Biophysical Journal
Article

Biophysical Society

Response of Single Cells to Shock Waves and Numerically Optimized Waveforms for Cancer Therapy

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ABSTRACT Shock waves are used clinically for breaking kidney stones and treating musculoskeletal indications. The mechanisms by which shock waves interact with tissue are still not well understood. Here, ultra-high-speed imaging was used to visualize the deformation of individual cells embedded in a tissue-mimicking phantom when subject to shock-wave exposure from a clinical source. Three kidney epithelial cell lines were considered to represent normal healthy (human renal epithelial), cancer (CAKI-2), and virus-transformed (HK-2) cells. The experimental results showed that during the compressive phase of the shock waves, there was a small (<2%) decrease in the projected cell area, but during the tensile phase, there was a relatively large (~10%) increase in the projected cell area. The experimental observations were captured by a numerical model with a constitutive material framework consisting of an equation of state for the volumetric response and hyper-viscoelasticity for the deviatoric response. To model the volumetric cell response, it was necessary to change from a higher bulk modulus during the compression to a lower bulk modulus during the tensile shock loading. It was discovered that cancer cells showed a smaller deformation but faster response to the shock-wave tensile phase compared to their noncancerous counterparts. Cell viability experiments, however, showed that cancer cells suffered more damage than other cell types. These data suggest that the cell response to shock waves is specific to the type of cell and waveforms that could be tailored to an application. For example, the model predicts that a shock wave with a tensile stress of 4.59 MPa would increase cell membrane permeability for cancer cells with minimal impact on normal cells.

INTRODUCTION

A shock wave is a type of acoustic wave characterized by the presence of a rapid-pressure jump governed by the interaction of nonlinear effects that steepen the waveform and attenuation mechanisms that smooth the waveform (1). Shock waves have been medically used for decades in a procedure called lithotripsy, in which shock waves fragment kidney stones. Although lithotripsy is a mature technology, there are concerns about bioeffects, including renal hemorrhage and scarring with a permanent loss of functional renal volume (2,3). Although damage is predominantly thought to be induced by cavitation (4,5) even in environments where cavitation is minimized, damage has been reported in cells (6) and tissues (7), suggesting a direct impact of shock waves on cells.

Shock waves have also been employed for orthotripsy, which is the treatment of musculoskeletal disorders, such as plantar fasciitis, tendon pain, and nonunions or delayed unions of long-bone fractures (8). The mechanism by which shock waves have an effect on musculoskeletal conditions is not understood. One of the hypotheses is that the disruption of the tissue by shock waves results in "microtrauma," which then induces neovascularization that is believed to improve blood supply and tissue regeneration. The increased permeability of the vessel wall may also promote the healing process (8).

Cancer therapy is another field in which shock waves have been investigated (9–11). It has been reported that besides mechanically rupturing cells, shock waves may enhance the sonoporation effect that temporarily increases the membrane permeability to allow molecules in the surrounding medium to diffuse into cells (9). This provides a mechanism for shock-wave-mediated therapeutic drug delivery and gene transfer. Furthermore, some experimental results have shown a positive influence of shock waves on suppressing tumor growth and selectively killing malignant cells (10,11). The mechanisms by which shock waves affect cancer cells are not well understood.

Submitted February 27, 2017, and accepted for publication September 28, 2017.

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Editor: Philip LeDuc.
https://doi.org/10.1016/j.bpj.2017.09.042

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All of these applications motivate the need for a better understanding of the interaction between shock waves and cells. The goal of this work is to develop a numerical model for the response of a single cell to shock waves that is calibrated and validated against ultra-high-speed imaging of single-cell deformation under the action of shock waves. The differences in cell response to shock waves due to cell type is also examined. The numerical model employs a three-dimensional (3D) continuum model of an individual cell modeled with a combined equation of state (EoS) and hyper-viscoelastic material framework. The validated numerical model was then used to analyze the development of the stress and strain fields under the compressive and tensile phases of the shock wave, from which insights into the mechanisms of cell destruction and sonoporation were obtained. Two shock-wave profiles are proposed to specifically target cancer cells for enhanced sonoporation or rupture while minimizing impact on normal healthy cells.

MATERIALS AND METHODS

The experimental rig consisted of a shock-wave source coupled to a tissue-mimicking gel in which cells were embedded. The gel contained cell media to maintain cell viability. Three kidney epithelial cell lines representing cancer cells, normal healthy cells, and virus-transformed cells were studied. An ultra-high-speed camera (SIMX 16; Specialised Imaging, Tring, United Kingdom) with a 20× objective (UMPLFLN20XW; Olympus, Tokyo, Japan) was used to image individual cells. Before the cell experiments, the camera and shock source were coaligned with a needle hydrophone.

For each experiment, the camera was focused onto a cell in the focal region of the shock waves. A reference image was taken before the delivery of shock waves. A shock wave was delivered, and the camera was triggered to capture 16 frames at a rate of 3.3 Mfps (interframe time of 300 ns with an exposure time of 200 ns). Each cell was imaged with three different shock-wave energy settings. The imaging experiment was repeated on eight different target cells for each of the three cell lines investigated. Further details of the experimental system are described in the Supporting Material.

