Cell viability and cytotoxicity assays: Biochemical elements and cellular compartments

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Abstract

Cell viability and cytotoxicity assays play a crucial role in drug screening and evaluating the cytotoxic effects of various chemicals. The quantification of cell viability and proliferation serves as the cornerstone for numerous in vitro assays that assess cellular responses to external factors. In the last decade, several studies have developed guidelines for defining and interpreting cell viability and cytotoxicity based on morphological, biochemical, and functional perspectives. As this domain continues to experience ongoing growth, revealing new mechanisms orchestrating diverse cell cytotoxicity pathways, we suggest a revised classification for multiple assays employed in evaluating cell viability and cell death. This classification is rooted in the cellular compartment and/or biochemical element involved, with a specific focus on mechanistic and essential aspects of the process. The assays are founded on diverse cell functions, encompassing metabolic activity, enzyme activity, cell membrane permeability and integrity, ATP content, cell adherence, reduction equivalents, dye inclusion or exclusion, constitutive protease activity, colony formation, DNA fragmentation and nuclear splitting. These assays present straightforward, reliable, sensitive, reproducible, cost-effective, and high-throughput approaches for appraising the effects of newly formulated chemotherapeutic biomolecules on the cell survival during the drug development process.

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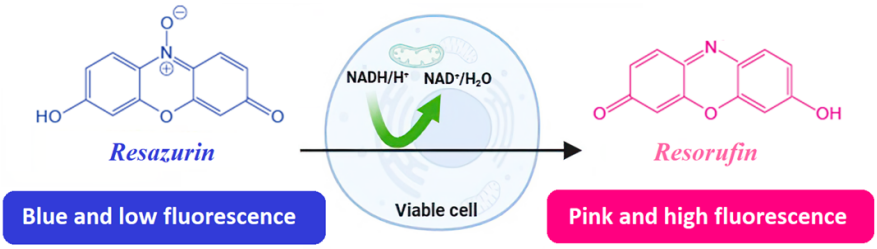

