21st Century Publisher

21 ST CENTURY PATHOLOGY

21st Century Pathology

Review Article

Open Access

The Comet Assay: A Straight Way to Estimate Geno-Toxicity

Summayya Fatima, Sabitha Yadam*

Ceincia Life Sciences, Sardar Patel Nagar, Nizampet, Hyderabad, India

***Corresponding Author:** Sabitha Yadam, Ceincia Life Sciences, Sardar Patel Nagar, Nizampet, Hyderabad, India;

E-mail: ceincialifesciences@gmail.com

Received: 05 April 2023; **Accepted:** 24 April 2023; **Published:** 27 April 2023

Abstract

Comet assay or single cell gel electrophoresis is a simple yet quite effective technique used in quantitative assessment of DNA damage and repair. This assay is widely used to evaluate the in-vitro single and double strand DNA damage/breaks. Comet assay covers the broad area of mutagenesis which includes Geno-toxicity, environmental toxicology/risk assessment, human biomonitoring along with clinical studies. Now a days comet assay is frequently used to study the effect of ionizing radiation (cancer cells), nanotoxicology along with estimating the sensitivity for various drugs on plant and animal cells. This assay allows us to investigate deep down in the area of molecular sciences. This assay is highly considered due to its efficiency, cost effectiveness, high reproducibility. Here we are reviewing comet assay, describing about its principle, materials/methodology, types and applications.

Keywords: *Comet assay; Geno-toxicity; DNA breaks*

Introduction

Cook P, et al. (1976) published a paper of nuclear structure based on their investigation where they lysed the cells using non-ionic detergents and high molar sodium chloride [1]. After the treatment they got nucleoid (nuclear matrix/DNA, RNA and proteins) which were then subjected to ethidium bromide treatment (an intercalating agent) relaxing the DNA loops. Rydberg B, et al. (1978) successfully made an initial attempt to quantify DNA strand breaks by embedding the cells on agarose slides and lysing them using mild alkaline conditions. This technique was successfully developed by Ostling O, et al. (1984) [1]. The cell lysis and electrophoresis were carried out under neutral conditions, acridine orange dye was used to stain the DNA and obtained image looked like a "comet" having an intact head consist of DNA and tail comprise of broken pieces of DNA hence the assay was named. Later in the year of 1988 this assay was modified by Singh NP, et al. [2]. They performed the assay in alkaline conditions which detected alkaline labile sites and also single stranded breaks re-joining (in X-ray irradiated human lymphocytes). From 1990 comet assay gain popularity and has been used broadly in various medical and research fields.

International Workshop on Geno-toxicity Test Procedure (IWGTP) and Organization for economic co-operation and development in the year of 1999 have developed in-vitro and in-vivo single cell gel electrophoresis assay, instructed and highlighted the optimal version of comet assay. A meeting held on September, 2011, Turkey, by scientists during the international comet assay workshop has launched a project group "COMNET-WORK". which mainly focused on protocol standardization and authentication of comet assay as a reliable technique for DNA damage detection for human healthcare.

Principle

Comet assay exploits the ability of denatured or cleaved pieces of DNA to migrate out of the cell under the influence of applied electric current (electrophoresis).

First cells are embedded into agarose gel at room temperature and are immobilized on comet slides.

The slides are then dipped into lysis solution (removes the membrane and histone protein from DNA) which break open the cell.

DNA is subjected to denaturation under alkali or neutral conditions (alkali treatment denatures and unwinds the DNA).

After the lysis treatment cells are washed with the distilled water to remove all the salts. Before applying electric-current the cells are immersed in an electrophoresis solution (for 20 minutes) which has its own adjusted PH that depend on the type of damage which is being investigated. The electric field of 1V/cm is supplied for around 20 minutes, undamaged part of nucleoid remains as comet head while the damaged DNA create the comet tail, results can be analysed with a software or by traditional cell counting technique.

To enhance the specificity and sensitivity nucleoids are incubated with bacterial endonucleases that scan for any DNA damage and convert lesions to breaks thus increasing the concentration of DNA in comet tail. The after-treatment incubation helps us in monitoring the DNA repair by measuring the damage at various intervals. The statistical analysis of the result should also be considered.

Types

There are two types of comet assay.

Neutral comet assay

Mainly used to investigate double stranded breaks which are produced mostly because of ionizing radiations. In this type of method DNA is preserved as double stranded structured.

Alkaline comet assay

Most widely used technique and much more sensitive than neutral variant, in this method DNA is subjected to denaturation which reveals the single as well as double strand DNA breaks. Along with this we can also identify alkali labile sites, DNA-DNA, DNA-protein and cross-linking sites. As the advent and applications of comet assay have been increasing dramatically, scientists came up with modified versions of the comet assay which are in use to study the unexplored areas of DNA and to uncharted the mechanism of base excision repair (BER) and nucleotide excision repair (NER) [3].

Enzyme specific comet assays: Uracil DNA glycosylase is been used in comet assay to detect the uracil in DNA, form-amidopyrimidine- glycosylase for studying the ring-opened purines, 8-oxoguanine, endonucleases III, and V for oxidized pyrimidines, AP sites and CPDs [4].

Production of nano-materials and their uses in comet assay play an important role to assess the human health as we expose to different kinds of nano sized particles on the daily basis which can cause a wide of disease like from minor skin irritation to lethal cancers.

Comets are visualized Using different DNA stains, the most common one is silver staining. Earlier ethidium bromide and Propodium iodide were used (but due to carcinogenic property of Etbr it is been avoided). Use of silver staining after propidium staining gives us very good results. Along with these now a days Giemsa staining, Bio-rad silver staining, SYBR green are popularly used [5].

Why do we perform comet assay?

To evaluate Geno-toxicity.

To predict Geno-toxicity.

To study damage repair mechanism.

To study the action of drugs in clinical trials.

To screen the DNA damage repair inhibitors.

Comet assay being a sensitive technique to measure the Geno-toxicity can give a range of results which depends on factors that influence the pathways of DNA damage and repair. Such factors include age, diet, life style (exercise, sleeping habits), environmental pollution, smoking, alcohol consumption, gender, prolonged medication, exposure to chemicals or radiation [6].

Applications

Comet assays are broadly used in cellular and molecular biology fields to investigate the DNA damage. Great use of comet assay comes in cancer studies where different anti-cancer drugs are screened to evaluate their levels of Geno-toxicity qualitatively and quantitatively. Cells are exposed to different mutagens, chemicals, nano-particles and irradiated with radiation to allowed the damage to happen and their results are studied. Alternatively, DNA repair kinetics can also be assessed. Ability of comet assay to distinguish the genotoxic from non-genotoxic chemicals (in-vivo) is widely utilized.

