Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Origami Paper-Based Analytical Device for DNA Damage Analysis

Chemicals and reagents. TUNEL Apoptosis Detection Kit (FITC) was purchased from YEASEN (Shanghai, China). The forward primers (actbF: 5'-GGCCCATCCATCGTTCACAG-3') and reverse primers (actbR: 5'-CGAGAGTTTAGGTTGGTCGTTCG-3') were synthesized by TaKaRa Bio Inc. (Dalian, China). Human alkyladenine DNA glycosylase (AAG), formamidopyrimidine-DNA glycosylase (FPG), Nuclease P1 and apurinic/apyrimidinic endonuclease (APE) were acquired from New England Biolabs (Beijing, China). Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), 0.25% Trypsin-EDTA and Calf Intestinal Alkaline Phosphatase (CIAP) were obtained from Thermo Scientific (Shanghai, China). N7-Methylguanine (7meG) was purchased from Macklin (Shanghai, China). 8-oxo-7,8-dihydro-2-deoxyguanosine (8oxodG) was purchased from Aladdin (Shanghai, China). MTT Cell Proliferation and Cytotoxicity Assay Kit was purchased from BBI Life Sciences (Shanghai, China). Triton X-100 cell lysis buffer was obtained from NOVON (Beijing, China). TIANAmp Genomic DNA Kit was purchased from TIAN GEN (Beijing, China). Deoxyribonuclease II (DNase II), phosphodiesterase I and all other chemicals were purchased from Sigma-Aldrich.

Fabrication of oPAD. The paper device was designed using a Microsoft PowerPoint. Wax was printed onto Whatman Grade 1 using a solid wax printer (Xerox Color Qube 8570), followed by heating at 120°C for 1 min. This produced a patterned paper with a circular testing zone (0.4 cm in diameter). 5 μL of Triton X-100 cell lysis buffer was applied onto panel A, and all-TUNEL reagents containing 1 μL of 10 × TdT reaction buffer (TRB), 1 μL of 350 nM TdT, 2 μL of 100 μM FdU in 10% (w/v) pullulan solution were printed onto panel B. After dried at RT, a transparent film was formed onto the paper. The paper device was stored in a desiccant container.

TUNEL reaction on paper. ZFL cells (~ 10^6) were first exposed to 50 mM H_2O_2 for 30 min. Genomic DNA was extracted by a DNA extraction kit according to the manufacturer's instructions. 1 μL of genomic DNA (2 ng), 1 μL of 10 × TRB, 1 μL of 350 nM TdT, and 2 μL of 100 μM FdU (final volume: 10 μL) were added onto the

paper chip. The mixture was incubated at room temperature (RT) for 20 min before adding 5 μL of 0.5 M EDTA (pH 8.0) to stop the TUNEL reaction. After washed twice with 20 μL of 1 × PBS, the paper well was imaged, and the fluorescence intensity was quantified by ImageQuant software. In terms of reaction kinetics on paper, 1 μL of genomic DNA (0.2 ng) was tested according to the protocol described above.

Detection of 3' OH ends by standard TUNEL assay. The H_2O_2-treated ZFL cells were fixed with 4% paraformaldehyde for 20 min, and then incubated with 20 ug/mL proteinase K in 1 × PBS for 10 min. These cells were mixed with 35 nM TdT and 20 μM FdU in 1 × TRB, and incubated at 37 °C for 60 min. Followed by washing, and staining with DAPI for 15 min, these cells were imaged using a confocal laser scanning microscope.

Detection of 3' OH ends by oPAD. For measuring 3' OH ends in genomic DNA, 1 μL of DNA with different amounts (0.17 pg, 1.7 pg, 17 pg, 170 pg, 1.7 ng, 17 ng, and 170 ng) was applied to panel A. By folding the device (panels B and A are folded together and onto panel C), 10 μL of ddH_2O was added onto panel B to rehydrate the TUNEL reagents. The released components will migrate to panel A driven by vertical capillary action. Following incubation at RT for 20 min, panel A was washed twice with 20 μL of 1× PBS. The fluorescence intensity was quantified by ImageQuant software.

