

Investigation of mechanical impact effects on biological cells with scanned image microscopy

B. R. Tittmann,^{*} S. Jayaraman, C. Miyasaka, J. Welsch, W.C. Hymer, N. Nicholas
The Pennsylvania State University, University Park, PA, USA 16802

ABSTRACT

The objective of this study is to observe the behavior of the living cells after introducing impact, caused by an aluminum bullet shot out from an air gun, onto them via tungsten/polymer plate and culture liquid. An air gun type of apparatus shoots an aluminum bullet, wherein the shape of the bullet is substantially a sphere (diameter: 5 mm), and wherein the velocity of the bullet is controlled by the amount of air used for shooting. The aluminum bullet shot out from the air gun impacts onto the polymer/tungsten plate, located above the living cells grown on the bottom of the container (i.e., thin semi-transparent polymer membrane), which is located on the surface of a 200 kHz Panametrics transducer. The container is supported by a polymer member to prevent movement from shock caused by the bullet impact. The plate generates an acoustic wave (i.e., shock wave) by the mechanical impact (i.e., bullet impact) which is then converted into an electrical signal by the transducer. The amplitude of the electrical signal is measured and monitored by the digital oscilloscope. The transducer is calibrated by hydrophone with its peripheral equipment including computer software. The output voltage from transducer was monitored by the digital oscilloscope. The injury and recovery of the specimen are evaluated by scanned image microscopes. Furthermore, quantitative data showing the injury and recovery of the specimen can be obtained with the electromagnetic measurement.

Keywords: Scanned image microscopy, MDCK cells, blunt impact, confocal laser microscopy, air-gun pellet impact

1. INTRODUCTION

Human responses to blunt impact relate to a variety of fields ranging from sports injuries to biological cellular injuries. Tests were conducted on MDCK cells. The cells were grown, and maintained by giving proper “food” (e.g., chemically defined cell culturing media containing a complex mixture of vitamins, sugars, serum proteins and the like). The cells were also grown on appropriate substrates under the controlled conditions of pH and gas (i.e., mixture of O₂ (95%) and CO₂ (5%)). These preparations made it possible to compare the responses caused by the different factors in the following experiments.

An air-gun based system that shoots an aluminum pellet was used to simulate the blunt impact. A Panametrics 200 kHz Videoscan transducer was used to measure the force generated by the pellet impact. Hydrophone experiments were performed to calibrate the electrical output of the transducer to physical force. Impacted MDCK cell specimens were then “fixed” and imaged using a confocal laser microscope. It was observed that the cell layer thickness initially increased in height (60 minutes), after the blunt impact and decreased later in time (90 minutes). For a typical specimen, the measured height after fixing (30 minutes) was 7 μm which then increased to 9.875 μm after 60 minutes and then decreased to 4.875 μm, after 90 minutes.

2. EXPERIMENTAL PROCEDURE

An air gun (Figure 1) was used to shoot a substantially spherical (diameter – 5 mm) aluminum pellet, wherein the velocity of the bullet is controlled by the amount of air used for shooting. The aluminum bullet impacts the polymer/tungsten plate, located above the living cells grown on the bottom of the container (i.e., thin semi-transparent polymer membrane), which is located on the surface of a 200 kHz Panametrics transducer, which converts the shock wave into an electrical signal. The container was supported by a Plexiglas member to prevent movement from shock caused by the bullet impact. The peak-to-peak amplitude of the electrical signal was measured and monitored by a Tektronix digital oscilloscope. (Figure 2)