Comet assay in cancer

Comet assay plays a crucial role in investigating the DNA damage and repair in different types of tumour cells (includes tumour cell lines and tumour cells extracted from cancer patients). In cancer, predicting the tumour cell response to radiation (radiotherapy) would give valuable information in the area of clinical oncology. There are several techniques available but Micro-array is in the field of research but this technique has got some limitations that can make the results complicated. Previously colonogenic cell survival was used to evaluate the survival percentage of tumour cells after radiotherapy, but this technique is time consuming and may take weeks to get the results that is why alkaline comet assay has been widely used on different tumour types like bladder, head, neck, breast, and some metastatic tumours. Cells that are obtained from tumour biopsy can be easily assessed using comet assay. The action of anti-cancer drugs (used for chemotherapy) like Cisplatin, Mitomycin can be screened in cancer cell lines.

This assay is also used to measure the basal level of DNA damage in peripheral blood lymphocytes (PBL) of cancer patients. PBL obtained from cancer patients are studied prior and after treatment of chemotherapy and radiotherapy, also by exposing them to several chemicals (mainly mutagens) to determine the genomic sensitivity to damage and repair in different samples [7,8].

Tumour hypoxia is one of the major factors that affect the clinical decision making, treatment and survival hopes of the patients [9]. Eppendorf microelectrode and comet assay can be used on several tumour cells (excised from biopsy) to measure the hypoxic conditions [10]. But comet assay is preferably used when compare

to Eppendorf microelectrode because presence of necrotic cells in the sample may alter the outcoming result but this will not become a problem in comet assay due to which this technique has given more preference [11]. Comet assay is been used for patients with breast, neck and head cancer to assess tumour hypoxia [11,12]. Eventually there is no such ideal technique to measure the hypoxic state of the tumour cell, but comet assay has proved to be a worthful measure to quantify the tumour related hypoxia at cellular basis [9].

Alkaline comet assay used to study the radiosensitivity in three bladder cancer cell lines. The studies have shown a contrary relation between comet tail moment and clonogenic survival, this tells us that alkaline comet assay can be used as a potential tool to predict the response of radiotherapy in single cell lines [13]. Using a panel of 6 bladder cancer cell lines to evaluate the extend of comet formation (in response to radiation) using alkaline comet assay best reflects the radiosensitivity of bladder cancer cell line, these results were supported by two parallel and independent studies using bladder tumour cells and colorectal tumour cells [14,15].

Genetic and other information is considered in order to customized the therapies and medication to individual patient [16,17]. Response of the patient to chemotherapeutics is the major concern. Several therapeutics like Melphalan, Mechlorethamines (Alkylating agents), doxorubicin in breast cancer and cisplatin in colon cancer lines have been tested in-vitro using comet assay. The results obtained by testing different chemo-drugs demonstrate the heterogeneity of various cancer types which says that chemotherapeutics have a range of activities even in the same type of cancer [18]. Given below is the table that highlights the types of cancer where comet assay is used for monitoring genomic damage (Table 1).

Table 1: Detection of Geno-toxicity in different types of cancer using comet assay.

Types of cancer	Geno-toxicity detection by comet assay
Breast cancer	✓
Colon cancer	✓
Bladder cancer	✓
Neck and head cancer	✓
Gastric cancer	✓
AIDS related cancer	✓

Comet assay in neurological and autoimmune disorders

This assay serves as an important tool for detecting the causative factors and clinical conditions of neurodegenerative disorders like Alzheimer, Parkinson and auto-immune diseases like Rheumatoid arthritis, Male sterility, Diabetes mellitus.

Oxidative stress plays an important role in pathogenesis and progression of neurodegenerative diseases, DNA damage was found to be significantly higher in patients with Parkinson and multiple sclerosis when compare to healthy control [19,20]. Here the cells in the brain are more prone to oxidative damage due to high levels of polyunsaturated fatty acids, antioxidants [21,22]. Assessment of DNA damage is obligatory for clinical and research purpose. Occurrence of non-communicable diseases (diabetes, Chronic obstructive pulmonary diseases and cancer) and the death risks can be predicted by evaluating the DNA damage in circulatory lymphocytes using comet assay.

Systemic lupus erythematous (auto immune disorder) is characterized by DNA breakage and the lymphocytes obtained from SLE patients displayed extensive genomic alterations [23,24].

Hyperglycemia and comet assay

Comet assay has its application in assessing the DNA damage in pregnant women suffering from hyperglycemia, in a condition like this when mother has hyperglycemia there always remains a tendency that this will affect the neonates. Usually, hyperglycemic mothers have high oxidative stress, if it gets uncontrolled leads to DNA damage. So, the babies born to Diabetic mothers have high risk of developing Hypoglycemia, sepsis and respiratory distress syndrome [25].

Metformin is a boon for type II diabetes but the studies on human lymphocytes treated with different doses of metformin results in the increase of sister chromatid exchange. Metformin significantly increases the tail length and frequency of micronucleus but does not affect the tail intensity much, this shows the mild Geno-toxicity of the drug [26].

There are several ways of drug administration one of them is

nanomaterial drug administrating system. Scientist suspect that this delivery system might have got the cellular or genomic toxicity (restricted information is available) where DNA damage in the given sample of the subject can be estimated using comet assay [27]. In the era of modern technology where the demand of nanomaterials in everyday life is exiling at industrial and commercial levels which definitely has negative impact on the health of individuals. To examine the nano-Geno-toxicity of the nanomaterials (to estimate the mutagenic and carcinogenic activities) comet assay is widely favoured [28,29]. Nano particles like fullerenes, single walled carbon nanotubes, carbon black, titanium dioxide are been experimented for their genotoxic activities in human lymphocytes, human bronchial epithelial cells, A549 (type 2 alveolar like human lung adenocarcinoma cell line), human-B cells lymphoblastoid using comet assay [30,27].

Gasoline and it's Geno-toxicity

People who are working at petrol pumps/auto mobile gas stations are exposed to the vapours of gasoline during their working hours [31]. According to experimental studies petrol pump workers constantly suffer from memory loss, sleep disturbance, headaches and fatigue, another study show that they excrete high levels of phenol in their urine (when they are exposed to fumes of gasoline). There are more than 100 types of hydrocarbons (paraffin, olefins, naphthalene, benzene, toluene, benzene, methyl benzene and xylene...etc) all these chemicals can adversely affect the health of humans [32]. Benzene is a carcinogenic agent (also has clastogenic property), on inhalation it gets absorbed in lungs. In humans it is known to suppress the production of DNA, RNA, several cellular proteins, decreasing the number of peripheral lymphocytes, platelets and interrupt the process of hematogenesis. It also causes sister chromatid exchange and chromosomal damage (aberrations) [33-35]. All these studies showed the high potential of petroleum compounds to cause DNA damage (genotoxic) in exposed individuals [36].

Sewage water and it's Geno-toxicity

Sewage water contains several organic molecules, deleterious compounds (contaminants), heavy metals (lead, cadmium, zinc) which are reported more in urban areas due to high pollution [37,38]. Sewage workers have high tendency to develop cancer as they are exposed to these hazardous elements [39,40]. Alkaline comet assay is conducted on blood samples obtained from exposed subjects to study the impact of dissolved and suspended solids of sewage water and the results have shown a significant variation in concentration of lead and cadmium in blood, DNA damage was also been noticed. Another study showed that municipal workers who deals with the sewage water treatment and disposal suffers from anaemia as their blood iron levels decreases [41-43].

