An integrated instrument for rapidly deforming living cells using rapid pressure pulses and simultaneously monitoring applied strain in near real time

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Because many types of living cells are sensitive to applied strain, different *in vitro* models have been designed to elucidate the cellular and subcellular processes that respond to mechanical deformation at both the cell and tissue level. Our focus was to improve upon an already established strain system to make it capable of independently monitoring the deflection and applied pressure delivered to specific wells of a commercially available, deformable multiwell culture plate. To accomplish this, we devised a custom frame that was capable of mounting deformable 6 or 24 well plates, a pressurization system that could load wells within the plates, and a camera-based imaging system which was capable of capturing strain responses at a sufficiently high frame rate. The system used a user defined program constructed in Labview[®] to trigger plate pressurization while simultaneously allowing the deflection of the silicone elastomeric plate bottoms to be imaged in near real time. With this system, up to six wells could be pulsed simultaneously using compressed air or nitrogen. Digital image capture allowed near-real time monitoring of applied strain, strain rate, and the cell loading profiles. Although our ultimate goal is to determine how different strain rates applied to neurons modulates their intrinsic biochemical cascades, the same platform technology could be readily applied to other systems. Combining commercially available, deformable multiwell plates with a simple instrument having the monitoring capabilities described here should permit near real time calculations of stretch-induced membrane strain in multiple wells in real time for a wide variety of applications, including high throughput drug screening. © 2010 American Institute of Physics. [doi:10.1063/1.3520135]

I. INTRODUCTION

The response of living cells to mechanical forces has been studied in detail, and many types of mechanosensitive cells have been identified. Hypotheses based on these studies can account for changes in cellular signaling triggered by diverse modalities of mechanical stimuli.^{1,2} Mechanical deformation and the corresponding cellular responses elicited by deformation vary depending upon the type of cell being loaded. For instance, the mechanical compression of primary chondrocytes affects ATP release,³ ion channel formation,⁴ activates transcription factors such as NF κ B⁵ and can activate kinases such as MAP kinase,⁶ focal adhesion kinase,⁷ and ERK,⁸ as well as Interleukin-1 signaling.⁹ More long term, mechanical loading can also alter the expression of collagen^{10–16} and enzymes^{7,17} involved in tissue remodeling. Typical frequencies encountered in cell loading in these cases are on the order of 1–10 Hz.¹⁴ In bone, mechanical strain influences osteoblast responses through other biochemical signaling cascades,^{18–22} which include signaling through the Wnt signaling mode,^{23–27} and activation of ERK^{8,21} and FAK.⁷ In

connective tissue, it is known that tendons and ligaments are susceptible to mechanical influences^{9,28–30} and similar cellular cascades to those listed above are triggered by the mechanical loading in these tissues. Our focus here was to improve and extend the capability of a simple *in vitro* model of traumatic brain injury where a controlled pulse of compressed gas is used to reproducibly deform neurons and astrocytes cocultured on an elastomeric membrane.^{31,32} To study the responses of cells such as these in a controlled and reproducible manner, a well-regulated and robust loading mechanism is required, combined with a way to accurately measure the displacement produced by the applied load. Different strategies used to date for applying such strain to living cells have been based on applied pressure,^{29,30,33} evacuation, the controlled displacement of cams,³⁴ or vibration,³⁵ and these perturbations have typically been applied to cells maintained in tissue culture. While these approaches have been productive, they suffer from certain imperfections. For instance, the cams used in compression-based models can lose contact with their specimen,³⁴ and while the incorporation of pressure sensors can rule out that leaks in pressure based systems, and allow applied pressure to be measured,³⁶ accurate assessment of the rate and magnitude of applied strain is not readily achievable with the systems that are currently available. While

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FIG. 1. (Color online) An overview of the apparatus, including the digital camera shown on the left for capturing images, and the well plate assembly and manifold system shown on the right. An example of a deformation captured using this system is visible on the monitor of the PC. The bar loading system used was clamped onto a 24 well plate and six Tygon[®] tubes were used to simultaneously pressurize six wells, as shown on the right hand of the figure.