The high-speed images were filtered and then processed to extract the boundary of the cells from the images using methods described in the Supporting Material. The deformation of the cell was determined by comparing the cell boundary during shock-wave passage to the cell boundary in the reference image; we note that a separate reference image was used for each camera channel to avoid channel-to-channel differences. Quantitative analysis of the perimeter and area change was performed based on the extracted cell boundaries.

Shock-wave pressure profiles

Fig. 1 shows pressure waveforms measured at the focus of a clinical shock-wave source (Minilith SL1-0G; STORZ, Tuttlingen, Germany) at three different energy settings (levels 4, 6, and 8) using a bespoke fiber-optic probe hydrophone embedded in a tissue-mimicking phantom (12). In each case, the shock wave consists of a compressive phase (duration around 1.5 μs) followed by a tensile phase (duration around 2.1 μs). As the energy level increased, three effects were observed: an increase in the peak positive pressure, a decrease in the shock-rise time (time duration for the shock front pressure to rise from 10 to 90% of the maximal shock pressure), and a gradual increase in the peak negative pressure. These are characteristic behaviors of a focused nonlinear acoustic wave (13).

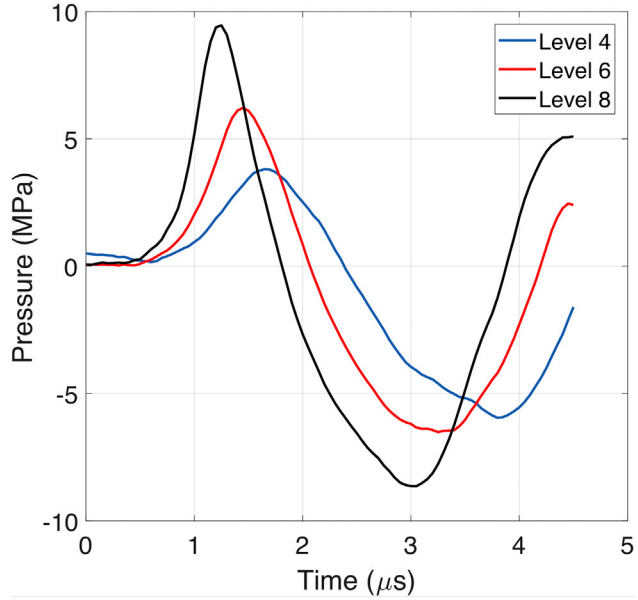

FIGURE 1 Measured focal shock waves in a tissue-mimicking phantom for source energy level 4 (*blue*), level 6 (*red*), and level 8 (*black*). To see this figure in color, go online.

Single-cell deformation under a shock wave

Fig. 2, *a–c* show representative images of an individual healthy human kidney (human renal epithelial (HRE); Lonza, Basel, Switzerland) cell during the compressive phase of a shock wave at energy level 8. The cell boundary was extracted, as described in the Supporting Material, and it can be seen that the cell is translated and the contour is slightly compressed in this phase of the shock wave. Fig. 2, *d–f* show the cell during the tensile phase, and it can be seen that the boundary has expanded and also become more diffuse in the image. As described in the Supporting Material, the effects of variability in the imaging, segmentation, and acousto-optic interactions were analyzed and found not to mask the cell deformation under shock waves.

The projected cell area inside the cell boundary was calculated for every image, and Fig. 3, *a*, *d*, and *g* show the relative area change-time curve (see Eq. 10 in the Supporting Material) for HRE cells for the three shock wave energy settings. It can be seen that the cells initially undergo a small compression (<2% area decrease) followed by a large expansion that increases with the increase of shock-wave energy levels (up to 13% area increase at shock-wave energy level 8). The timing of cell deformation was found to be consistent with the compressive phase and tensile phase of the shock wave; however, the sixfold increase in cell area changes between tension and compression was not consistent with the fact that the magnitude of the tensile stress was comparable to that of compression with similar loading rates. These data suggest that the cells are stiffer during compression than under tension.

The experiments were repeated with virus-transformed immortalized kidney cells (HK-2; ATCC, Manassas, Virginia) (Fig. 3, *b*, *e*, and *h*) and kidney cancer cells (CAKI-2; ATCC, Manassas, Virginia) (Fig. 3, *c*, *f*, and *i*). Both the cancer cells and immortalized cells exhibited the same qualitative behavior as that of the healthy cells (a small response to the compressive phase and a large response to the tensile phase of the shock wave). Further, the cell-area change increased with the shock-wave energy level setting, and the difference in the maximal area increase among the three cell types also became more distinguishable; see Supporting Material. At energy level 8, the maximal area increase was 13% in HRE cells (Fig. 3 *g*), 17% in the HK-2 cells (Fig. 3 *h*), and 9% in CAKI-2 cells (Fig. 3 *i*). The difference was statistically significant for HK-2 and CAKI-2 cells with a p-value of less than

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