Application of Comet Assay to determine the DNA Damage in the model organism *Caenorhabditis elegans*

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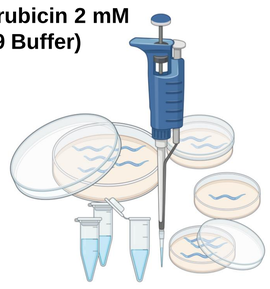
Introduction

The comet assay is a widely used method for measuring DNA damage and can be conducted under neutral or alkaline conditions. It is a well-established method in cell culture and in some rodent models. However, the application in the model organism *Caenorhabditis elegans* (*C. elegans*) is limited. In this study, we aim to use *C. elegans* for genotoxicity testing, bridging the gap between *in vitro* and *in vivo.*

We treated L4 staged worms with oxidative agent *tert*-butyl hydroperoxide (tBOOH, 0.75 mM for 1h), alkylating agent methyl methanesulfonate (MMS, 0.75 mM for 1 hour) and topoisomerase-II inhibitor doxorubicin (2mM for 1 hour). After treatment, single-cell suspension was obtained and alkaline comet assay was performed with 5 minutes of alkaline unwinding and 10 minutes of alkaline electrophoresis. Images were taken and a reliable scoring system was established. Scoring was performed blinded, by two independent researchers.

Experimental design

- tBOOH 0.75 mM
- MMS 0.75 mM
- Doxorubicin 2 mM (in M9 Buffer)



1. Treatment of L4 staged worms

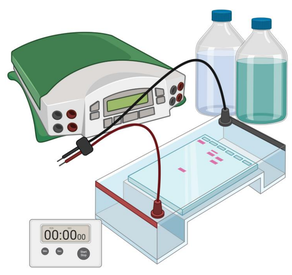
- Incubation with fresh buffer
- Incubation with papain
- Homogenization and filtering

2. Preparation of single-cell suspension

3. Slide preparation

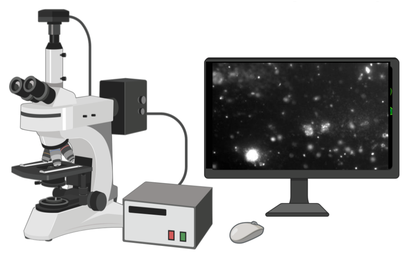
4. No lysis

- 5 min of alkaline unwinding
- 10 min of electrophoresis



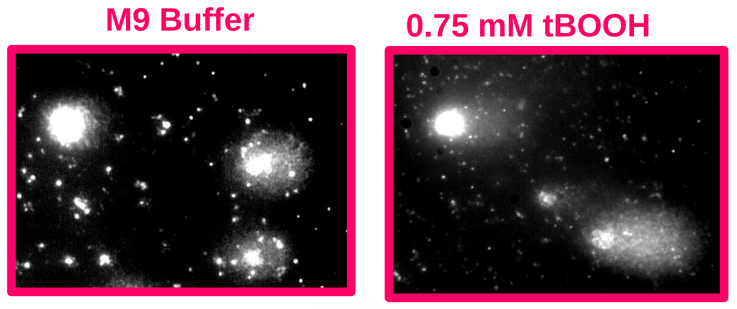
5. Alkaline Unwinding and Electrophoresis

50 comets were scored per sample



6. Staining, Visualisation and Scoring

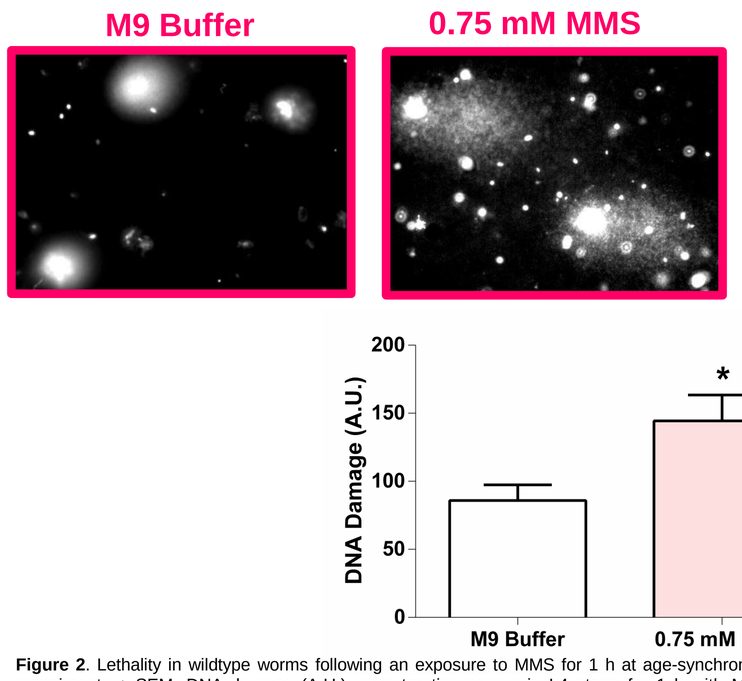
Results of comet assay with 0.75 mM tBOOH



* p ≤ 0.05 vs. M9 Buffer

Figure 1. Lethality in wildtype worms following an exposure to tBOOH for 1 h at age-synchronized L4 larvae stage. The data presented are mean values of n ≥ 4 experiments ± SEM. DNA damage (A.U.) upon treating worms in L4 stage for 1 h with tBOOH and performing comet assay. Slides were scored blinded by 2 independent researchers.