systems have been designed that measure strain or deflection using depth gauges, a molding resin to replicate the bulging membranes,^{37,38} static imaging of pressurized disks³⁷ or optical tracking of cell strain by induced pressure,³⁹ these systems do not in general result in real or near real time data. In addition, it is also desirable, from a data analysis standpoint, to translate displacements measured during perturbation experiments into functional strains. While previous work has resolved strain using finite element analysis⁴⁰ and calculations that assume spherical deformation with loading,^{39,41} the degree of strain itself affects whether a spherical or an elliptical geometry is most appropriate to assume, as elliptical caps form at low pressures, and more spherical caps at higher pressure.³⁶

We discuss here the design, construction, and evaluation of an integrated strain device that uses pressure to displace the bottoms of deformable well plates upon which living cells are cultured, and where applied strain can in turn be optically determined. The design allows increased throughput, can be easily integrated with commercial plate readers after cell strain has been applied, and allows both strain and pressure to be measured dynamically as they are captured. We have investigated neuronal responses to mechanical strain as an *in vitro* model of traumatic brain injury using this system,⁴² but a similar approach can be applied to other cell systems, including those where lower loading rate conditions are needed.

II. MATERIALS AND METHODS

A. Physical structure of the device

A rigid frame (74.9 cm × 19.1 cm × 20.3 cm) was made from three 3.8 cm thick pieces of polymethylmethacry-

late stock (PMMA). Two aluminum angle brackets of length 57.8 cm and height and width of 5.1 cm were separated by 1.9 cm to create an optical path for a camera, and to create a stable mounting for the well plates. Slotted grooves facilitated proper positioning of the multiwell plate onto this frame, and four stops were placed in the slots to fix specific mounting locations for the well plate that were within the optical path of the camera, one for each row of 6 wells within the 24 well plate configuration. A threaded screw was placed between the pressure bar, which was sealed with a gasket, and a tapped screw hole in the mount so that the gasket tightly sealed each well separately once the well plate was inserted. Each multiwell plate was tested by loading one row of the well plate at a time, and then loosening the screw and sliding the well plate down one stop, allowing the gasket to seal the second row, and so on until all four rows of the 24 well plate were loaded.

The system was configured to accommodate two different loading scenarios. The loading system used initially consisted of a single plastic pressure plug connected to a compressed air or N₂ tank through a regulator, with a solenoid switch used to set the pulse period, as described previously.³⁷ A higher throughput loading unit was then made using the bar design described earlier as it allowed pulses of air or N₂ to be simultaneously applied to all six wells of a row. After the first row was pulsed, the plate was easily translated so that the next row of wells was aligned under the pressure bar, and this was then repeated. The period of the gas pulse used was set at 50 ms, and pressure ranged from 18 to 40 psi (0.12–0.28 MPa). For “proof of concept,” a commercially available, six well test plate specifically designed for deforming tissue-cultured cells (FlexCell International Corp,

FIG. 2. (Color online) Details of the pressurization system. The 24 well plate fits onto the stand and the pressure bar is then placed on top of the well plate where it is firmly affixed using the cross nuts. A rubber membrane placed onto the bottom of the pressure bar acts to seal the wells.

McKeysport, NC) was used. This plate closely resembled conventional six well plastic plates except that the bottom of each well was replaced with a precast, deformable silicone membrane.

The deflection of the silicone membrane upon which cells were cultured was captured orthogonal to the loading direction using a Basler A504K 12-bit digital camera (Advanced Digital Vision, Inc., Natick, MA) equipped with an Nikkor 60 mm f/2.8G ED lens mounted along a 57.8 cm optical track and attached by angle brackets. The camera was mounted to capture orthogonal deflections of the plate bottoms at 250 frames per second, and with a resolution of 1280×1024 . An external light source (Advanced Illumination

AL116) ensured that there was sufficient illumination for photography.

The overall system was controlled using Labview[®] software (National Instruments, Austin, TX), combined with a high performance camera link (PCIe-1429), and an M-Series data acquisition board (PCI 6221; National Instruments). The camera link allowed fast integration of orthogonal images at a capture frequency of 200 Hz. Camera operation was controlled in LabView[®], and frames capturing membrane deflections were saved as AVI movie files that could, in turn, be viewed offline to calculate deflection rates. A picture of the functioning unit is shown in Figs. 1 and 2. The camera/lens system is on the left, while the multiwell plate, pressure manifold and mounting bracket are on the right. A commercial PC (Dell Computer Corp.) equipped with an appropriate PCI interface and A/D boards is also shown.

