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Effect of pH and Duration of Electrophoresis on Comet Tail

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The comet assay is widely used for measuring DNA damage and DNA repair. After embedding the cells in agarose, cells are lysed to yield supercoiled nucleoids. In case of DNA strand breaks, the supercoiling of DNA is relaxed and after electrophoresis, moved towards to anode and forms a comet tail. Together with increasing DNA strand breaks, we observe an increase in the percentage of DNA in the tail. However, a question has remained unanswered whether the comet tail contains DNA fragments or loops. We have now revisited this question.

To develop an understanding of what might be in the comet tail, we have conducted alkaline and neutral comet assay by using human lymphoblastoid cell line TK6. The TK6 cells were either treated with the alkylating agent methyl methanesulfonate (MMS, 100µM for 4h) to induce at this low concentration presumably single strand breaks leading to loop formation or with the unspecific protein kinase C inhibitor staurosporine (1 µM for 4 h) to induce apoptotic cells yielding DNA fragments and ghost cells. After treatment, we conducted alkaline as well as neutral comet assay with the same samples under different electrophoresis durations (0, 10, 20, 30, 40, and 60 min). We observed an increasing percentage of DNA in the tail as well as tail length over time in electrophoresis with both substance treatments. There was no saturation of this increase, which may be an indication of the presence of DNA fragments in the tail of comets after treatment with both substances. In addition, we have observed a faster migration of apoptotic small DNA fragments, which leads to a reduced number of visible comets and loss from the comet tail area in staurosporine-treated samples. There seem to be subtypes of cells after staurosporine treatment, possibly dependent on the cell cycle phase of treatment, with some cells dissolving or disappearing into very small non detectable DNA fragments and others harboring visible comets. In addition, we could show that the results of the neutral comet assay didn't differ from the alkaline comet assay, which indicates that both endpoints are able to detect single and double-strand breaks.

Altogether, the tail of comets might contain both loops and fragments after treatment with the genotoxin methyl methanesulfonate, whereas staurosporine yields apoptotic cells with DNA fragments.

Keywords:

Comet assay, DNA fragments, DNA loops, alkaline comet assay and neutral comet assay.