Metal Geno-toxicity

For all living being heavy metals like cadmium (cd), lead (pb), mercury (Hg), arsenic (As) and chromium (Cr) are often hazardous as they show persistent profile in ecosystem [44]. The increase usage of heavy metals causes continuous release of their metallic by-products in environment which is a matter of global concern as these metals and their compounds are major threat to human/animal/plant health. Arsenic induces Geno-toxicity as it damages the DNA, inhibits the repair mechanism and introduce chromosomal aberrations, sister chromatid exchange (SCEs). "As" compounds induces gene amplification, oxidative stress and arrest the cell cycle. Arsenic compounds interrupt nucleotide excision repair as they interact with the zinc finger DNA binding domains [45].

Cadmium influences cell proliferation, apoptosis, bind to cellular proteins, up regulate cytokines, proto-oncogenes, degrade proteins and inhibit cellular respiration which can cause potential chromosomal deletion [46-49].

Chromium and its compounds have genotoxic activities (which depends on valency and oxidation state) chromium compounds are potent oxidizing agents as they get absorbed by lungs, GI tract and skin. Reduction of Cr generates free radicals which reacts with the DNA, proteins, effecting the cell membrane and genomic integrity and also introduce DNA single strand breaks, intra strand cross-links hence are genotoxic [50-53].

Mercury is also a genotoxic agent which mainly act by the production of reactive oxygen species. Lead interferes with calcium dependent inter, intra cellular signal transduction and signaling. The genotoxic activities of these heavy metals can be examined by Comet assay [54,55].

In-Vivo metal Geno-toxicity

Mice are widely used as sensitive models to study the pathophysiology and Geno-toxicity (metals compounds, drugs). Potassium dichromate is known to induce DNA single and double stranded Breaks. This study is carried out by using alkaline comet assay technique. Here different doses of potassium dichromate (0.59 to 76.0) mg/kg per body weight administered orally and whole blood samples were collected. Peripheral leukocytes are used as biomarkers to study the consequences of potassium dichromate in human subjects. The results showed significant increase in comet tail length (the results were totally depended on the concentration of dose), higher the concentration more will be the tail length [56].

DNA damage by nickel chloride: Swiss albino mice were administered with doses of Nickel chloride orally in different concentration (3.4, 6.8, 13.6, 27.2, 54.4, and 108.8 mg/kg according to body weight). Whole-Blood samples were collected (1st and 2nd week post treatment with ACA), results showed an increase in comet tail length representing DNA damage [57].

Copper sulphate induces single stranded DNA breaks, different

doses of Cu2So4 were administered to study genome toxicity and its repair mechanisms. Blood samples were taken out post administration and comet assay were performed which showed an increase in comet tail length, samples were then cultured and Incubated in RPMI media (2 hrs), a decrease in tail length was observed [58].

Germ cell Geno-toxicity and comet assay

It is necessary to conduct the studies on mutagen and their classification. Comet assay is potentially used (in-vivo, ex-vivo, and in-vitro) to detect the impact of mutagens and carcinogens on different somatic cells. But it is also possible to use this assay to study Geno-toxicity in gonadal cells mostly spermatic cells (female germ cell-ovum is more complicated to obtained that's why work is preferably done with male germ cells). Many variants of comet assay (alkaline and neutral) are used but there is a need of protocol standardization and results validation [59].

Comet assay is not always be a suitable technique to assess DNA lesions (in mammalian sperms), so to compensate this, a modified version of comet assay was developed which uses protein extracts (obtained using Hela cells) and this approach gives obvious results showing that this modified version can become a promising tool to detect sperm Geno-toxicity [60].

Cryopreservation is widely used and a well-known method to store germ cells. Comet assay can be used to evaluate the viability of semen (Crassostrea gigas) before and after cryopreservation. This method involves fluorescent staining to study DNA integrity. The results showed increase in tail length. which can be taken as a major Parameter for DNA damage detection. Hence cryopreservation has got negative effects on the quality of germ cells [61].

Two-tailed comet is used in fertility and other andrological studies in humans and in different species. Here samples are subjected to electrophoresis twice providing denaturation and non-denaturion conditions.

1st electrophoresis-non denaturing conditions-detection of double strand breaks.

2nd electrophoresis-denaturing and alkaline conditions-detection of single strand breaks.

The result creates two tailed comet that helps in evaluation of SSBs and DSBs at the same time in a given cell. Mammalian sperm cells are assessed for DNA damage using two tailed Comet assay [62].

Smoking associated Geno-toxicity and comet assay

For this study whole blood and peripheral blood lymphocytes were examined, using comet assay they have demonstrated that smoking can damage DNA. Use of whole blood is considered as a better way than peripheral blood lymphocytes because the procedure to isolate lymphocytes may induce oxidative damage in the cells that causes background level of DNA damage altering the results [63].

Comet assay is less useful in detecting the lower levels of chronic exposure to any genotoxic compounds and hence it is understandable that smokers do not show clear effect in comet assay. Smoking has DNA-damaging effect on PBL as evaluated by comet assay but this effect was more pronounced when smoking was investigated as a genotoxic exposure and expressed to lower extend in the sample of populations considering smoking as a potential factor in an occupational investigation [64,65].

Comet assay in combination with other techniques are used to determine the environmental Geno-toxins by exploiting lower organisms like earth warm, mussels (Eco-Geno-toxicity in aquatic organisms) etc. COMET-FISH technique is developed for detecting the gene specific or sequence specific DNA damage and repair which became a potential diagnostic tool [66].

Plant Geno-toxicity and comet assay

Comet assay is used in the study of plant toxicity caused by chemicals like pesticides, insecticides, weedicides, heavy metals, nano-particles. This helps us in assessing the factors responsible for DNA damage, oxidative stress, cell death, cell cycle interruptions and degree of gene expression [67]. The earliest report of plant comet assay comes from late 90s, Ginchner T, et al. (2009) who reviewed technical data of plant comet assay [68]. Presence of cell wall in plants can be a major problem that prevent the use of comet assay and to avoid this problem, Cerda H, et al. (1993) developed an efficient technique to isolate the nuclei (using mechanical extraction) and this technique was improvised further in later days [69]. Depending up on the type of study or type of plant, a modified form of comet assay is used. Plants response to biotic and abiotic agents which may alter the cellular and molecular mechanisms of plant cells eventually leading to genomic damage. Secondary metabolites are known to have protective activities against abnormal conditions but the varied concentration of these compounds can influence the oxidative stress and regulate the cell death pathways. One study showed that alkaloids extracted from N. tazetta show growth inhibition in Oryza sativa, Brassica rapa [70]. High levels of epinodosin may halt the cell cycle progression by inhibiting mitotic division. Alkaline comet assay showed that saponins can induce double strand breaks in DNA plus some phytohormones like salicylic acid may display genotoxic activity [71]. Plants are very sensitive to the action of alkylating agents, dyes, chelating agents, antibiotics, aromatic compounds, hydrogen peroxide etc. Endosulfan and azo dyes are widely used in pesticides and textile industries, the mode of action of these chemicals is demonstrated in A. cepa, white clover using comet assay and results showed dose dependent-

DNA damage [72,73]. Beside this effect of contaminated matrices (sewage water, remains of animals, industrial leakages, fly ashes) on plants can also be assessed by comet assay as plant Geno-toxicity is an important biomarker for environmental pollution [74,75].