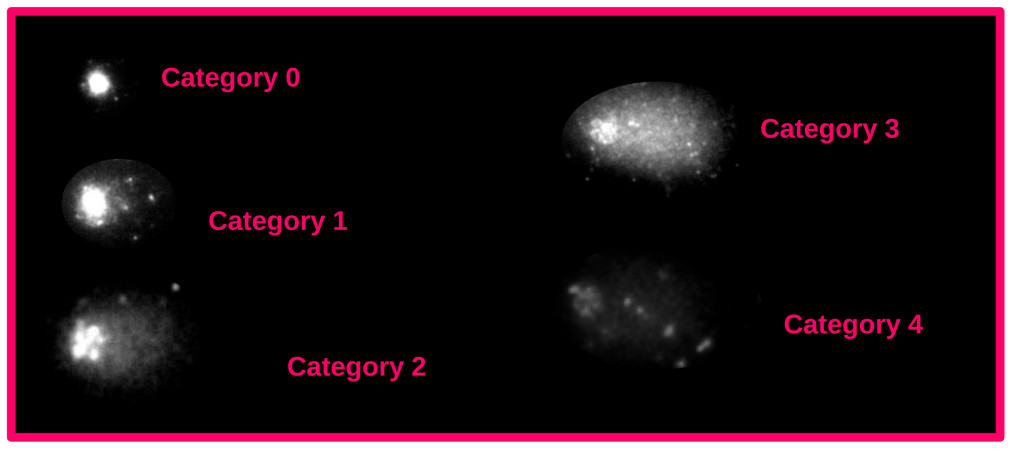
Results of comet assay with 0.75 mM MMS



* p ≤ 0.05 vs. M9 Buffer

Figure 2. Lethality in wildtype worms following an exposure to MMS for 1 h at age-synchronized L4 larvae stage. The data presented are mean values of n ≥ 4 experiments ± SEM. DNA damage (A.U.) upon treating worms in L4 stage for 1 h with MMS and performing comet assay. Slides were scored blinded by 2 independent researchers.

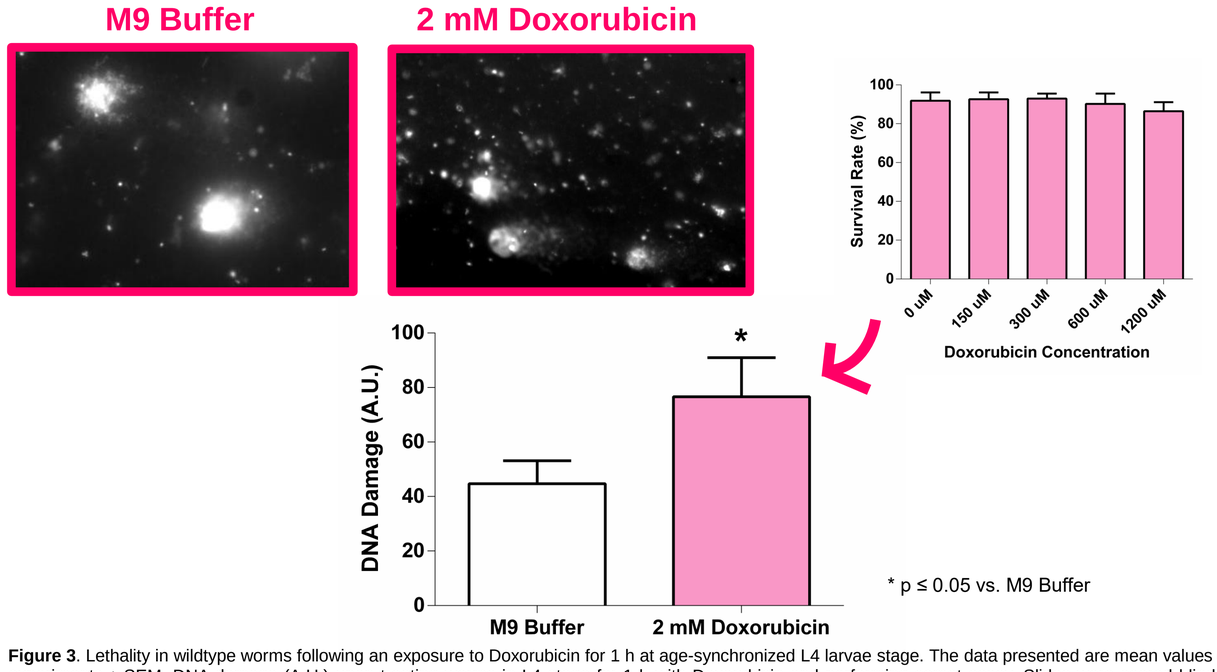
Comet Categories



$$\text{Arbitrary Unit (A.U.)} = N_{\text{category 0}} * 0 + N_{\text{category 1}} * 1 + N_{\text{category 2}} * 2 + N_{\text{category 3}} * 3 + N_{\text{category 4}} * 4$$

N - number of comets per category
Arbitrary unit represents DNA damage

Results of comet assay with 2 mM Doxorubicin



* p ≤ 0.05 vs. M9 Buffer

Figure 3. Lethality in wildtype worms following an exposure to Doxorubicin for 1 h at age-synchronized L4 larvae stage. The data presented are mean values of n ≥ 4 experiments ± SEM. DNA damage (A.U.) upon treating worms in L4 stage for 1 h with Doxorubicin and performing comet assay. Slides were scored blinded by 2 independent researchers.

Conclusion

The alkaline comet assay was successfully performed using worms and a five-class scoring system was established for the worm comet assay. Worms treated with tBOOH, MMS and doxorubicin showed increased DNA damage, which was evident to two independent researchers.

The findings showed that visual scoring is reliable. Furthermore, we intend to challenge our system with a longer time of incubation for further validation of the method.