TOWARDS A HIGH-THROUGHPUT COMET ASSAY

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Introduction: Comet assay is a sensitive, versatile and inexpensive technique used in genotoxicity testing in fields such as pharmaceuticals for human use and food additives. The objective of this study was to increase comet assay throughput decreasing the time of electrophoresis through the application of a higher voltage than the one used in the standard protocol.

Materials and methods: A standard comet assay protocol, applying 20 minutes electrophoresis at 1.1 V/cm, was followed in untreated and methyl methanesulfonate (MMS)-treated TK6 cells. Afterwards, different protocols using different times of electrophoresis (2.5 minutes, 5 minutes, 10 minutes, 15 minutes and 20 minutes) and the maximum voltage that the power supply could reach were tested (~ 3 V/cm). In all experiments, temperature and voltage, from power supply as well as from an external voltmeter, were recorded. Finally, another electrophoresis at the selected timepoint was run in order to compare results with the standard protocol.

Results: An electrophoresis time-dependent increase in the % DNA in tail was observed in MMS-treated cells subjected to a high electric field. This increase was also observed in untreated cells after 10 minutes of electrophoresis. Some difficulties were found in comet scoring after 10, 15 and 20 minutes of electrophoresis. Voltage and temperature varied during the electrophoresis. Results similar to the ones obtained with the standard protocol were observed between 5 and 10 minutes of electrophoresis so, a 7-minute electrophoresis at high voltage was the selected timepoint to compare with the standard comet assay. In 300 µM MMS-treated cells, a 35.3 % DNA in tail was obtained after 7 minutes at high voltage in comparison with 41.2 % DNA in tail for the standard protocol. Both negative controls had low % DNA in tail values.

Conclusions: A short and strong electrophoresis can be use as a strategy to shorten the comet assay protocol.

Keywords: Comet Assay; Electrophoresis; DNA damage; genotoxicity