Food Geno-toxicity and comet assay

Now a days additives are more specifically used in package food to preserve the taste, flavor, aroma and to enhance the self-life. Frequent consumption of packaged food indirectly increases the concentration of additives in body so it became necessary to evaluate their effects on genetic material for the betterment of human health (this process is carried out by WHO in association with FAQ). Geno-toxicity of food additives can be determined by comet assay in bacterial cells, peripheral lymphocytes, plant cells, mice and in other organisms. Artificial Food colors- red tar food colors like amaranth, allura red, acid red dye been tested (in-vivo) in pregnant mice. Acid red dye showed no Geno-toxicity but amaranth and allura red showed positive results indicating DNA damage in colon and lungs. Over all there are 7 dyes, new coccine, amaranth, tartazine, erythrosine, rose Bengal, allura red, phloxine showed genomic damage in colon, urinary bladder, gastro-intestinal tract along with this antioxidants-BHA, BHT, fungicides-thiabezadole, artificial sweeteners like sucralose, saccharin are also known to induce DNA damage in GI tract [76,77].

Geno-toxicity in Prokaryotes and in lower organisms

X- ray radiation and its Geno-toxicity were studied for the first time in E. coli by Singh N, et al. [78]. Modified form of bacterial neutral comet assay was used to investigate the activity of antimicrobial compound (CB clay) to assess double strand breaks in E. coli [79]. Bacterial cells are treated with water, ethanol, kanamycin, CB clay and bleomycin, the results showed a significant increase in tail length due to DSBs in cells which are treated with CB clay where as in other controls the difference in comet tail length was insignificant.

Saccharomyces cerevisae and other species like S. pombe became model organism to study the impact of engineered nanoparticles, heavy metals, contaminated water through comet assay [80,81]. Unicellular aquatic algae are been assessed to monitor the toxicity degree of their habitat. Pseudokirchneriella subcapitata (fresh water green algae), Nanocloris occulata, Chlamydomonas showed DNA damage to toxic chemicals of herbicides and fungicides, impact of UV radiation was also studied by a modified version of comet assay [82,83].

Comet assay in lower protozoan and invertebrates: chemicals like phenol, hydrogen peroxide, formaldehyde, melamine, dechlorane plus have been tested on tetrahymena, and the results showed increase in DNA damage [84]. Dreissena polymorpha, Mytilus edulis, Unio tumidis (fresh water mussel) are studied for the detection of DNA damage response of peracetic acid, styrene, carbon allotropes, NSAIDS, polybrominated diphenyl ether by comet assay [85-87].

Earth worms who are also known as friends of farmers as their presence can indicate the quality of soil. Coelomocytes, eleocytes, sperm cells of earth worm (Eisenia fetida) have used to estimate the impact of industrial contaminants, waste water contaminants (contain mercury, lead, Nickle cholride), radiation, hydrogen peroxide and several other compounds using comet assay, the obtained results indicated high potent of Geno-toxicity [88-90].

Sleep deprivation and comet assay

Sleep plays king size role in physical health and mental wellbeing because as we sleep our body starts to heal that will recharge and refresh you so that you can have a kick start morning. Sleeping disorders or sleep deprivation is associated with chronic diseases like respiratory, cardiac diseases, diabetes, obesity [91]. An experimental study conducted on individuals who do night shifts to detect whether sleep deprivation can damage DNA or not. Peripheral lymphocytes were isolated from whole blood sample of subjects and when alkaline comet assay and form-amido pyrimidine DNA glycosylase assisted comet assay were performed the obtained results showed a significant increase in DNA damage (single, double strand breaks, DNA lesions, oxidized purines) and decrease in DNA repair activity [92]. Another study conducted using comet assay to evaluate genomic integrity in sleep deprived female Zucker rats where obese and normal rats were taken as subjects. Comet results showed increase in genomic damage in liver cells (obese rats) and in brain cells (almost all subjects irrespective of obese status) [93].

Comet assay in bacteriophage

Bacteriophage are bacterial infecting agents, as soon as they infect the bacteria, they tend to take control over the genetic machinery of host by integrating into their genome. They can either show lytic cycle or lysogenic cycle. In lytic cycle they multiply very fast increasing their number as a result at one point cell burst out releasing newly produced virion (here cell act as virion making factory). Plaque assay is used to measure the quantity or degree of infection but as this technique is time consuming, a modified and novel comet assay was then used for the detection of cell lysis. Released genetic content can be visualized by using ethidium bromide staining. This way of using modified comet assay to assess bacteriophage lytic activity has gain popularity as it is a sensitive technique which works efficiently when compare to other assays [94].

Comet assay in toxicological pathology

To study histopathology, in-vitro Geno-toxicity assays are used for any tissue level Geno-toxicity but to get accurate results, in addition to in-vitro assays in-vivo Geno-toxicity assays such as

Comet assay is also used. In clinical trials, Drug associated tissue site Geno-toxicity is studied using Comet assay and together these assays ensure the safety of the compound which is to be released in the market [95]. Comet assay is also used to check the in-vivo environment of cells or tissues which is an important step prior to transplantation. Comet assay allows to explore different types of epithelial cells (epithelial cells are the major components of many organs) such as buccal, nasal epithelial cells, corneal and tear duct epithelial cells. More than 50% of cancer are of epithelial origin that is why it is mandatory to evaluate DNA damage in these cells/tissues [96].

Covid associated Geno-toxicity and comet assay

Corona virus/covid-19 emerged as pandemic spreading havoc all over the world which is caused by severe acute respiratory syndrome coronavirus 2 viral strain (SARs-COV2) and was a major concern to mankind. It is a single positive sense RNA enveloped virus having structural and non-structural proteins, mainly effect the adaptive immunity and organ functioning particularly lungs that's why patients suffering from covid-19 show symptoms of organ failure (lungs) and display very weak immunity response. Before conducting any experimental/clinical studies all the parameters like hospital, lab, clinical data have to be strongly considered. For fighting the infection organism mainly rely on immune system. Studies done on the samples collected from patients who are admitted to critical care unit of hospital on different days showed a decrease in percentage of T-cells which make patients immunodeficient and more susceptible to secondary infections elevating mortality rates. Alkaline comet assay studies are done to assess the level of DNA damage and a modified version of comet which involves the use of endonuclease enzyme to detect oxidative pyrimidines, form-amido pyrimidine DNA glycosylase for oxidized purines (as oxidative stress tend to damage DNA) in covid patients and obtained results gave positive response to Geno-toxicity mediated by corona virus. It is also been confirmed that viral spike proteins inhibit the Non homologous End Joining and Homologous Joining repair mechanism which further levitate the rate of DNA damage [97,98].

