

Does time of lysis really affect the *in vitro* alkaline comet assay results?

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Single-cell gel electrophoresis (the comet assay) is now the method of choice for measuring several kinds of DNA damage in cells and tissues. It has several applications such as in genotoxicity testing, human biomonitoring and ecogenotoxicology. Although this assay has been in use for almost 30 years, due to its versatility it is still under development. Various organisations and regulatory bodies have an interest in monitoring chemicals for genotoxicity with this method; in fact, a new version of the “*In vivo* mammalian alkaline comet assay” draft OECD guideline for the testing of chemicals was published just a few months ago. However, little effort has been made to standardise the *in vitro* version of the comet assay. Agarose concentration, alkaline unwinding time and electrophoresis conditions have been identified as critical points affecting the alkaline comet assay outcome. Nevertheless, there is no scientific publication reporting the effect that modifying the time of lysis would have. Here we tried 10 different times of lysis in control HeLa cells and HeLa cells treated with different concentrations of either methyl methanesulfonate (MMS) or H₂O₂. We also tested 7 different times of lysis in the alkaline comet assay combined with formamidopyrimidine DNA glycosylase (FPG) in untreated HeLa cells and cells treated with the photosensitiser Ro 19-8022 (Ro) plus light. Leaving the gels on ice before lysis appears to increase the DNA damage detected in MMS-treated cells, but this effect was not observed in H₂O₂ or Ro plus light-treated cells. Besides, it was observed that MMS or H₂O₂-induced DNA damage can be detected in the absence of lysis, with similar results from 0 min to 1 h of lysis. Nevertheless, no enzyme-sensitive sites were detected in the absence of lysis in Ro plus light-treated cells, presumably because the enzyme is not capable of entering intact cells. In this case, a 5-min-lysis step was enough to detect enzyme-sensitive sites. Finally, as longer times of lysis (i.e. more than 1 h) increase the sensitivity of the assay, different times of lysis might be employed under circumstances requiring enhanced sensitivity.