Mechanism for stimulating cell shape changes with vibration, a treatment to reduce pain

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Introduction

In 1926, Drs. Bunting and Eades (Bunting and Eades, 1926) discovered that mechanical forces, particularly the direction that stiches are sewn to hold a cut together, could change the way that a cell moves. Since that time, the field of cell biology has been moving toward the idea that it doesn't always take a molecule to change the way a cell moves. It could be as simple as the natural forces around the cell (Gibson and Gibson, 2009). From a clinical point of view, application of force is often used to treat patients, such as pain and acute migraines (Juto and Hallin, 2014).

Here, we asked, why vibration works to treat pain? A literature search revealed few hints and most of them were directed to vibration changing cell shape (Ito et al., 2011). Last year, my lab predecessors ran a pilot project using a vortex to apply vibration to a variety of epithelial cells. They took picture of the cells and collected total proteins. They next used vertical electrophoresis to split the proteins according to their size. The results from these set of experiments showed that vibration caused the epithelial cells to round up and increase an 80kD protein that was later identified as calpain, an 80kD calcium-dependent, protease involved in cell mobility (Dourdin et al., 2001; Lebart and Benyamin, 2006; Potter et al., 1998).

We propose that calpain is intricately involved with the cell's change of shape, which would lead the cell programmed to act as a barrier (epithelial) to turn into a nomadic, fixing (mesenchymal) cell (Mogilner and Keren, 2009). **Our hypothesis is that vibration induces an epithelial-mesenchymal transition by increasing expression of calpain.**

Here, we designed a set of experiments using calpain inhibitor predicting that if calpain is inhibited then vibration would no longer induce a cell shape change. Thereby, supporting our hypothesis that calpain is the mechanism by which vibration changes cell shape.

Materials & Methods

Cells. HeLa (ATCC-CCL-165), a human cervical epithelial carcinoma cell line was grown in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% Fetal Bovine Serum and antibiotics at 37˚C with 5% CO_2.

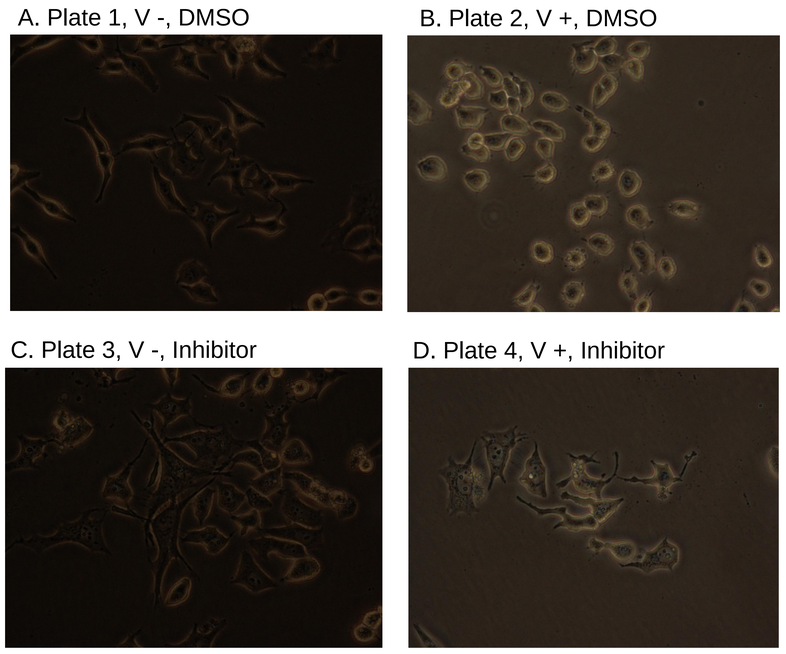
Inhibition. HeLa were pre-treated 100 µM PD150606, a calpain inhibitor (Calbiochem) for 20 hours.

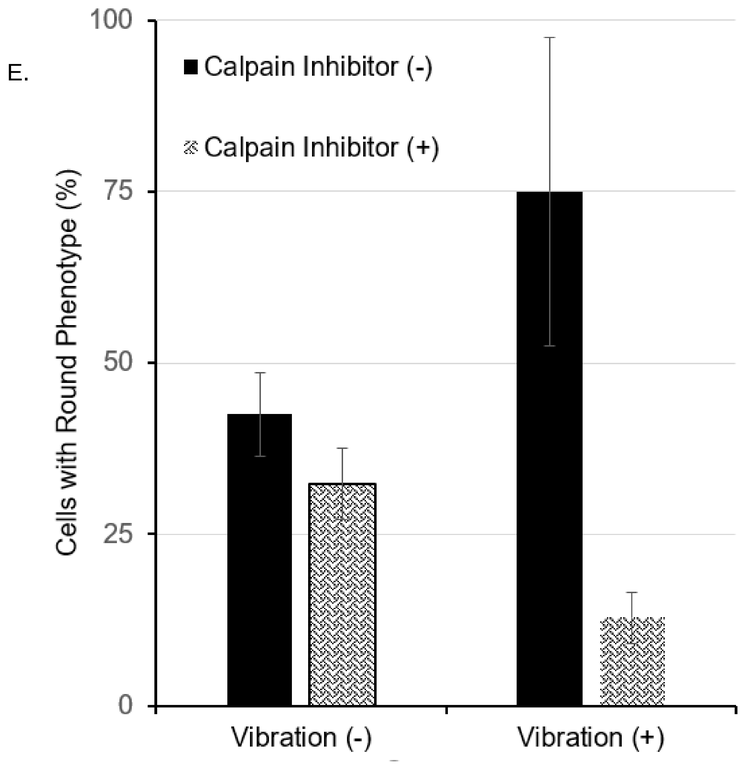
Vibration. HeLa were washed with PBS and either placed on a vibration resistant table [vibration (-)] or exposed to vibration with an analog vortex mixer (VWR) for 15 minutes at 1,200 rpm [vibration (+)].

Microscope. Micrographs were documented using CellSens imaging software using an inverted, Olympus CKX41 outfitted with phase contrast, fluorescence (excitation 470/40; emission 525/50) and an InfinityHD digital camera.

Results

Figure 1: Vibration induces a calpain-related cell shape change. Representative phase contrast micrographs of cells without vibration (A) and with vibration (B) in the absence or presence of calpain inhibitor (C and D) at 200x total magnification. Cells (≥50) were counted as either round or not. As previously reported, rounded phenotype induced by vibration. Data shown as average percentage round cells (±sd) (E). * = $p < 0.05$ Vibration (+) vs. Vibration (-).





Conclusions

The study presented here shows that epithelial cells exposed to vibration are less likely to adapt a round shape in the presence of calpain inhibitor. **We, therefore, supported our hypothesis that vibration induced calpain-related cell shape changes.** Understanding how a particular cell type reacts to vibration is essential to understanding why vibration therapy helps reduce pain.

The next step for our research would be to perform a Calpain Activity Assay to determine whether variation in protein levels is associated with a change in calpain activity.

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Acknowledgments

Jennifer Donnelly and the Department of Biology for their dedicated support of research and Grand View University for providing and maintaining research facilities.