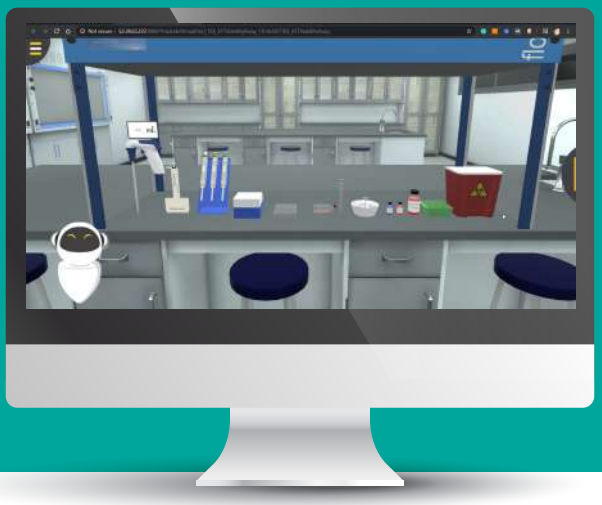
Biology | Toxicology

In Vitro Viability Assay Using Tetrazolium Salt XTT



General Aim

This experiment aims at testing the viability of cultured cells after exposure to the geometric concentration of different nanoparticles.

Method

In Vitro Colorimetric Analysis of Cell Viability by XTT Assay

Learning Objectives (ILOs)

- Successfully handle the required instruments and consumables needed in the experiment.
- Check the confluence and count cells under the microscope.
- Dilute the cells to a specific count suitable for seeding in the 96-well plate.
- Calculate the concentration of tested chemicals and prepare the calculated doses in the cell culture medium.
- Aspirate the old medium and add the new medium containing the tested chemicals in the appropriate wells.
- Add the XTT solution to cells and read the results using the microplate reader after incubation of cells. Read the results of XTT and calculate the viability percent of cells exposed to different doses of tested chemical(s).

Theoretical Background/Context

- Cytotoxicity is the quality of being toxic to cells. Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers, as in Nanotechnology, can either look for cytotoxic nano-based materials, if they are interested in developing a nanomedicine that targets rapidly dividing cancer cells, for instance; or they can screen "hits" from initial high-throughput nanoparticle screens for unwanted cytotoxic effects before investing in their development as a nanomedicine.

Theoretical Background/Context (Cont')

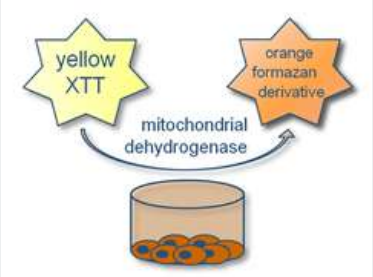
- Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components. Alternatively, membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside.
- Protease biomarkers have been identified that allow researchers to measure relative numbers of live and dead cells within the same cell population. The live-cell protease is only active in cells that have a healthy cell membrane, and loses activity once the cell is compromised and the protease is exposed to the external environment. The dead-cell protease cannot cross the cell membrane, and can only be measured in culture media after cells have lost their membrane integrity.
- Cytotoxicity can also be monitored by measuring the reducing potential of the cells using a colorimetric reaction, or using ATP content as a marker of viability. Such ATP-based assays include bioluminescent assays in which ATP is the limiting reagent for the luciferase reaction. A label-free approach to follow the cytotoxic response of adherent animal cells in real-time provides the kinetics of the cytotoxic response rather than just a snapshot like many colorimetric endpoint assays.

Principle of Work

- The mitochondria in cellular cytoplasm have many enzymes. Among these, a specific enzyme system named 'succinate-tetrazolium reductase' system belongs to the respiratory chain of the mitochondria and is only active in viable cells. This experiment aims to test the activity of such enzymatic system and measures this activity in nanoparticles-treated cells in comparison with the control untreated-cells.
- The use of tetrazolium salts, including XTT (2, 3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carbox-anilide), to assay cell proliferation, cell viability, and/or cytotoxicity is a wide-spread, established practice.
- Cleavage of the tetrazolium salt to formazan occurs via the succinate-tetrazolium reductase system in the mitochondria of metabolically active cells.

Principle of Work (Cont')

- The reaction is attributed mainly to mitochondrial enzymes and electron carriers, but a number of other non-mitochondrial enzymes have been implicated.



- XTT, a yellow tetrazolium salt, is cleaved to a soluble orange formazan dye, which can be measured by absorbance at 490 (or 450) nm in a microplate reader.