Beside so many advantages there are few limitations of this Assay:

1. DNA damage caused by fixed mutations cannot be evaluated by this method.
2. Experimental variations can give altered results.
3. Genomic damage caused by epigenetic mechanisms cannot be studied by comet assay.

Conclusion

From the time of invention to till now various studies in the biology and chemistry related to genomic Toxicity and eco-toxicity have been potentially carried out which involve the use of comet assay since then this assay is widely used in the area of cellular and molecular biology to conduct genotoxic studies which can help us in understanding the physiology and mode of progression of several diseases. It is like opening the pandora box and as we are going deeper and deeper more challenges and mysteries are welcoming us. Likewise depending upon the type of study some modifications can be made to standardized the protocol which may allow us to conduct the study in different ways but more research and deep analyzation is required in order to further modernize the assay.

Conflict of Interest

The authors declare no conflict of interest.

Fundings

None.

References

1. Piperakis SM. Comet assay: a brief history. Cell biology and toxicology. 2009 Feb;25:1-3. https://doi.org/10.1007/s10565-008-9081-y
2. Azqueta A, Slyskova J, Langie SA, O'Neill Gaivão I, Collins A. Comet assay to measure DNA repair: approach and applications. Frontiers in genetics. 2014 Aug 25;5:288. https://doi.org/10.3389/fgene.2014.00288
3. Lu Y, Liu Y, Yang C. Evaluating in vitro DNA damage using comet assay. JoVE (Journal of Visualized Experiments). 2017 Oct 11(128):e56450. https://doi.org/10.3791/56450
4. Muruzabal D, Collins A, Azqueta A. The enzyme-modified comet assay: Past, present and future. Food and Chemical Toxicology. 2021 Jan 1;147:111865. https://doi.org/10.1016/j.fct.2020.111865
5. Bakuradze T, Richling E. Comparison of different DNA staining methods in the comet assay. Frontiers in Genetics. 2015;6(1):1-1. https://doi.org/10.3389/conf.fgene.2015.01.00007
6. Møller P, Knudsen LE, Loft S, Wallin H. The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. Cancer epidemiology biomarkers & prevention. 2000 Oct;9(10):1005-15.
7. McKenna, D. J., McKeown, S. R., & McKelvey-Martin, V. J. (2008). Potential use of the comet assay in the clinical management of cancer. Mutagenesis, 23(3), 183-190. https://doi.org/10.1093/mutage/gem054
8. Peters LJ, Brock WA, Johnson T, Meyn RE, Tofilon PJ, Milas L. Potential methods for predicting tumor radiocurability. International Journal of Radiation Oncology* Biology*

Physics. 1986 Apr 1;12(4):459-67. https://doi.org/10.1016/0360-3016(86)90053-2

9. Tatum JL. Hypoxia: importance in tumor biology, noninvasive measurement by imaging, and value of its measurement in the management of cancer therapy. International journal of radiation biology. 2006 Jan 1;82(10):699-757. https://doi.org/10.1080/09553000601002324

10. Milosevic M, Fyles A, Hedley D, Hill R. The human tumor microenvironment: invasive (needle) measurement of oxygen and interstitial fluid pressure. InSeminars in radiation oncology 2004 Jul 1 (Vol. 14, No. 3, pp. 249-258). WB Saunders. https://doi.org/10.1016/j.semradonc.2004.04.006

11. Olive PL, Durand RE, Le Riche J, Olivotto IA, Jackson SM. Gel electrophoresis of individual cells to quantify hypoxic fraction in human breast cancers. Cancer Research. 1993 Feb 15;53(4):733-6. https://doi.org/10.1089/ars.2013.5378

12. Le QT, Kovacs MS, Dorie MJ, Koong A, Terris DJ, Pinto HA, Goffinet DR, Nowels K, Bloch D, Brown JM. Comparison of the comet assay and the oxygen microelectrode for measuring tumor oxygenation in head-and-neck cancer patients. International Journal of Radiation Oncology* Biology* Physics. 2003 Jun 1;56(2):375-83. https://doi.org/10.1016/s0360-3016(02)04503-0

13. McKelvey-Martin VJ, Ho ET, McKeown SR, Johnston SR, J. McCarthy P, Rajab NF, Downes CS. Emerging applications of the single cell gel electrophoresis (Comet) assay. I. Management of invasive transitional cell human bladder carcinoma. II. Fluorescent in situ hybridization Comets for the identification of damaged and repaired DNA sequences in individual cells. Mutagenesis. 1998 Jan;13(1):1-8. https://doi.org/10.1093/mutage/13.1.1

14. McKeown SR, Robson T, Price ME, Ho ET, Hirst DG, McKelvey-Martin VJ. Potential use of the alkaline comet assay as a predictor of bladder tumour response to radiation. British journal of cancer. 2003 Dec;89(12):2264-70. https://doi.org/10.1038/sj.bjc.6601426

15. Dunne AL, Price ME, Mothersill C, McKeown SR, Robson T, Hirst DG. Relationship between clonogenic radiosensitivity, radiation-induced apoptosis and DNA damage/repair in human colon cancer cells. British journal of cancer. 2003 Dec;89(12):2277-83. https://doi.org/10.1038/sj.bjc.6601427

16. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environmental and molecular mutagenesis. 2000;35(3):206-21. https://doi.org/10.1002/(sici)1098-2280(2000)35:3<206::aid-em8>3.0.co;2-j

17. Tofilon PJ, Basic I, Milas L. Prediction of in vivo tumor response to chemotherapeutic agents by the in vitro sister chromatid exchange assay. Cancer research. 1985 May;45(5):2025-30.

18. Apostolou P, Toloudi M, Kourtidou E, Mimikakou G, Vlachou I, Chatziioannou M, Papasotiriou I. Use of the comet assay technique for quick and reliable prediction of in vitro response to chemotherapeutics in breast and colon cancer. Journal of Biological Research-Thessaloniki. 2014 Dec;21:1-7. https://doi.org/10.1186/2241-5793-21-14

19. Ercan A, Kulaksiz G, Dalmizrak O, Muftuoglu M, Ogus H, Cavdar L, Inan L, Ozer N. Mitochondrial respiratory chain enzyme activities, mtDNA variants and gene expression levels in idiopathic Parkinson's disease.