III. RESULTS

Using our improved system, we could set the duration and then trigger pulses of air or N₂ applied to the membranes of the multiwell plates while simultaneously measuring membrane deformation with our imaging system. As shown in Figs. 3 and 4, the plate bottoms were deformed with time, where the latter depicts this schematically. Maximal membrane deflection (in mm) was measured orthogonal to the direction of the applied stretch and was found to vary linearly with the degree of pressure exerted, as shown in Fig. 5. In theory, other factors besides applied pressure can affect elastomer deformation, including the thickness and modulus of elasticity of material. Imaging revealed that the elastomeric membrane formed an ellipse having the diameter of the well plate when pressurized. It is likely that the ellipse-like shape resulted from the varying radius of curvature of the membrane after pressure was applied. The perimeter of the ellipse that formed (p) under load is related to the lengths of the major and minor axes as calculated by the Ramanujan approximation (Eq. (1)) (Ref. 43) as:

$$p \approx \pi * [3(a + b) - \sqrt{(3a + b)(3b + a)}], \quad (1)$$

where a is the half of the minor axis in the direction of applied pressure, and b is the half of the major axis orthogonal to the loading direction, which in this case is the diameter of the inner lip of the well plate, or 9.5 mm.⁴³ One half of the

FIG. 3. (Color online) Three expanded video frames showing the membrane deformation of a single well captured while being pressurized. Corresponded to (a) $t = 0.172$ s, (b) $t = 0.184$ s, and (c) $t = 0.212$ s.

FIG. 4. Schematic representation of a membrane deflection observed in individual wells following typical loading conditions.

calculated perimeter, p , corresponds to the arc length representing the length of the stretched membrane. Strain is then determined simply as the length of the dependent arc (using Ramanujan's approximation), relative to the diameter of the well plate observed before stretching. An example of the dynamic deflection (in mm) observed in an elastomeric membrane that was pressurized by 0.18 MPa (28 psi) is shown in Fig. 6. As can be seen in this figure, the monitored deflection reached its maximum value within 50 ms. The rates of deflection and recovery were asymmetric, as the development of the deflection phase was more sigmoidal in character, while the trajectory of recovery appeared to be more linear over time. The corresponding strains associated with these dynamic deflections, as determined from the Ramanujan approximation for the arc of an ellipse,⁴³ are shown in Fig. 7 for a single pressure pulse. The total strains determined in this manner were similar to those reported previously by Geddes *et al.*³¹ A total of seven tests were completed using different levels of pressure. Table I tabulates the maximum pressures and the displacements achieved during each of these tests.

Note that strain that was delivered by our instrument was not a classical square wave, but a continuous function that reached a maximum over a short time compared to the

FIG. 5. (Color online) Maximum membrane deflections produced during the first 50 ms of standard applied pressure pulses. Increasing the applied pressure increased the average amplitudes of the measured deflections.

FIG. 6. A time dependent membrane deflection measured using our system in response to a 0.18 MPa pulse.

total cycle of loading and unloading. We noted that the dynamic strain curve obtained was more symmetrical in character. The values we obtained for the calculated strain rates were about 7 s^{-1} on loading and near 9 s^{-1} on unloading; these are in the same general range as the 10 s^{-1} rate cited by Geddes *et al.*³¹

We also examined the behavior of the system when multiple pulses were applied in succession to the silicone membrane. Responses to these multiple pulses are shown in Fig. 8. It can be seen from the figure that the loading and unloading profile had a sawtooth-like character that increased linearly with each successive pulse and resulted in somewhat larger deflections over time despite the same final pressure being applied during each pulse. The compliance of the silicone membrane can be seen to increase slightly with each pulse. The increased compliance we observed was not due to pressure

FIG. 7. The profile of time dependent strain resulting from an applied pressure of 0.18 MPa.

TABLE I. Calculated perimeters and percent strain in FlexCell six well plate.

