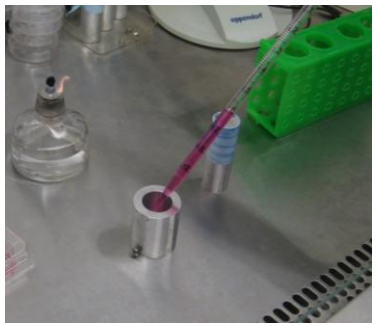


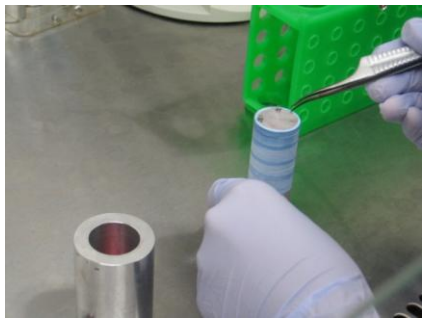
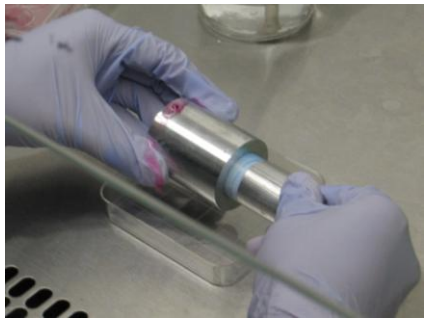
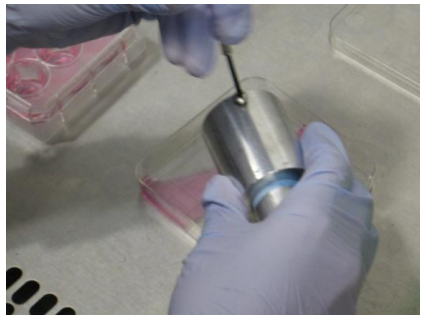
Video by Jonathan Hein

Impulsive Neuronal Cell Pressurization Experiment

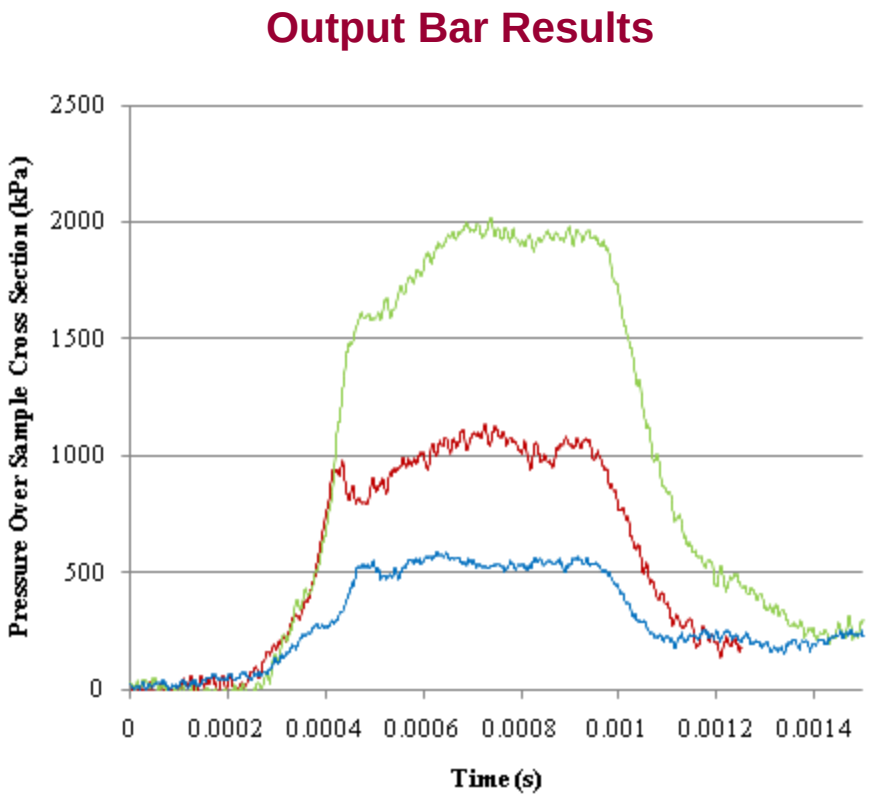
- Neuronal cells are cultured and differentiated onto glass slides.
- Glass slides are installed in chamber then sent up to the Kolsky bar lab.