20. Baydar M, Aydın S, undeger ulku, et al. Assessment of DNA Damage in the Peripheral Lymphocytes of Patients with Parkinson's Disease and Multiple Sclerosis Using the Comet Assay. Turkiye Klinikleri Journal of Medical Sciences.2013;33:936-945. https://doi.org/10.1016/j.toxlet.2011.05.402

21. Haider L, Fischer MT, Frischer JM, Bauer J, Höftberger R, Botond G, Esterbauer H, Binder CJ, Witztum JL, Lassmann H. Oxidative damage in multiple sclerosis lesions. Brain. 2011 Jul 1;134(7):1914-24. https://doi.org/10.1093/brain/awr128

22. Nakabeppu Y, Tsuchimoto D, Yamaguchi H, Sakumi K. Oxidative damage in nucleic acids and Parkinson's disease. Journal of neuroscience research. 2007 Apr;85(5):919-34. https://doi.org/10.1002/jnr.21191

23. Tung GK. DNA damage in a patient with Systemic Lupus Erythematosus and Nephropathy-A Case Report. International Journal of Biomedical Research. 2015;6.527:10. https://doi.org/10.7439/ijbr.v6i7.2213

24. Montalvão TM, Miranda-Vilela AL, Roll MM, Grisolia CK, Santos-Neto L. DNA damage levels in systemic lupus erythematosus patients with low disease activity: An evaluation by comet assay. Advances in Bioscience and Biotechnology. 2012 Nov 29;3(07):983-8. https://doi.org/10.4236/abb.2012.327121

25. Durga KD, Adhisivam B, Vidya G, Vishnu Bhat B, Bobby Z, Chand P. Oxidative stress and DNA damage in newborns born to mothers with hyperglycemia–a prospective cohort study. The Journal of Maternal-Fetal & Neonatal Medicine. 2018 Sep 17;31(18):2396-401. https://doi.org/10.1080/14767058.2017.1344630

26. Yuzbasioglu D, Mahmoud JH, Mamur S, Unal F. Cytogenetic effects of antidiabetic drug metformin. Drug and Chemical

Toxicology. 2022 Mar 4;45(2):955-62. https://doi.org/10.1080/01480545.2020.1844226

27. Vandghanooni S, Eskandani M. Comet assay: a method to evaluate genotoxicity of nano-drug delivery system. BioImpacts: BI. 2011;1(2):87. https://doi.org/10.5681/bi.2011.012

28. Cardoso S, da Silva CF, Severino P, Silva AM, Souto SB, Zieli□ska A, Karczewski J, Souto EB. Genotoxicity assessment of metal-based nanocomposites applied in drug delivery. Materials. 2021 Jan;14(21):6551. https://doi.org/10.3390/ma14216551

29. Evans SJ, Clift MJ, Singh N, de Oliveira Mallia J, Burgum M, Wills JW, Wilkinson TS, Jenkins GJ, Doak SH. Critical review of the current and future challenges associated with advanced in vitro systems towards the study of nanoparticle (secondary) genotoxicity. Mutagenesis. 2017 Jan 1;32(1):233-41. https://doi.org/10.1093/mutage/gew054

30. Ema M, Tanaka J, Kobayashi N, Naya M, Endoh S, Maru J, Hosoi M, Nagai M, Nakajima M, Hayashi M, Nakanishi J. Genotoxicity evaluation of fullerene C60 nanoparticles in a comet assay using lung cells of intratracheally instilled rats. Regulatory Toxicology and Pharmacology. 2012 Apr 1;62(3):419-24. https://doi.org/10.1016/j.yrtph.2012.01.003

31. Mehlman MA. Dangerous properties of petroleum□ refining products: Carcinogenicity of motor fuels (Gasoline). Teratogenesis, carcinogenesis, and mutagenesis. 1990;10(5):399-408. https://doi.org/10.1002/tcm.1770100505

32. Page NP, Mehlman M. Health effects of gasoline refueling vapors and measured exposures at service stations. Toxicology and industrial health. 1989 Dec;5(5):869-90. https://doi.org/10.1177/074823378900500521

33. International Agency for Research on Cancer. Occupational exposures in petroleum refining; crude oil and major petroleum fuels. IARC monographs on the evaluation of carcinogenic risks to humans. 1988;45.

34. Lan Q, Zhang L, Hakim F, Shen M, Memon S, Li G, Vermeulen R, Smith MT, Rappaport SM, Hayes R, Linet M. Lymphocyte toxicity and T cell receptor excision circles in workers exposed to benzene. Chemico-biological interactions. 2005 May 30;153:111-5. https://doi.org/10.1016/j.cbi.2005.03.015

35. Fishbein L. Benzene: uses, occurrence and exposure. IARC scientific publications. 1988(85):67-96.

36. Khisroon M, Gul A, Khan A, Ali N, Zaidi F, Rasheed SB, Akbar H. Comet assay based DNA evaluation of fuel filling stations and automobile workshops workers from Khyber Pakhtunkhwa province, Pakistan. Journal of Occupational Medicine and Toxicology. 2015 Dec;10:1-6. https://doi.org/10.1186/s12995-015-0069-2

37. Mdegela RH, Braathen M, Mosha RD, Skaare JU, Sandvik M. Assessment of pollution in sewage ponds using biomarker responses in wild African sharptooth catfish (Clarias gariepinus) in Tanzania. Ecotoxicology. 2010 Apr;19:722-34. https://doi.org/10.1007/s10646-009-0449-4

38. Hartwig A. Cadmium and cancer. Cadmium: from toxicity to essentiality. 2013:491-507. https://doi.org/10.1007/978-94-007-5179-8_15

39. Solano MD, de Lima PL, Luvizutto JF, Silva PR, de Aragão Umbuzeiro G, de Camargo JL. In vivo genotoxicity evaluation of a treated urban sewage sludge sample. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2009 May 31;676(1-2):69-73. https://doi.org/10.1016/j.mrgentox.2009.04.002

40. Francisco Lozano Luvizutto J, de Lourdes Marzo Solano M, Passareli D, Adriene da Silva Franchi C, de Aragão Umbuzeiro G, Lauro Viana de Camargo J. Subchronic toxicity evaluation of a treated urban sewage sludge. Journal of Toxicology and Environmental Health, Part A. 2010 May 28;73(13-14):916-25. https://doi.org/10.1080/15287391003745036

41. Miura N. Individual susceptibility to cadmium toxicity and metallothionein gene polymorphisms: with references to current status of occupational cadmium exposure. Industrial health. 2009;47(5):487-94. https://doi.org/10.2486/indhealth.47.487

42. Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ, Nawrot T. Cadmium stress: an oxidative challenge. Biometals. 2010 Oct;23:927-40. https://doi.org/10.1007/s10534-010-9329-x

43. Basu R, Talapatra SN, Mukhopadhyay A, Roy Goswami M, Ray SS, Chakrabarti P, Ram SS, Sudarshan M, Chakraborty A, Dasgupta A, Ghosh UC. Genotoxicity study with special reference to comet test in the blood cells of workers exposed to sewage water. Advances in Toxicology. 2014 Nov 23;2014. https://doi.org/10.1155/2014/251812

44. Järup L. Hazards of heavy metal contamination. British medical bulletin. 2003 Dec 1;68(1):167-82. https://doi.org/10.1093/bmb/ldg032

45. Senila M, Levei E, Cadar O, Senila LR, Roman M, Puskas F, Sima M. Assessment of availability and human health risk posed by arsenic contaminated well waters from Timis-Bega area, Romania. Journal of Analytical Methods in Chemistry. 2017 Oct 15;2017. https://doi.org/10.1155/2017/3037651

46. Rahimzadeh MR, Rahimzadeh MR, Kazemi S, Moghadamnia AA. Cadmium toxicity and treatment: An update. Caspian journal of internal medicine. 2017;8(3):135. https://doi.org/10.22088/cjim.8.3.135