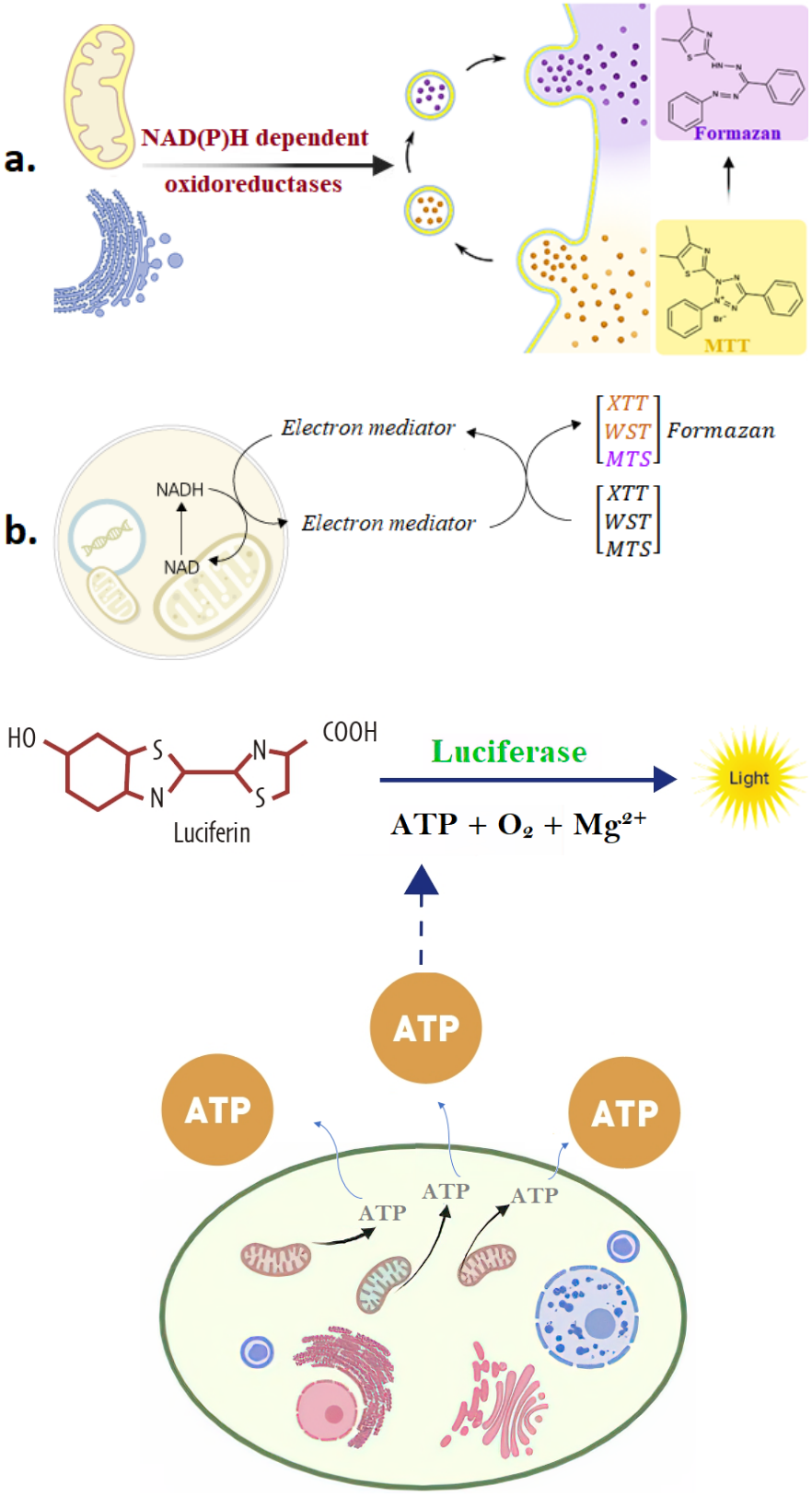

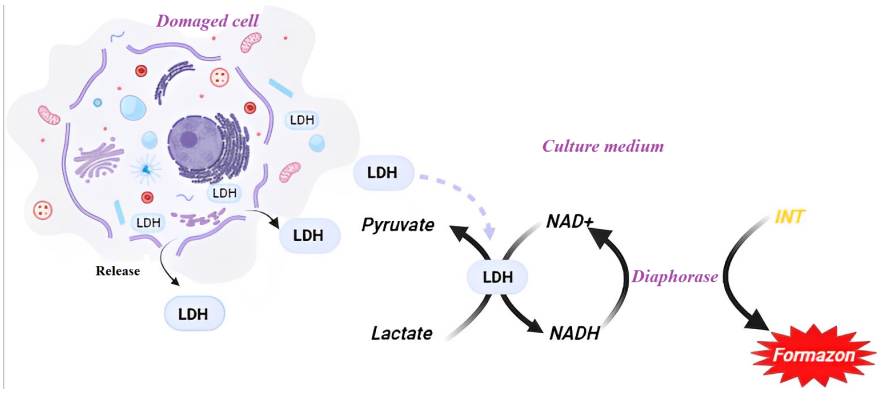

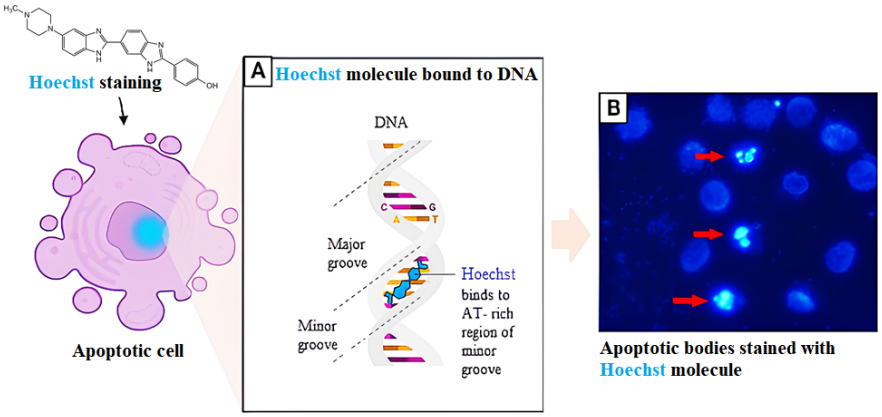

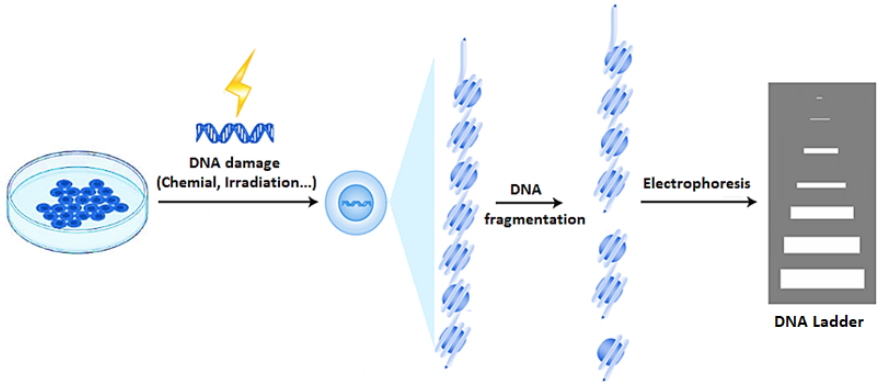

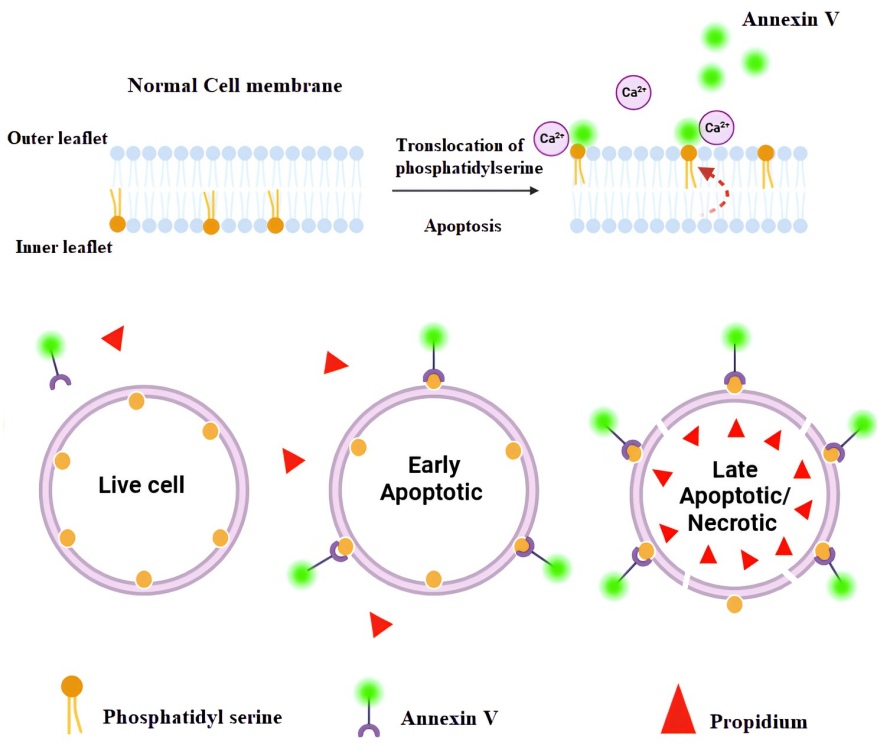

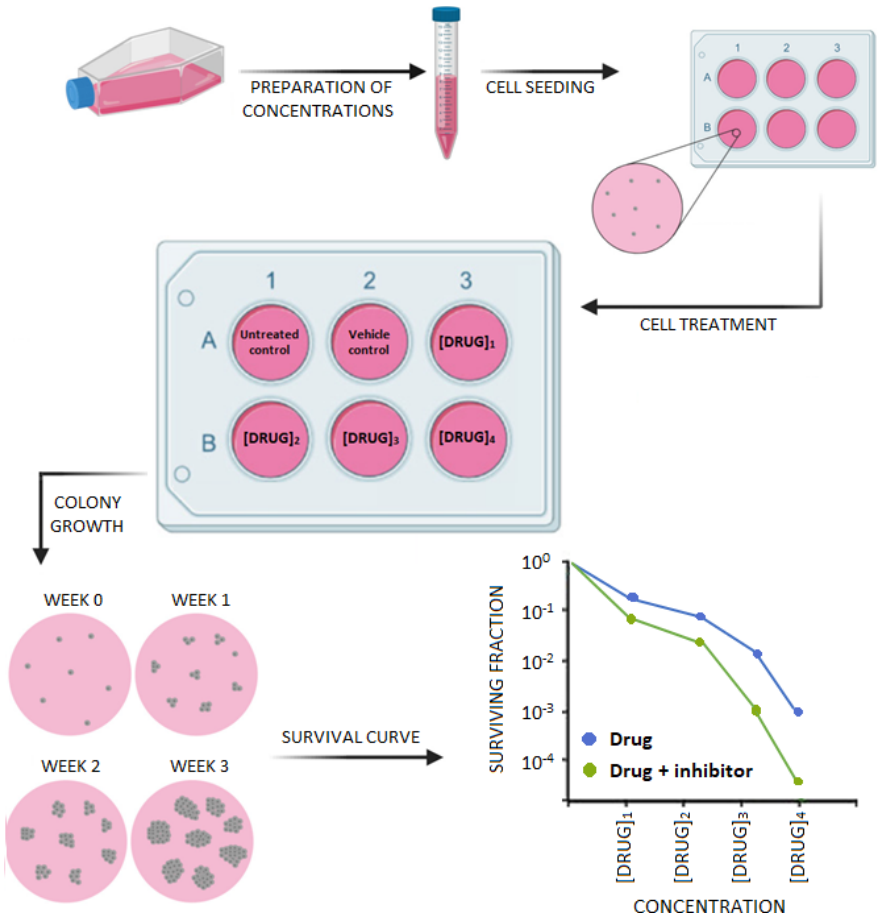

Figures captions

Fig. 1: Resazurin Assay. In the cytosol of a living cell, mitochondrial enzymes and cytochromes convert the oxidized blue form of resazurin into its reduced red form, known as resorufin.

Fig. 2: Tetrazolium salts assays. **a).** MTT is a yellow-colored compound with water-soluble properties, allowing it to be taken up by viable cells. This internalization process leads to the creation of an insoluble blue formazan within the cells. **b).** XTT, MTS, WTS are impermeable facing constraints in cellular absorption. As a result, reduction occurs on the cell surface