^{*}brt4@psu.edu; Phone 1 814 8652737 Fax 1 814 8653626



Figure 1. Air-gun based blunt impact system

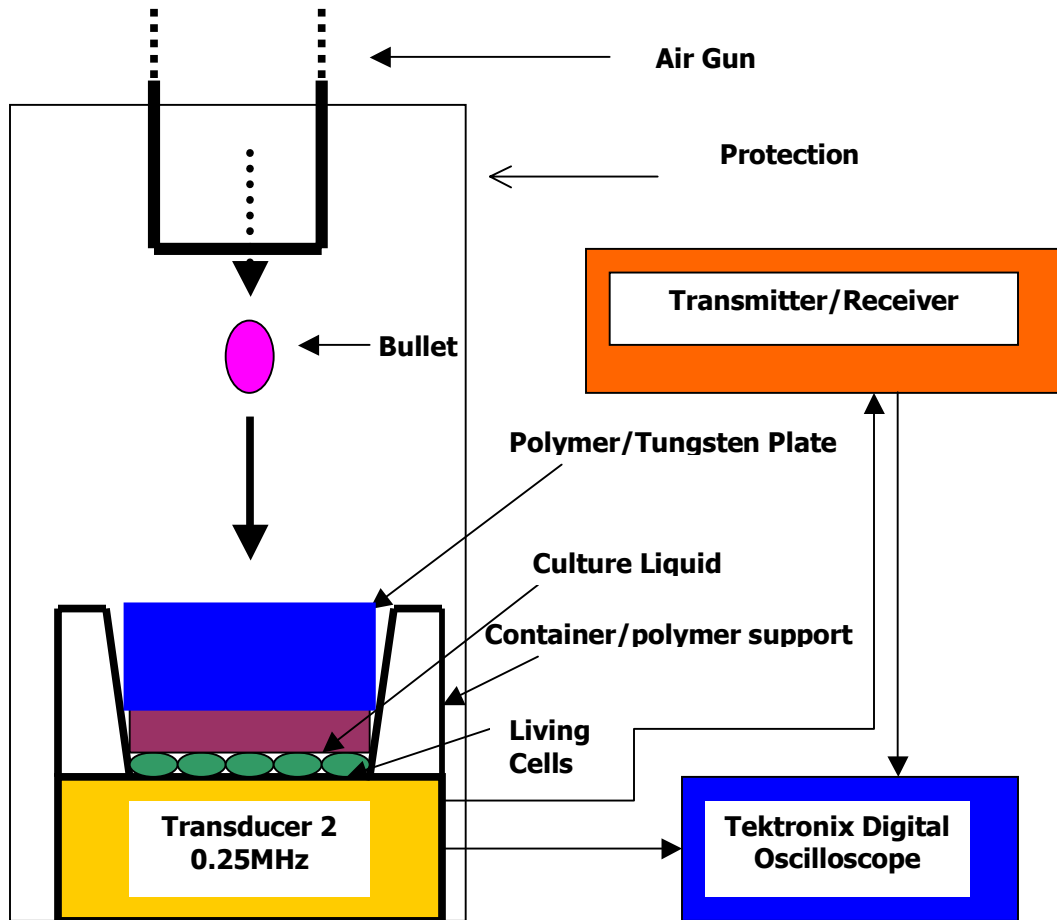


Figure 2. Schematic of the blunt impact system

The Air-gun pellet impacted specimens were then stained to be observed under a Confocal Laser Microscope. After impact and removal of the media, the specimens were rinsed with Phosphate Buffered Saline (PBS). 4% Para formaldehyde (in PBS) was added to fix the cells at room temperature. Cells were then washed 3 times for 5 minutes with PBS. Then, 0.1% Triton X-100 (in PBS) was added and 1% Bovine Serum Albumin (BSA) (in PBS) was added. To this, phalloidin at a 1:50 dilution and SYBR Green at a 1:5000 dilution in 1% BSA was mixed. The whole mixture was then incubated with Antibody/BSA solution for 20 minutes. Finally, the fixed cells were washed 3 times for 5 minutes with PBS.

2.1 Transducer calibration

The center frequency of the ultrasonic wave is substantially 200 kHz. Using the calibration curve (Figure 3), the free-field voltage sensitivity is 203dB at 200 kHz. As the result of the hydrophone calibration, we obtain the Equations 1 and 2.