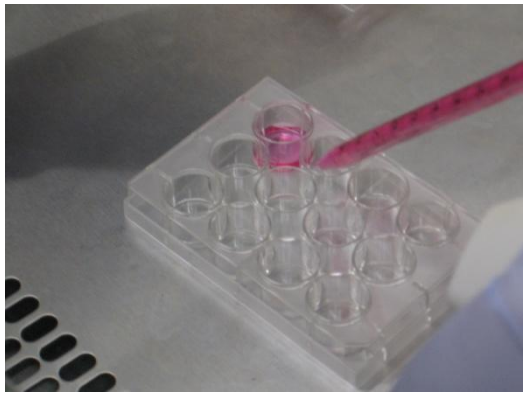




Three Typical Target Pressures

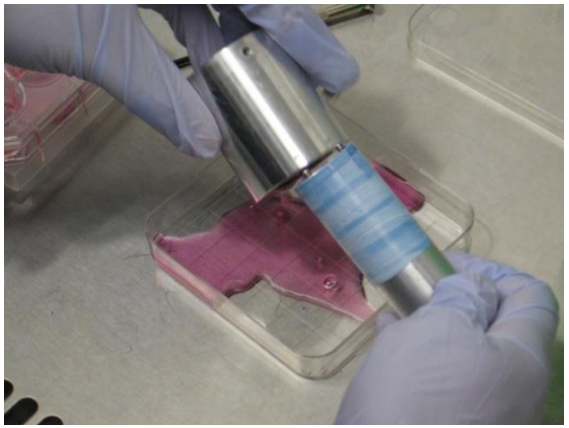


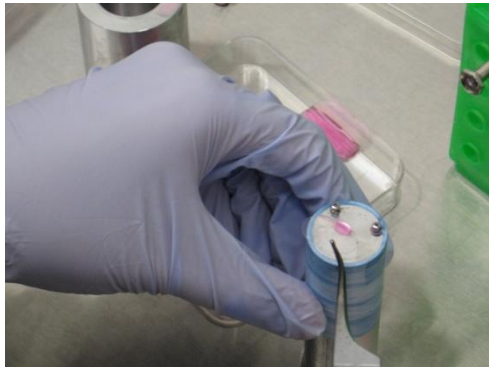
- For the biological study, three significantly different pressures are needed.
- It is thought that mild TBI may be induced at sub-MPa level.
- Tests are collected around these three pressures.

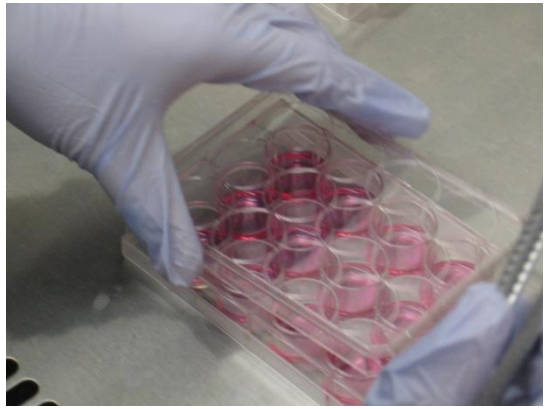
Impulsive Neuronal Cell Pressurization Experiment



- The cells are pressurized.
- Cells are removed and either incubated for 24h or are inspected immediately.