through electron transport, generating water-soluble formazan crystals. This characteristic facilitates direct dissolution in the cell culture medium for straightforward spectrophotometric measurements.

Fig. 3: ATP assay. This assay relies on quantifying ATP levels in living cells. The mechanism involves the conversion of the luciferin substrate by the enzyme luciferase into light, a reaction that takes place in the presence of ATP and oxygen.

Fig. 4: LDH assay. Diaphorase enzymes utilize the produced NADH to reduce a yellow tetrazolium salt into a spectrophotometrically detectable red formazan. The assay is particularly useful in studying cellular damage or injury, as increased LDH activity in the extracellular environment can be indicative of cell membrane disruption.

Fig. 5: Hoechst nuclear staining. Hoechst dyes bind to the minor groove of DNA, when excited by UV light at approximately 360 nm, they emit a broad spectrum of blue light, peaking around 460 nm.

Fig. 6: DNA ladder assay. A method used to detect apoptosis by detecting fragmented genomic DNA on an agarose gel using electrophoresis.

Fig. 7: PI and Annexin V labelling. useful markers to distinguish between live, apoptotic, and necrotic cells based on differences in membrane permeability. Apoptotic cells are labelled exclusively with Annexin V, whereas necrotic or late apoptotic cells are doubly labeled with Annexin V and PI.

Fig. 8: Clonogenic assay. The assay evaluates the long-term maintenance of cellular reproductive integrity. This approach involves analyzing drug dose–survival curves for a comprehensive assessment of drug resistance.

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