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Low-frequency Mechanical Vibration Inducing Apoptosis In Vitro Cancer Cells

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Protocol status: Working

We use this protocol and it's working.



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Abstract

Protocol for replication study researching mechanisms to induce cancer cell apoptosis without damaging healthy tissue.

The protocol describes how mechanical vibration, (mechanomedicine) applied for one hour at brief low-frequency (20 Hz) mechanical vibration on glucose consumption and survival (apoptosis, necrosis, HMGB1 release) results in significantly increased at 48 h after mechanical vibration compared with cells maintained in static culture.



Materials

Main Protocol Materials

- Cell Line:
 - Human epidermoid carcinoma cell line

- Cell Culture Medium:
 - MEM/DMEM with:
 - 3.7 g/L sodium bicarbonate
 - 4.5 g/L glucose
 - 4 mM glutamine
 - 10% fetal bovine serum (FBS) for initial culture
 - Serum-free DMEM for 48 h prior to experiments.

- Mechanical Vibration System:
 - Vibration transducer or other equipment capable of delivering sinusoidal wave with or without contact
 - Controller software: Pd-extended on PC
 - Vibration frequency: 20 Hz sinusoidal waves

- Assay Kits:
 - Apoptosis/Necrosis Detection Kit
 - Glucose Assay Kit
 - HMGB1 ELISA Kit

- Microscopy and Imaging:
 - Eclipse Ti-S inverted microscope
 - NIS-Elements D software for image capture
 - ImageJ software for quantification.

- Incubation Conditions:
 - 37°C, 5% CO₂ for culture
 - Vibration performed at 34°C inside humidified CO₂ incubator, medium temperature reaches 37°C during vibration.

Protocol Steps

1 Cell Preparation:

- 1.1 Seed cells at 1.5×10^5 cells per 250 μ L well in 12 central wells of a 96-well plate.
- 1.2 Culture for 48 h in serum-free medium at 37°C, 5% CO₂.

2 Mechanical Vibration Application:

- 2.1 Place the 96-well plate on the vibration transducer secured with double-sided tape.
- 2.2 Apply mechanical vibration at 20 Hz sinusoidal frequency for 1 hour inside a humidified CO₂ incubator at 34°C (medium reaches 37°C during vibration).

3 Post-Vibration Incubation:

- 3.1 Remove plates from vibration system.
- 3.2 Incubate cells without vibration for 0 (immediate), 24, or 48 hours at 37°C, 5% CO₂.

4 Apoptosis and Necrosis Assay:

- 4.1 At 0, 24, and 48 hours post-vibration, analyze cell death using Apoptosis/Necrosis Detection Kit.
- 4.2 Visualize cells by inverted microscopy and quantify apoptotic and necrotic areas using ImageJ.

5 Glucose Consumption Assay:



5.1 Collect culture supernatants at indicated times.

5.2 Measure glucose concentration using Glucose Assay Kit following manufacturer's protocol.

6 HMGB1 Release Measurement:

6.1 Collect and pool supernatants at indicated time points.

6.2 Perform HMGB1 ELISA according to manufacturer's instructions, including coating, washing, antibody incubations, and colorimetric detection at OD450.

7 Data Analysis:

7.1 Perform experiments in biological triplicates.

7.2 Present data as mean $\pm$ SD.

7.3 Use Student's t-test for group comparisons; significance at $p < 0.05$.



Protocol references

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