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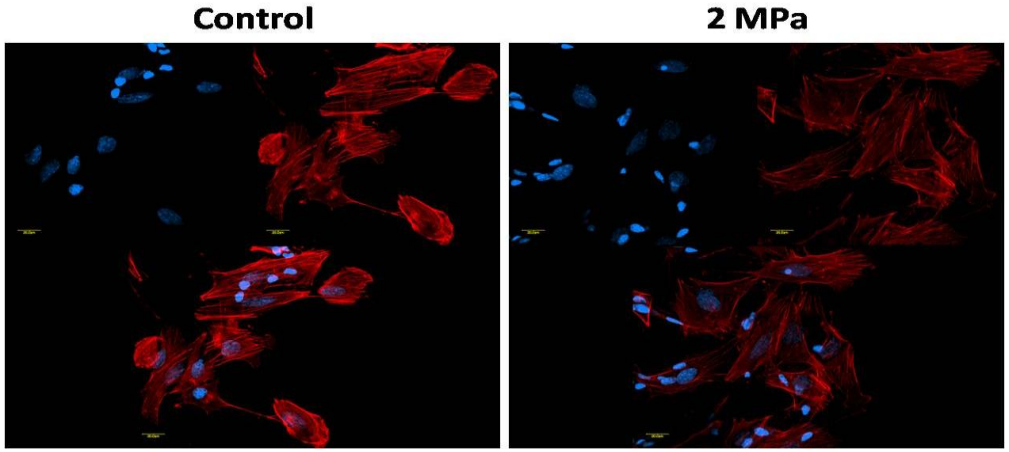
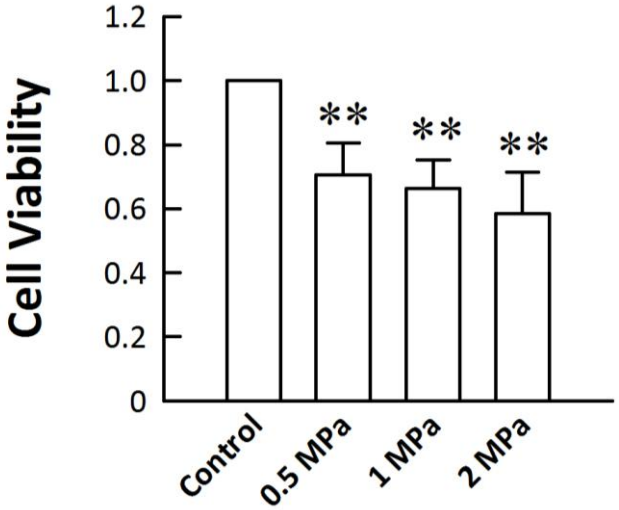
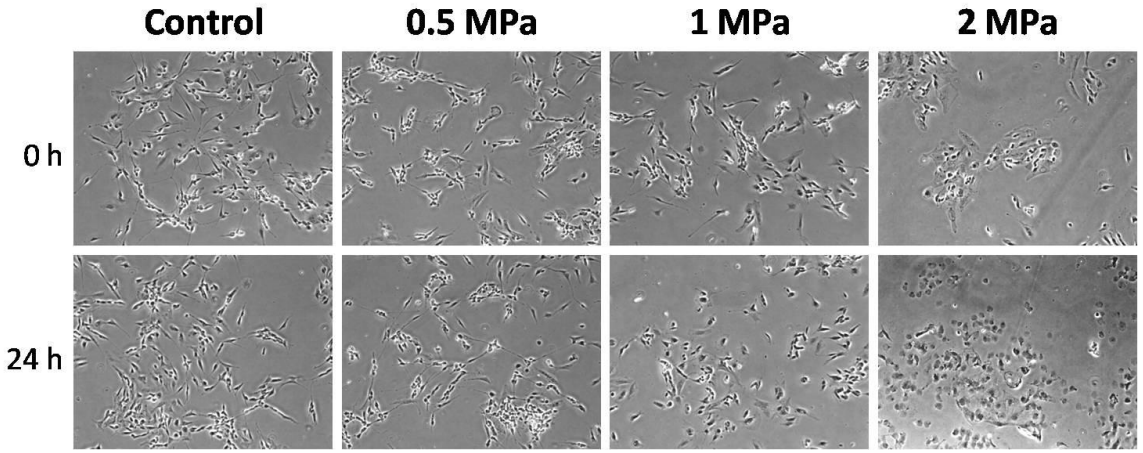


- Cells are inspected with actin staining. The damaged cells appear lighter red than the healthy cells. The nuclei show up as blue.
- A microscope inspection shows long-term cell death.
- MTT assays are used to determine the vitality of cells. Active mitochondria will process the chemicals to a wavelength that can be detected by a spectrophotometer.





SUMMARY

- A new Kolsky compression technique for impulsively pressurizing neuronal brain cells has been developed.
- In-vitro cell containment vessel for impulsive pressurize of brain cells has been designed and validated.
- With this new device, a single compression pulse with pulse duration over 1ms has been achieved.
- Sample stresses as low as 0.5 MPa can be accurately measured and repeated.

Future Work

- Continue experiments, especially in the lower pressure ranges
- Adapt experiment for testing blast wave mitigation technology

Thank You

Questions?