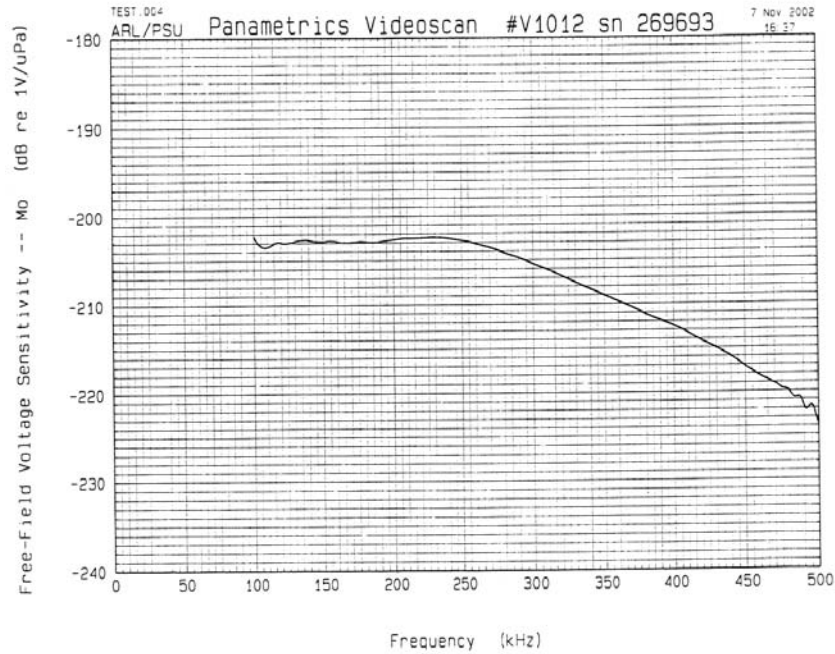


Figure 3. Calibration curve for the Panametrics transducer obtained using the hydrophone experiments

$$203 = 20 \log_{10} \frac{V}{V_0}$$

$$\therefore \frac{203}{20} = \log_{10} \frac{V}{V_0} \quad (1)$$

$$\therefore 10.15 = \log_{10} \frac{V}{V_0}$$

$$1V_0 = 1\mu Pa \quad (2)$$

Pressure (P) is expressed in the following Equation. (3)

$$P = V \times 10^{\frac{203}{20}} \mu Pa = (10^{4.15}) V Pa \cong (1.413 \times 10^4) V Pa \quad (3)$$

Area of the tungsten plate is $25\pi \text{ mm}^2$. Therefore,

$$F = (1.413 \times 10^4 \times 25\pi \times 10^{-6}) N/V \cong 1.1 N/V \quad (4)$$

2.2 Impact force estimation

It was calculated that for every Volt of output by the transducer, the corresponding force felt by the cell layer is 1.1 N. The amount of force generated by the air-gun could be controlled by the volume of air intake. During these experiments, three levels of air volume were used. Another method of varying the force was to use different distances the bullet had to travel before it impacted the specimen. This was accomplished using two Plexiglas tubes of different lengths. Still another way of varying the force was to use damping layers of foams. These were simple foams that are used for packaging material for shipping. All these different techniques enabled us to generate a wide range of impact force. Table 1 shows the air volume (in terms of number of pumps of the air-gun) and the electrical voltage output generated, and hence the mechanical force.

Table 1. Air volume and the corresponding force output, with and without the cell layers

Number of Pumps	Peak-to-peak Voltage (V)		Force (N)		Difference in voltages (V)
	Without cells	With cells	Without cells	With cells	
1	18.2	17.8	20.0	19.6	0.4
2	42.0	35.9	46.2	49.5	6.7
3	59.6	42.3	65.6	46.5	19.1

Figure 4 shows the electrical waveform obtained due to the impact. This particular one shows the waveform from a trial without the cell layers. The shapes of the waveforms look similar, irrespective of the presence of the cell layers.

