Methods

Media preparation

Every media used in this experiment for feeding or experimental conditions was prepared using the same method. All media were prepared to match L-15 10% Fetal bovine serum (FBS)[1] by using 1.23 g of Leibovitz's L-15, 0.6 mL antibiotic, 10 mL of FBS and 89 mL of deionized water. All reagents were stirred until the solution is homogenous. The pH of the media was determined with a calibrated pH meter and adjusted according to its purpose with drops of either 1.0 M HCl to acidify or 1.0 M NaOH to increase the pH as needed. Media for feeding flask, sub-culturing and for control conditions was adjusted to 7.4 pH (following physiological pH); media for the acidic treatment was adjusted to a pH of 6.9; and media for alkaline conditions was adjusted to a pH of 7.9. All media was filtered with a syringe of 0.22 μm and stored in 15 mL falcon tubes in a refrigerated environment. Every falcon tube of media was strictly used twice and discarded to avoid cross-contamination. Proper disposal is ensured by adding 70% ethanol to the media before discarding it.

Feeding and Sub-Culturing process

Cells were initially provided for the experiment in a T-25 flask and were fed every two days to ensure proper growth prior to applying treatment. The feeding process was performed aseptically and consisted of removing half of existing and used media. With that, a remaining of fresh media was added to a total overall volume of 4 mL of media. Therefore, flasks were then sub cultured once they were deemed with sufficient cell confluency, with around 80% or

more of the bottom of the flask. All media was removed from the flask and cells were washed twice with 4 mL of PBS. Trypsin (2 mL) was added to detach cell culture from the bottom of the T-25 flask followed by addition of 4 mL media to neutralize it. The contents were transferred to a falcon tube and
centrifuged at 300 rpm for 10 minutes. The supernatant was discarded, and the remaining pellet was resuspended with 1 mL of fresh media using disposable pipettes. Cells were then distributed among two T-25 flasks. In the case of this experiment, because of high confluence
of cells, one subculture process was divided into four flasks instead of one. In addition, once ready, one available flask was sub-cultured into nine well plates to allow the addition of further treatments.

Scratch Test Preparation

Fresh Media was prepared the day of performing the scratch test. Sub-culturing was
done from one flask containing around 70% confluency and distributed into nine wells in the well-plate (A1 - A3, B1 - B3, C1 - C3). Cells were allowed to grow on 7.4 pH media until cell confluency reached around 66%. Media was removed from wells and washed with PBS. Media with respective treatments were added in triplicates: acidic condition with pH of 6.9 into wells A1, A2 and A3 while alkaline conditions with pH of 7.9 into wells B1, B2 and B3. A control was also set up at physiological conditions with adjusted pH of 7.4 into spots C1, C2 and C3. For reference, before the subculture process, each well was marked with a cross line made with a blade. From then, a scratch was done on the plates using an Eppendorf pipette tip to ensure removal of the cells in that area. Usually, the scratch is performed based on the reference lines previously created. The well-plate was then monitored two to three times a day for 3 consecutive days to assess the healing of the scratch. Images with measurements related to the width of the scratch were taken to ensure proliferation progress.

References

1. Cell Physiology Laboratory Manual. Thompson Rivers University. Winter 2024.