

Effects of Mechanical Vibration on Prostate Cancer Bone Metastasis

Amel Sassi¹, Xin Song¹, Lidan You¹,

¹University of Toronto, Toronto, ON

Email of Presenting Author: amel.sassi@mail.utoronto.ca

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INTRODUCTION: Prostate cancer is one of the most common cancers across Europe and North America, and it is expected that 1 in 8 men will be diagnosed during his lifetime [1]. Prostate cancer preferentially metastasizes to bone, which leads to bone pain, fractures, and nerve compression. Notably, 80% of men who die from prostate cancer exhibit signs of bone metastases [2]. Such morbidity may be attributed to the interaction of prostate cancer cells with surrounding bone cells resulting in the disruption of bone structure and the acceleration of disease progression [3]. To mitigate these effects, exercise is often recommended to patients with prostate cancer due to the beneficial effects on bone remodeling. Specifically, mechanical loading such as physical activity is detected by the mechanosensing bone cell, osteocytes, which then send signals to other bone cells such as osteoblasts to promote bone growth [3]. However, physical activity may be challenging for elderly or bedridden patients. As such, vibration has emerged as a safe, effective, and easy to perform alternative therapy that has been shown to increase bone mineral density and significantly reduce fracture risks in the clinical environment [4]. Specifically, low magnitude high frequency (LMHF) has been shown to activate osteocytes and thereby reduce breast cancer cell migration [5]. Nevertheless, the effects of vibration on prostate cancer cell extravasation remain to be elucidated. Given that traditional 2D cell culture systems do not allow for cellular crosstalk to be fully explored, our novel bone-metastasis-on-a-chip platform enables bone cells and prostate cancer cells to be seeded into the device to uncover how vibration inhibits prostate cancer cell invasion. We hypothesize that LMHF vibration (0.3 g, 60 Hz, 1h/day) will decrease prostate cancer cell invasion into the bone through the activation of osteocytes.

METHODS: The bone-metastasis-on-a-chip model was fabricated using the methods outlined in Mei et al. (2019) [6]. The osteocyte channel was coated with 0.15 mg/mL type-1 rat-tail collagen for one hour. The microchannels were coated with 100 µg/mL fibronectin solution for 40 minutes at 4°C. The lumen channel was coated with a hydrogel solution with 10.7 mg/mL type-1 rat-tail collagen and 2.5 mg/mL of Matrigel and was aspirated after 30 seconds to form the lumen. The devices were then incubated in a water bath for 1 hour before adding the respective culture media into each channel. MLO-Y4 cells (osteocyte-like cells) were seeded into the osteocyte channel, while PC3s (prostate cancer cells) were seeded into the lumen channel. Microfluidic devices with the respective cells were placed on the custom-made vibration platform for 1 hour every day for 3 consecutive days. The platform produced vertical and sinusoidal motion set at 0.3 g and 60 Hz. Invasion distance for each of the side channels was quantified. Additionally, to assess the effects of vibration on apoptosis of both healthy cells and cancer cells, an apoptosis assay was carried out on PC3 and MLO-Y4 cells. PC3 cells were plated in 48-well plates and MLO-Y4 cells were plated in collagen coated 48-well plates. Cells were either vibrated under LMHF conditions for 3 days or remained static. Apoptosis was quantified by incubating with 5% APOPercentage dye for 30 minutes, followed by PBS wash. Four random images per well were captured under the light microscope. Percentage of apoptotic cells was determined by dividing the average number of apoptotic cells by the average total cell count for each well. Prism nine was used for statistical analysis. A student t-test was performed to determine statistical significance ($p < 0.05$) between the static and vibration conditions.

RESULTS: Preliminary results indicate that LMHF vibration (0.3 g, 60 Hz, 1h/day for 3 days) significantly reduced migration distance by approximately 52% ($n = 14$, $p < 0.0001$, figure 2). Additionally, PC3 cells ($n = 4$) showed a significant increase in the percentage of apoptotic cells for vibrated cells compared to static cells ($p = 0.0359$, figure 3A). MLO-Y4 cells ($n = 4$) showed no significant difference in apoptosis following vibration (figure 3B).

DISCUSSION: A significant reduction in prostate cancer migration following LMHF vibration treatment was observed, suggesting that vibration may be effective at reducing or preventing the incidence of prostate cancer bone metastases. While current data only represents migration of prostate cancer cells and adjacent osteocytes, transendothelial extravasation will be observed by seeding HUVECs (endothelial cells) into the lumen channel prior to seeding prostate cancer cells. It is expected that this will enhance physiological relevance and allow us to observe extravasation in real time using our novel bone-metastasis-on-a-chip model. Furthermore, apoptosis of PC3 cells as opposed to the MLO-Y4 and HUVECs is promising since it may indicate that vibration can reduce the overall tumour burden while conferring no harmful effects on surrounding cells.

SIGNIFICANCE/CLINICAL RELEVANCE: The observed reduction in migration distance and increased apoptosis in prostate cancer cells suggest that LMHF vibration could be used as a non-invasive alternative therapy to reduce prostate cancer bone metastases while sparing healthy cells from harmful effects.

REFERENCES:

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FIGURES:

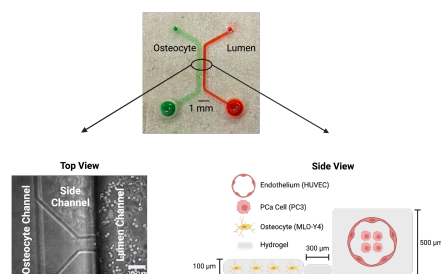


Figure 1. Microfluidic platform with osteocyte channel dyed green and lumen channel dyed red and the top and side view of each microfluidic device.

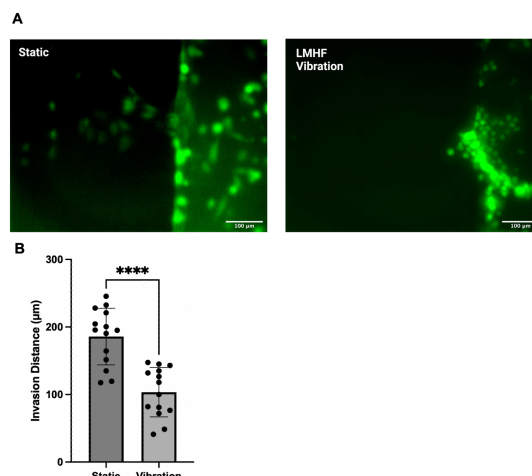


Figure 2. (A) Fluorescent image of prostate cancer cell invasion in microfluidic devices. (B) Histogram demonstrating invasion distance over 3 days under LMHF vibration and static conditions. Data are represented as mean \pm SD, **** = $p < 0.0001$.

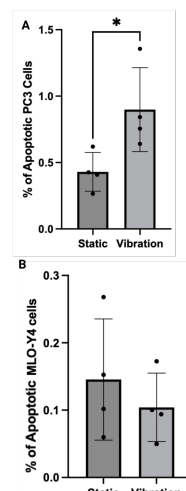


Figure 3. A) Percentage of apoptotic A) PC3 cells and B) MLO-Y4 cells experiencing static or LMHF vibration conditions. Data are represented as mean \pm SD, * = $p < 0.05$ ($p = 0.0359$).