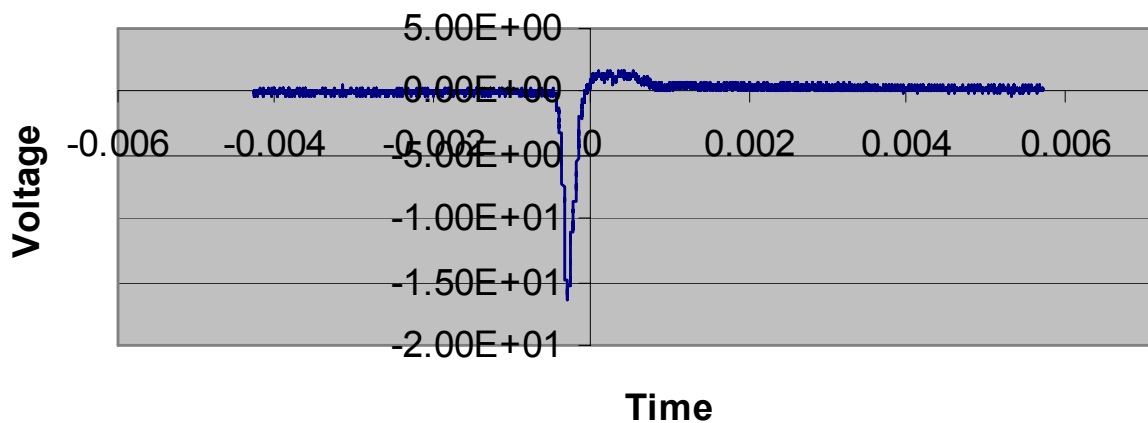


Figure 4. Waveform of the shock wave generated (1 pump)

3. RESULTS

The amount of force with which the Aluminum pellet impacts the cell layers could be controlled in three ways: a) Amount of air intake, b) Length of Plexiglas tube (the two variants will be called, short and long), and c) Use of damping material such as foam. The mean output voltage obtained using the long tube is: 1 pump -12.0 V; 2 pumps – 36.6 V and 3 pumps – 50.4 V. There is an average decrease of about 6.0 V (and hence 6.6 N of force) when the length of the tube is changed. Figure 5 shows waveform obtained while using the long tube, no cells and one pump air volume.

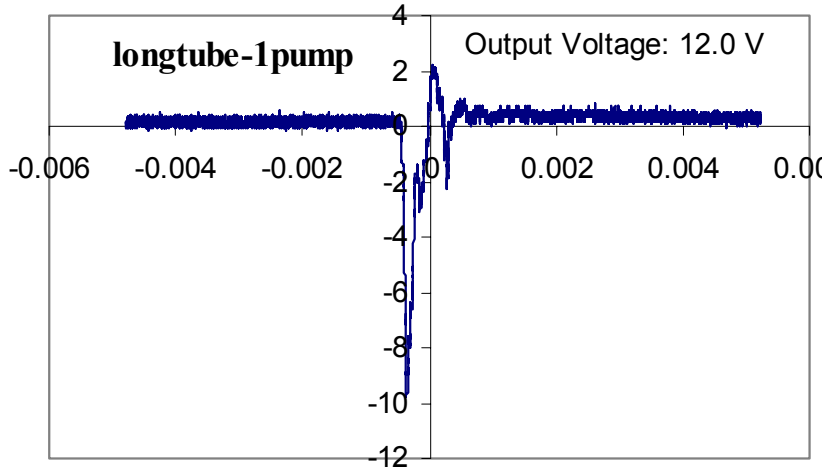


Figure 5. Output waveform using a long tube without cells, one pump of air

Figure 6 shows the waveforms obtained when impacting the cell layers. The arrangement contained the damping material as well. Figure 6(a) shows the waveform while using the short tube and figure 6(b) for long tube. In both cases, only one pump air volume was used. It can be observed that the foam material acted as an effective damping mechanism. In the setup using the short tube, the reduction in amplitude is about 7.0 V (from 17.8 V to 10.8 V). That is a reduction of approximately 8 N of force on average. In case of the long tube, it is about 3 N. Therefore, length of Plexiglas tube, air volume and damping material play vital roles in obtaining wide range of force from the air-gun setup.

