**Results/Discussion**

Our *in vitro* results showed that increase of 32-47% , 46-126% ($p<0.05$) and 32-109% ($p<0.05$, except for 1 MHz ultrasound application) in corneal permeability for Tobramycin, Sodium Fluorescein, and Dexamethasone Sodium Phosphate, respectively, was achieved at different ultrasound-treatment parameters as compared to sham-treated samples. For *in vivo* study, increase in drug concentration in aqueous humor samples was 2.8 times ($p<0.05$) at frequency of 400 KHz and 2.4 times ($p<0.01$) using 600 KHz frequency, both at 0.8 W/cm$^2$ intensity and 5 min of ultrasound application, as compared to sham treated samples. Histologic analysis showed some structural changes that were limited to epithelial layers of cornea and were observed at all applied ultrasound parameter combinations.

In conclusion, ultrasound application provided enhancement of drug delivery, increasing the permeability of the cornea, and has a potential to provide effective and safe method for ocular drug delivery in treatment of eye infection and inflammation. Ultrasound appeared to be most effective at lower frequencies and higher intensities.

**IUS1-PA4-5**

## A Nano-Mechanical Study on the Influence of Ultrasound Exposure on Cellular Elasticity

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**Background, Motivation and Objective**

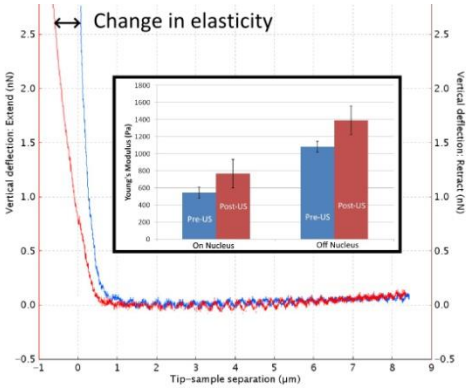
Ultrasound (US) has for some time been identified as a method of effecting change at the cellular level. Long established in the medical field as an indispensable imaging modality, currently however there is much promise for its [non-thermal] therapeutic potential, such as in targeted drug delivery where it can be exploited to mediate the transport of molecules into or across tissues and membranes. Of key importance for the therapeutic application of US is a safe dose threshold, particularly in light of recent publications highlighting the genotoxic potential of US via the induction of DNA double strand breaks through purely mechanical mechanisms (Y. Furusawa et al.). In a previous preliminary study we sought to examine the mechanical effects of US on live cells, where we observed marked increases in cellular elasticity post US exposure.

**Statement of Contribution/Methods**

Compelled by the results of our preliminary work, here we have undertaken live-cell investigations on the MCF-7 human breast cancer cell line, expanding on the experimental parameter space previously used in order to examine the dependency of mechanical induced effects with incident US power and exposure times. Sonication was performed using Sonidel KP-SS2Y 2mm transducer driven by a Nepagene KTAC-4000 and quantitative cell elasticity measurements were done pre and post US exposure using a JPK NanoWizard scanning force microscope (JPK Instruments, Berlin, Germany). Force-distance curves were recorded (Figure 1) using 10µm spherical indenter's and Young's modulus values were determined by using a Hertz model fit.

**Results/Discussion**

Correlating with our early data we observe marked increases in cellular elasticity both on and off nucleus with US exposure (see histogram figure 1). By varying US power and exposure times we note in some instances a divergence in the induced elasticity change between the on and off nucleus region, where, at low powers and shorter exposure times the off nucleus region exhibits a tendency to maintain an elasticity value close to its pre exposure value while the nucleus will exhibit a higher elasticity.




**IUS1-PA4-6**

## Ultrasound as a Repulsive Cue for Neuronal Development: Real-Time Morphological Observations In-Vitro

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**Background, Motivation and Objective**

A core event in neuronal development is the outgrowth of neurite branches from a neuron's cell body. It is known that, by presenting physical or chemical cues to the extracellular environment, neuronal development can potentially be modulated. In this work, we seek to investigate the use of ultrasound in modifying neuronal development behavior. We hypothesize that ultrasound, when applied at low intensity and pulsed settings, can possibly serve as a physical cue that operates like mechanical stimulation.

**Statement of Contribution/Methods**

Our study was carried out on a customized platform that allows real-time imaging of neuronal morphology. Pulsed ultrasound (1 MHz frequency, 20 or 100 cycle pulse lengths, 500 or 2500 Hz PRF) was applied through a waveguide at a 45 degree angle with respect to the microscope's field of view, which is aligned to the center of a 100mm polystyrene cell dish. Three different peak acoustic pressures (measured in-situ) were used: 0.13, 0.40, 0.84 MPa (correspond to SPTA intensities of up to 1.168 W/cm2 for our given pulsing parameters). In this work, N2a mouse neuroblastoma was used as the cell model; they were first induced to differentiate into neuronal-like cells using retinoic acid (for 24 h), after which they were seeded onto the cell dish for real-time morphological observations. Ultrasound exposure was applied over a 10 min period, and the resulting morphological behavior was recorded using a video camera (from start of exposure to 100 min after exposure). A subset of experiments was conducted in the presence of Gd3+ stretch-sensitive ion channel blockers or EGTA calcium ion chelators to examine the potential involvement of mechanotransduction.

**Results/Discussion**

Neurite retraction and cell body shrinkage were found over the exposure period (see figure). The effect was dependent on acoustic intensity: peak acoustic pressure was found to be a more important contributing factor than pulse duration. The morphological changes were found to be non-destructive; indeed, post-exposure neurite outgrowth and

neuritogenesis were respectively observed in neurite-bearing and neurite-less cells (see figure). The morphological changes were suppressed if stretch-activated ion channels were blocked or if calcium ions were chelated. Overall, our results show that ultrasound can potentially influence how neuronal cells develop.