Optimization of Comet Assay Staining Conditions for the Analysis of Cellular DNA Damage

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DNA oxidative stress, caused by an increased production of free radicals, is associated with illnesses such as Parkinson's Disease, Alzheimer's Disease and skin cancer. One of the most common methods of assessing DNA damage in individual cells is through single cell gel electrophoresis (comet assay), capable of both detecting and quantifying DNA damage. This study focuses on the optimization of the concentration of Propidium Iodide (P.I.) used to stain the DNA during this assay. Briefly, human keratinocytes (HaCaTs) were irradiated with different doses of UVB (0, 0.5 and 1 J/cm^2) on ice prior to undergoing the alkaline comet assay. In the process, HaCaTs were stained with various concentrations of P.I. (2.5, 3.0, 3.5 and 4.0 µL/mL). Comet Assay IV software (Perceptive, UK) was used to score and quantify DNA damage in 300 individual cells from three trials. For HaCaTs exposed to both 0 and 1.0 J/cm^{2}UVB, altering the P.I. concentration showed no significant difference in DNA damage detected (8% and 62%, respectively). For those irradiated with 0.5 J/cm^{2}UVB, a P.I. concentration of 2.5 µL/mL detected 4% less DNA damage (52%) compared to the three other P.I. concentrations (56%). In addition, using 3.5 µL/mL and 4.0 µL/mL of P.I. should be avoided because the high intensity of the stain under a fluorescence microscope interferes with the software's ability to differentiate between damaged and non-damaged DNA. These results suggest that 2.5 µL/mL P.I is the optimum concentration for staining the DNA during the comet assay.