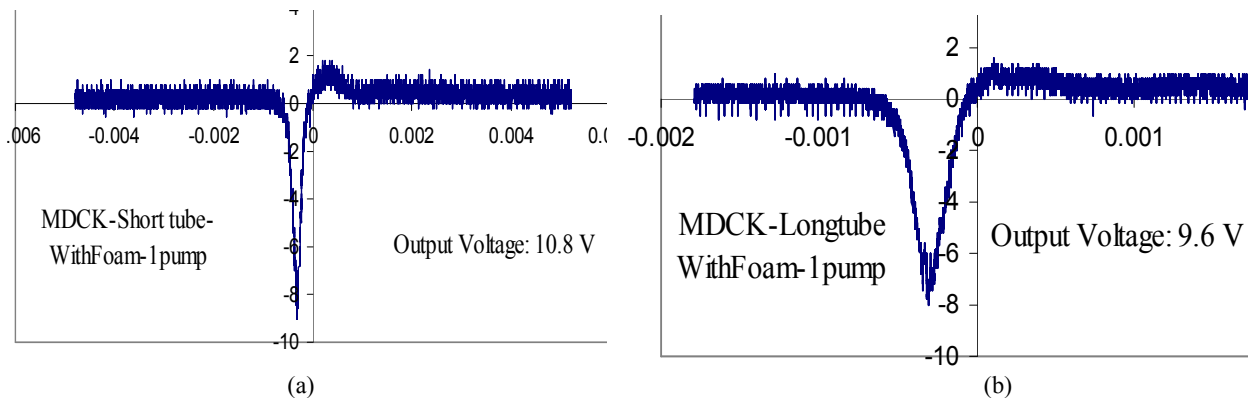


Figure 6. Waveforms (Amplitude vs. Time) of trials with cells and damping material (foam) using (a) Short tube and (b) Long tube

The MDCK cells were imaged after impact using the confocal laser microscope. The cells were fixed and stained after impact. This process takes about 30 minutes. It was observed that the cell layer thickness initially increased in height (60 minutes), after the blunt impact and decreased later in time (90 minutes). For a typical specimen, the measured height after fixing (30 minutes) was 7 μm which then increased to 9.875 μm after 60 minutes and then

decreased to 4.875 μm , after 90 minutes. Figure 7(a) shows the confocal laser image of a sample that was not impacted. Figures 7(b) and 7(c) show images of impacted cell layers after 60 and 90 minutes respectively.

