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This essay was submitted by Vijaykumar Naidu to the essay competition held by TechResource in Mumbai, India and won the second prize. TechResource holds the copyright to the essay

The comet assay- A sensitive genotoxicity test.

Comet assay also referred to as Single Cell Gel Electrophoresis (SCGE) and Micro Gel Electrophoresis (MGE) was first introduced by Ostling and Johanson in 1984 as a technique used for direct visualization of DNA damage in individual cells on a microscope. It is used for assessing DNA damage, and has applications in the field of Genotoxicity, Human bio- monitoring, Oncology and Molecular epidemiology, Eco-Genotoxicology and also in other fundamental research.

The technique works by combining DNA gel electrophoresis method with fluorescent microscopy to observe migration of single, broken or damaged DNA strands from individual agarose embedded cells when an electric current is passed through it. The entire procedure does not take more than 24 hours to complete. Here, the cell suspension is mixed with 0.5% agarose and dropped on a pre coated 1% agarose slide. This step is followed by treating the slides in an alkaline lysis buffer, which removes all the proteins, RNAs, enzymes and other cell components leaving only the DNA behind. Neutral method can also be used which is very sensitive. More stringent lysis condition results in loss of more than 95% of the cellular protein which allows broken duplex molecules to migrate. This adaptation provides the basis for the neutral method which detects only double stranded DNA breaks whereas alkaline method is used to detect single strand breaks.

After lysis buffer treatment, the slides are incubated for some time in the electrophoresis buffer so that the osmotic balance is retained between the embedded cells in the agarose and the buffer. A current of 40mA is then passed through the slides for about 25 minutes. The relaxed and broken smaller size and weight DNA fragments migrate further towards the anode resembling a tail like structure and the undamaged DNA being larger in size does not migrate faster forming a head. On analysis, a comet or a “halo” like structure is observed and hence the name “COMET” assay. The length of the tail or the comet is measured to determine the extent of DNA damage.

In this technique there are a few limiting steps, like due to the short electrophoresis period not all the smaller fragments might have migrated to ensure reproducible results. Also interpretation is not always reliable due to no direct relation between DNA damage caused by a chemical effect and the biological impact of this effect. During gel electrophoresis, cells behave differently when they are actively replicating their DNA, thereby questioning the inherent sensitivity of the technique. Like during electrophoresis, under alkaline conditions, replication forks acts like single strand breaks and hence S-phase migrates quicker compared to neutral condition treatment where the S-phase DNA acts as replication bubbles which retards migration.

Inspite of these limitations, this technique is widely used for its simplicity, versatility, speed and economy. Usually fluorescent DNA dyes like Ethidium Bromide or Propidium Iodide is used for staining. This method can be too costly so, instead of fluorescent DNA dye, Silver stain or Giemsa stain also gives good results.

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