ENDOTHELIAL CELLS EXPOSURE TO MECHANICAL VIBRATIONS

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Introduction

The mechanobiology of vascular cells plays a key role in vascular remodelling [1] and evidence showed that turbulent hemodynamics influence endothelial cells (ECs) functions *in vitro* [2]. Recent numerical simulations investigated the effect of mechanical stresses on vessels remodelling [3], but the biological effect on cellular functions remains still unclear. Therefore, this preliminary study aims to investigate the effects of vibrations on ECs exploiting an experimental setup capable of exposing cells to mechanical vibrations obtained by a 50 Hz sound-generated pressure wave.

Methods

To expose cells to vibrations, an aluminium support structure was built, equipped with a speaker connected with a two-channel digital power amplifier capable of receiving an input sound recording of 50 Hz (Figure 1a). Human umbilical vein endothelial cells (HUVECs) were seeded and cultured in two gelatin-precoated 150 mm cell culture dishes at a density of $1 \, x \, 10^6$ cells. After 4 days, a cell culture dish was mounted on the support structure to make it receive vibrations and incubated for 48 hours, while the other one was maintained in static conditions as normal control in another cell incubator.
The sounds emitted by the speaker and the vibrations of the cells were recorded at the beginning of the experiment, after 24 and 48 hours respectively with an in-house device consisting of an Adafruit Pulse Density Modulation microphone managed by an Arduino Nano 33 BLE board and with an accelerometer (LSM9DS1) mounted on the Arduino platform (Figure 1b), fixed to the plate center. Data were collected with Coolterm and analyzed in MATLAB R2022a. Specifically, sound recordings were analyzed in time and frequency while information of cell culture dish velocity and displacement during time were obtained by the integration of the acceleration data following appropriate high-pass filtering steps.

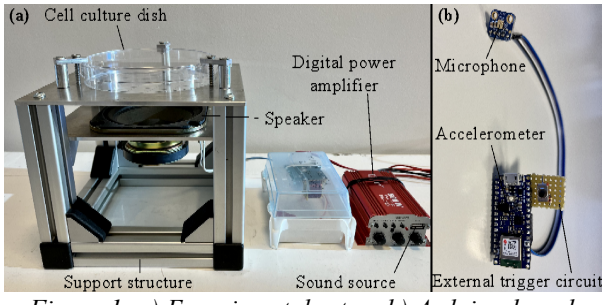

Figure 1: a) Experimental setup. b) Arduino-based device for sound and vibration measures.

At the end of the experiment the morphology of the stimulated and the static control cells was investigated by phase-contrast microscopy. Then, cell counting in the two conditions was performed to evaluate the effect of vibrations and Alamar Blue assay was performed on the samples to determine the HUVECs' viability.

Results

The sound received by cell culture dish was characterized over time by the main frequency of 50 Hz and its odd harmonics. Assumed a normal distribution of velocity and displacement, mean velocity obtained from acceleration data was 0.04 m/s (4σ) and the associated $2\sigma = 71 \, \mu m$ indicates the movement of the plate given by vibrations in the direction perpendicular to the dish. The Fourier transform of the acceleration data revealed the same vibration frequency of the sound. Phase-contrast analysis of the stimulated and unstimulated cells showed that sound-induced vibrations changed HUVECs typical morphology and determined their detachment from the substrate (Figure 2). This was confirmed by the number of death cells found in the culture medium of vibrations-exposed HUVECs, about 20% higher than the static control. Alamar Blue assay reported a decrease in cell viability greater than 50% for HUVECs exposed to the mechanical stimulus, suggesting that vibrations importantly affected cell functions.

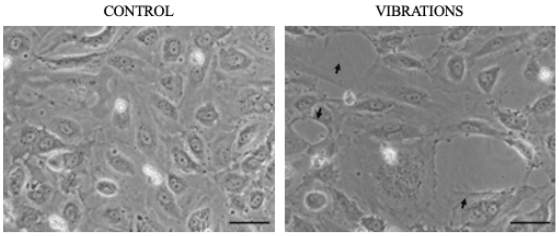

Figure 2: Phase-contrast microscopy magnification 20X, scale bars 50 µm.

Discussion

Preliminary results underlined a reduction in ECs viability when they are stimulated with a 50 Hz frequency for 48 hours. Our framework showed a great potential for the investigation of vascular remodelling and the related cell dysfunctions responsible for vascular diseases with a simple but reliable system.

References

1. Koseki et al, Transl. Stroke Res., 11:80-92, 2020.
2. Gimbrone et al, Circ. Res., 118: 620–636, 2016.
3. Souche et al, J. Biomech., 145, 2022.