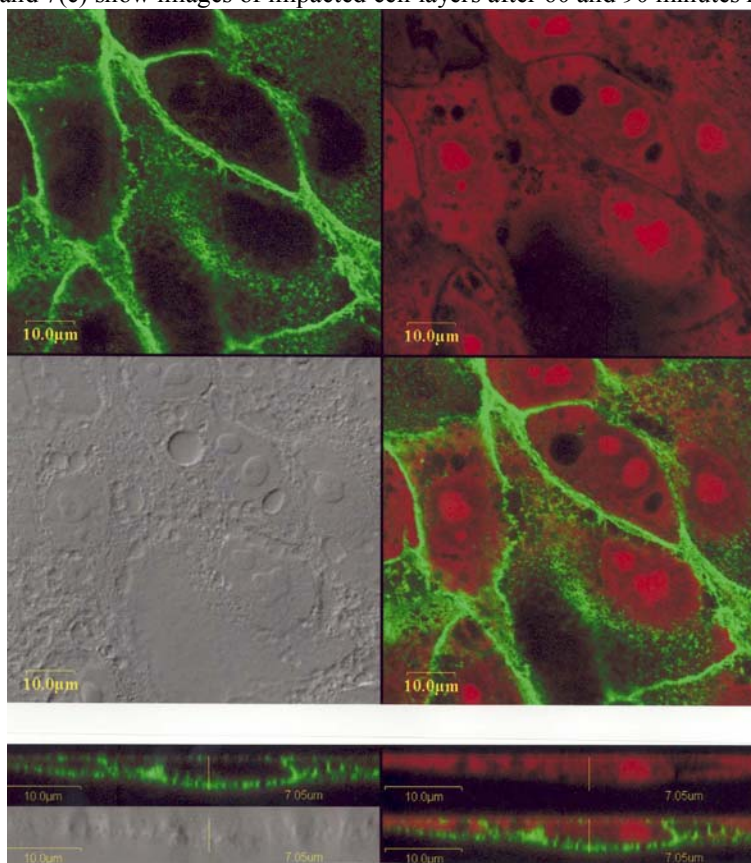


Figure 7(a). Confocal laser microscope image of MDCK cells prior to blunt impact

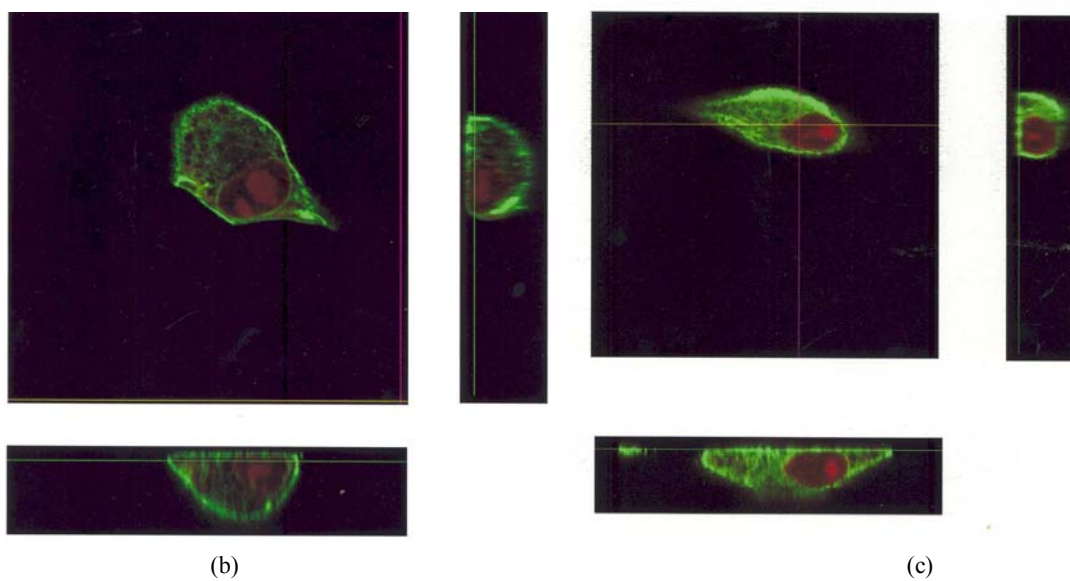


Figure 7 (b) Images of the MDCK cells after the blunt impact showing early swelling and 7(c) shrinking in height later

4. CONCLUSIONS

The system provides a real blunt impact onto the living cells, and generates useful data on the behavior (i.e., injury and recovery) of the living cells after the impact. The study has helped in devising methods of generate a wide range of blunt impact force using variety of techniques such as differing the length of Plexiglas tube support, using damping material such as foam and as well as the volume of air used by the air-gun. It was seen using the confocal laser microscope images that the MDCK cells initially swell in size after the blunt impact but shrink with time.

One of the problems was that confocal images could not be obtained immediately after the impact. Since the staining procedure took close to 30 minutes, probably a lot of vital information on the immediate response of the cells, to the blunt impact is lost. A better way of observing the cells that can capture the immediate response would be another step in the right direction towards understanding he blunt impact response of the cells. Efforts are also underway to construct a model that could simulate the behavior of the cell junctions under blunt impact.

REFERENCES

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