47. Ashizawa A, Faroon O, Ingerman L, Jenkins K, Tucker P, Wright S. Toxicological profile for cadmium.

48. Davison AG, Taylor AN, Darbyshire J, Chettle DR, Guthrie CJ, O'Malley D, Mason HT, Fayers PM, Venables KM, Pickering CA, Franklin D. Cadmium fume inhalation and emphysema. The Lancet. 1988 Mar 26;331(8587):663-7. https://doi.org/10.1016/s0140-6736(88)91474-2

49. Elinder CG, Järup L. Cadmium exposure and health risks: recent findings. Ambio. 1996 Aug 1:370-3.

50. Wakeman TP, Yang A, Dalal NS, Boohaker RJ, Zeng Q, Ding Q, Xu B. DNA mismatch repair protein Mlh1 is required for tetravalent chromium intermediate-induced DNA damage. Oncotarget. 2017 Oct 10;8(48):83975. https://doi.org/10.18632/oncotarget.20150

51. Modrich P. Mismatch repair, genetic stability, and cancer. Science. 1994 Dec 23;266(5193):1959-60.

52. Singh J, Pritchard DE, Carlisle DL, Mclean JA, Montaser A, Orenstein JM, Patierno SR. Internalization of carcinogenic lead chromate particles by cultured normal human lung epithelial cells: formation of intracellular lead-inclusion bodies and induction of apoptosis. Toxicology and applied pharmacology. 1999 Dec 15;161(3):240-8. https://doi.org/10.1006/taap.1999.8816

53. Costa M. Toxicity and carcinogenicity of Cr (VI) in animal models and humans. Critical reviews in toxicology. 1997 Jan 1;27(5):431-42. https://doi.org/10.3109/10408449709078442

54. Valko MM, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Current medicinal chemistry. 2005 May 1;12(10):1161-208. https://doi.org/10.2174/0929867053764635

55. Kocadal K, Alkas FB, Battal D, Saygi S. Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: A review. Human & Experimental Toxicology. 2020 Jan;39(1):3-13. https://doi.org/10.1177/0960327119874439

56. Devi KD, Rozati R, Banu BS, Jamil K, Grover P. In vivo genotoxic effect of potassium dichromate in mice leukocytes using comet assay. Food and chemical toxicology. 2001 Aug 1;39(8):859-65. https://doi.org/10.1016/s0278-6915(01)00019-9

57. Danadevi K, Rozati R, Banu BS, Grover P. In vivo genotoxic effect of nickel chloride in mice leukocytes using comet assay. Food and chemical toxicology. 2004 May 1;42(5):751-7. https://doi.org/10.1016/j.fct.2003.12.013

58. Banu BS, Ishaq M, Danadevi K, Padmavathi P, Ahuja YR. DNA damage in leukocytes of mice treated with copper sulfate. Food and chemical toxicology. 2004 Dec 1;42(12):1931-6. https://doi.org/10.1016/j.fct.2004.07.007

59. Speit G, Vasquez M, Hartmann A. The comet assay as an indicator test for germ cell genotoxicity. Mutation Research/Reviews in Mutation Research. 2009 Jan 1;681(1):3-12. https://doi.org/10.1016/j.mrrev.2008.03.005

60. Baumgartner A, Cemeli E, Anderson D. The comet assay in male reproductive toxicology. Cell biology and toxicology. 2009 Feb;25:81-98. https://doi.org/10.1007/s10565-007-9041-y

61. Gwo JC, Wu CY, Chang WS, Cheng HY. Evaluation of damage in Pacific oyster (Crassostrea gigas) spermatozoa before and after cryopreservation using comet assay. Cryoletters. 2003 May 1;24(3):171-80.

62. Cortés-Gutiérrez EI, López-Fernández C, Fernández JL, Dávila-Rodríguez MI, Johnston SD, Gosálvez J. Interpreting sperm DNA damage in a diverse range of mammalian sperm by means of the two-tailed comet assay. Frontiers in genetics. 2014 Nov 27;5:404. https://doi.org/10.3389/fgene.2014.00404

63. Hoffmann H, Högel J, Speit G. The effect of smoking on DNA effects in the comet assay: a meta-analysis. Mutagenesis. 2005 Nov 1;20(6):455-66. https://doi.org/10.1093/mutage/gei064

64. Speit G, Witton-Davies T, Heepchantree W, Trenz K, Hoffmann H. Investigations on the effect of cigarette smoking in the comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2003 Dec 9;542(1-2):33-42. https://doi.org/10.1016/j.mrgentox.2003.08.002

65. Hoffmann H, Speit G. Assessment of DNA damage in peripheral blood of heavy smokers with the comet assay and the micronucleus test. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2005 Mar 7;581(1-2):105-14. https://doi.org/10.1016/j.mrgentox.2004.11.015

66. Glei M, Hovhannisyan G, Pool-Zobel BL. Use of Comet-FISH in the study of DNA damage and repair. Mutation Research/Reviews in Mutation Research. 2009 Jan 1;681(1):33-43. https://doi.org/10.1016/j.mrrev.2008.01.006

67. Koppen G, Toncelli LM, Triest L, Verschaeve L. The comet assay: a tool to study alteration of DNA integrity in developing plant leaves. Mechanisms of ageing and development. 1999 Oct 1;110(1-2):13-24. https://doi.org/10.1016/s0047-6374(99)00038-x

68. Gichner T, Znidar I, Wagner ED, Plewa MJ. The use of higher plants in the comet assay. The comet assay in toxicology. 2009

Aug 27:98-119. https://doi.org/10.1039/9781847559746-00098

69. Koppen G, Angelis KJ. Repair of X‐ray induced DNA damage measured by the comet assay in roots of Vicia faba. Environmental and molecular mutagenesis. 1998;32(3):281-5. https://doi.org/10.1002/(SICI)1098-2280(1998)32:33.3.CO;2-I

70. Hu Y, Li J, Yang L, Nan W, Cao X, Bi Y. Inhibition of root growth by narciclasine is caused by DNA damage-induced cell cycle arrest in lettuce seedlings. Protoplasma. 2014 Sep;251:1113-24. https://doi.org/10.1007/s00709-014-0619-y

71. Yan S, Wang W, Marqués J, Mohan R, Saleh A, Durrant WE, Song J, Dong X. Salicylic acid activates DNA damage responses to potentiate plant immunity. Molecular cell. 2013 Nov 21;52(4):602-10. https://doi.org/10.1016/j.molcel.2013.09.019

72. Liu W, Zhu LS, Wang J, Wang JH, Xie H, Song Y. Assessment of the genotoxicity of endosulfan in earthworm and white clover plants using the comet assay. Archives of environmental contamination and toxicology. 2009 May;56:742-6. https://doi.org/10.1007/s00244-009-9309-8

73. Türko lu . Determination of genotoxic effects of chlorfenvinphos and fenbuconazole in Allium cepa root cells by mitotic activity, chromosome aberration, DNA content, and comet assay. Pesticide Biochemistry and Physiology. 2012 Jul 1;103(3):224-30. https://doi.org/10.1016/j.pestbp.2012.06.001