Test number	Pulse number	Deflection (mm)	Pressure (psi)	a (mm)	b (mm)	p (mm)	$p/2$ (mm)	Strain (%)
1	1	7.5	28.0	15.1	19.0	107.4	53.7	0.41
3	1	9.9	40.0	19.8	19.0	122.0	61.0	0.61
	2	10.7	40.0	21.4	19.0	127.2	63.6	0.67
	3	10.7	40.0	21.4	19.0	127.2	63.6	0.67
	4	11.5	40.0	23.0	19.0	132.3	66.2	0.74
4	1	11.9	45.0	23.8	19.0	134.9	67.5	0.78
	2	12.3	45.0	24.6	19.0	137.5	68.8	0.81
	3	11.9	45.0	23.8	19.0	134.9	67.5	0.78
5	1	6.4	20.0	12.7	19.0	100.6	50.3	0.32
	2	6.8	20.0	13.5	19.0	102.8	51.4	0.35
	3	6.4	20.0	12.7	19.0	100.6	50.3	0.32
6	1	7.3	25.0	14.7	19.0	106.2	53.1	0.40
	2	7.5	25.0	15.1	19.0	107.4	53.7	0.41
	3	7.1	25.0	14.3	19.0	105.1	52.5	0.38
	4	7.5	25.0	15.1	19.0	107.4	53.7	0.41
7	1	12.7	45.0	25.4	19.0	140.2	70.1	0.85
	2	12.7	45.0	25.4	19.0	140.2	70.1	0.85
	3	12.7	45.0	25.4	19.0	140.2	70.1	0.85

leaks during loading, but likely reflected the viscoelasticity of the elastomeric membrane. When multiple pulses were employed, the degree of strain, and the corresponding strain rate were thus dynamical functions. We observed increased deflection amplitude with subsequent pulsing in all samples that were subjected to repetitive strains. This result could be significant, as multiple pressure pulses of this type have been applied to cultured neurons to model repetitive brain injuries.⁴⁴ Any “synergism” ascribed to the use of multiple pulses to damage neurons, therefore, may well need to take into account the technical irregularities of the system to ensure that data are interpreted properly.

FIG. 8. Multiple deflections produced in response to a series of 40 psi (0.28 MPa) pressure pulses. The data show that measured deflections can be well captured by the optical system but exhibit some irregularities. The line shown is intended as a visual aid (see text).

IV. SUMMARY AND CONCLUSIONS

The goal of our work here was to extend an existing model system by incorporating computer control, adapting the system to allow more than one well to be stretched at a time, and providing simultaneous, dynamical image capture in order to determine convex deflections of the membrane in the form of an arc or an ellipse when observed orthogonally in real time. The approach here used the Ramanujan approximation for an eccentric ellipse to determine arc lengths related to strain and strain rate from captured images of membrane deflection. If strain and strain rate are determining factors for cellular function and viability, our approach allows these variables to be more directly controlled and measured. While the Ramanujan approximation is different than other analyses, those based on membrane mechanics and strain energy release assume a bulge geometry that may not always be appropriate to the system being studied. For example, there are regimes of strain that are better modeled using an ellipse as opposed to a sphere.³⁶

When elastomeric membranes were subjected to repeated pressurizations using only a short (0.2 ms) rest period, noticeably higher compliance was observed. We are not aware of any earlier work detailing irregularities of this type in stretch models of injury. Comparing the strains and strain rates we measured here to earlier deformation studies confirm that the rates we obtained correspond well to those shown to induce cellular damage in neurons and other tissues.

In conclusion, we have developed a novel, computer controlled platform based on an existing (and widely-used) stretch model for injuring cultured neurons in culture, which can now: (1) apply stretch to larger numbers of experimental wells (the standard model could only use commercial six well plates); (2) includes dynamic imaging to simultaneously monitor membrane deflection in up to 6 wells at

a time, and (3) allows digital calculation of both applied strain and strain rates. We propose that this system is well suited for development to versions that may allow much higher throughput for drug screening applications (e.g., use of 96 well plates), as well and can be readily adapted to the study of many other types of mechanically sensitive cells and tissues.

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