74. Gichner T, Lovecká P, Kochánková L, Macková M, Demnerová K. Monitoring toxicity, DNA damage, and somatic mutations in tobacco plants growing in soil heavily polluted with polychlorinated biphenyls. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2007 Apr 20;629(1):1-6. https://doi.org/10.1016/j.mrgentox.2006.11.013

75. Santos CL, Pourrut B, Ferreira de Oliveira JM. The use of comet assay in plant toxicology: recent advances. Frontiers in Genetics. 2015 Jun 30;6:216. https://doi.org/10.3389/fgene.2015.00216

76. Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchi K, Sasaki YF. DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicological Sciences. 2001 May 1;61(1):92-9. https://doi.org/10.1093/toxsci/61.1.92

77. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2002 Aug 26;519(1-2):103-19. https://doi.org/10.1016/s1383-5718(02)00128-6

78. Singh NP, Stephens RE, Singh H, Lai H. Visual quantification of DNA double-strand breaks in bacteria. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1999 Oct 19;429(2):159-68. https://doi.org/10.1016/s0027-5107(99)00124-4

79. Solanky D, Haydel SE. Adaptation of the neutral bacterial comet assay to assess antimicrobial-mediated DNA double-strand breaks in Escherichia coli. Journal of microbiological methods. 2012 Nov 1;91(2):257-61. https://doi.org/10.1016/j.mimet.2012.08.009

80. Azevedo F, Marques F, Fokt H, Oliveira R, Johansson B. Measuring oxidative DNA damage and DNA repair using the yeast comet assay. Yeast. 2011 Jan;28(1):55-61. https://doi.org/10.1002/yea.1820

81. Chatterjee N, Luo Z. Cr-(III)-organic compounds treatment causes genotoxicity and changes in DNA and protein level in Saccharomyces cerevisiae. Ecotoxicology. 2010 Apr;19:593-603. https://doi.org/10.1007/s10646-009-0420-4

82. Martinez RS, Di Marzio WD, Sáenz ME. Genotoxic effects of commercial formulations of Chlorpyrifos and Tebuconazole on green algae. Ecotoxicology. 2015 Jan;24:45-54. https://doi.org/10.1007/s10646-014-1353-0

83. David RM, Winter MJ, Chipman JK. Induction of DNA strand breaks by genotoxicants in the alga Chlamydomonas reinhardtii. Environmental Toxicology and Chemistry: An International Journal. 2009 Sep;28(9):1893-900. https://doi.org/10.1897/08-349.1

84. Lah B, Malovrh S, Narat M, Cepeljnik T, Marinsek‐Logar R. Detection and quantification of genotoxicity in wastewater‐treated Tetrahymena thermophila using the comet assay. Environmental Toxicology: An International Journal. 2004 Dec;19(6):545-53. https://doi.org/10.1002/tox.20062

85. Parolini M, Binelli A. Cyto-genotoxic effects induced by three brominated diphenyl ether congeners on the freshwater mussel Dreissena polymorpha. Ecotoxicology and environmental safety. 2012 May 1;79:247-55. https://doi.org/10.1016/j.ecoenv.2012.01.008

86. Mamaca E, Bechmann RK, Torgrimsen S, Aas E, Bjørnstad A, Baussant T, Le Floch S. The neutral red lysosomal retention assay and Comet assay on haemolymph cells from mussels (Mytilus edulis) and fish (Symphodus melops) exposed to styrene. Aquatic Toxicology. 2005 Nov 10;75(3):191-201. https://doi.org/10.1016/j.aquatox.2005.08.001

87. Labieniec M, Biernat M, Gabryelak T. Response of digestive gland cells of freshwater mussel Unio tumidus to phenolic

compound exposure in vivo. Cell Biology International. 2007 Jul;31(7):683-90. https://doi.org/10.1016/j.cellbi.2006.12.005

88. Rajaguru P, Suba S, Palanivel M, Kalaiselvi K. Genotoxicity of a polluted river system measured using the alkaline comet assay on fish and earthworm tissues. Environmental and Molecular Mutagenesis. 2003;41(2):85-91. https://doi.org/10.1002/em.10134

89. Di Marzio WD, Saenz ME, Lemière S, Vasseur P. Improved single‐cell gel electrophoresis assay for detecting DNA damage in Eisenia foetida. Environmental and Molecular Mutagenesis. 2005;46(4):246-52. https://doi.org/10.1002/em.20153

90. Zang Y, Zhong Y, Luo Y, Kong ZM. Genotoxicity of two novel pesticides for the earthworm, Eisenia fetida. Environmental Pollution. 2000 May 1;108(2):271-8. https://doi.org/10.1016/s0269-7491(99)00191-8

91. Arnardottir ES, Nikonova EV, Shockley KR, Podtelezhnikov AA, Anafi RC, Tanis KQ, Maislin G, Stone DJ, Renger JJ, Winrow CJ, Pack AI. Blood-gene expression reveals reduced circadian rhythmicity in individuals resistant to sleep deprivation. Sleep. 2014 Oct 1;37(10):1589-600. https://doi.org/10.5665/sleep.4064

92. Cheung V, Yuen VM, Wong GT, Choi SW. The effect of sleep deprivation and disruption on DNA damage and health of doctors. Anaesthesia. 2019 Apr;74(4):434-40. https://doi.org/10.1111/anae.14533

93. Tenorio NM, Ribeiro DA, Alvarenga TA, Fracalossi AC, Carlin V, Hirotsu C, Tufik S, Andersen ML. The influence of sleep deprivation and obesity on DNA damage in female Zucker rats. Clinics. 2013;68:385-9. https://doi.org/10.6061/clinics/2013(03)OA16

94. Khairnar K, Sanmukh S, Chandekar R, Paunikar W. A simple and novel modification of comet assay for determination of bacteriophage mediated bacterial cell lysis. Journal of virological methods. 2014 Jul 1;203:33-8. https://doi.org/10.1016/j.jviromet.2014.02.028

95. Maronpot RR, Hobbs CA, Hayashi SM. Role of pathology peer review in interpretation of the comet assay. Journal of Toxicologic Pathology. 2018;31(3):155-61. https://doi.org/10.1293/tox.2018-0019

96. Rojas E, Lorenzo Y, Haug K, Nicolaissen B, Valverde M. Epithelial cells as alternative human biomatrices for comet assay. Frontiers in genetics. 2014 Nov 28;5:386. https://doi.org/10.3389/fgene.2014.00386

97. Jiang H, Mei YF. SARS–CoV–2 spike impairs DNA damage repair and inhibits V (D) J recombination in vitro. Viruses. 2021 Oct;13(10):2056. https://doi.org/10.3390/v13102056

98. Rauch A, Dupont A, Goutay J, Caplan M, Staessens S, Moussa M, Jeanpierre E, Corseaux D, Lefevre G, Lassalle F, Faure K. Endotheliopathy is induced by plasma from critically ill patients and associated with organ failure in severe COVID-19. Circulation. 2020 Nov 10;142(19):1881-4. https://doi.org/10.1161/CIRCULATIONAHA.120.050907