For measuring 3' OH ends in cells, ZFL cells (~ 10^6) were first treated with 50 mM H_2O_2 for 30 min. 10 μL of cell samples with different numbers (1, 10, 10^2, 10^3, 10^4, and 10^5) were added onto panel A. Followed by lysis at RT for 5 min, the paper chip was washed twice using 50 μL of 1× PBS. By folding the device as described above, 10 μL of ddH_2O was added onto panel B to rehydrate TUNEL reagents. The TUNEL reaction was allowed to proceed at RT for 20 min on panel A before washed twice with 20 μL of 1× PBS. The fluorescence signal was recorded and analyzed by ImageQuant software.

Detection of 7meG sites by oPAD. For the fabrication of oPAD, 5 μL of 10% (w/v) pullulan mixtures containing 10 U AAG and 10 U of APE were printed onto the dried pullulan film containing TUNEL reagents on panel B. ZFL cells (~ 10^5) were first exposed to 1 mM DMS at 0°C for 30 min. The protocol for measuring 7meG in cells was similar to the one described above.

Analysis of genotoxicity of environmental toxicants. ZFL cells (~10^6) were exposure to different concentrations of ZnO (3 μM, 12 μM, 48 μM, and 192 μM), K_2CrO_4 (75 μM, 150 μM, 300 μM, and 600 μM) and paraquat (120 μM, 240 μM, 480 μM, and 960 μM) at 37 °C for 4 h. These cells (~ 5× 10^5) were then applied to panel A according to the protocol described above.

Dissociation of ZFL cells from primary tissue of zebrafish. The liver tissue of zebrafish was first cut into 3 to 4 mm pieces using a sterile scissors. After washing twice with 1 × PBS, 400 μL of 0.25% trypsin-EDTA was added, and incubated at room temperature (RT) for 2 min. The obtained cell suspension was mixed with 800 μL of DMEM, and filtered through a sterile nylon mesh to separate the cells and tissue fragments. The obtained mixture was then centrifuged at 2,000 g at 4 °C for 10 min. The purified ZFL cells were re-suspended in DMEM containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, and used in the following study.

DNA extraction efficiency of paper. 5 μL of ZFL cells (1, 10, 10^2, 10^3, 10^4, and 10^5) were applied onto panel A for cell lysis. After 5 min, the paper chip was washed twice with 50 μL of 1 × PBS. The extracted DNA was quantified by RT-PCR. Thermal cycles were typically performed as follows: 95 °C for 3 min, n cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s; 72 °C for 10 min.

Comet assay. The treated ZFL cells were firstly mixed with PBS containing 0.7% agarose at 37 °C, and pipetted onto the agarose-covered (PBS with 1% normal agarose) slides. After incubation at 4 °C for 10 min, the slides were immersed into the lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium lauroyl sarcosine, 1%Triton-X-100, 10% DMSO, pH = 10) at 4 °C for 90 min under dark conditions. After washed twice with ddH_2O, electrophoresis was carried out in

alkaline electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH = 13) at 25 V, 300 mA for 20 min at 4 °C. The slides were washed three times with ddH_2O and immersed into the neutralization buffer (0.4 M Tris, pH 7.5) for 15 min. The genomic DNA was stained with GelRed for 60 min, and imaged using a fluorescence microscope. The degree of DNA damage was assessed by the distance between the center of the head and the tail, defined as the olive tail moment (OTM).

Detection of modified bases using LC-MS^2.

5 μg of extracted alkylated DNA was first mixed with 10 U AAG in 100 μL of 1× TRB at 37 °C for 60 min. 7meG was collected by centrifugation through a 3 K membrane (NANOSEP OMEGA, Pall Incorporation) at 5,000 g for 30 min. 10 μg of genomic DNA extracted from H_2O_2/Fe^{2+}-treated cells was mixed with 10 U Nuclease P1, 5 U DNase I, 0.002 U phosphodiesterase I and 10 U CIAP in 100 μL of 1× TRB at 37 °C for 60 min. 8oxodG was obtained by centrifugation through a 3 K membrane at 5,000 g for 30 min. 7meG and 8oxodG were then analyzed by QQQ LC/MS^2 (Agilent 6410 B) with a XTERRA® MS C18 column (2.1×100 mm, 3.5 μm) at a flow rate of 0.25 mL/min at 40 °C. 5 mM NH_4OAc and CH_3CN were used as the mobile phase. The gradient elution started with 5% CH_3CN, increasing to 40% CH_3CN in 10 min, then held for 2 min. It returned to 5% CH_3CN in 0.01 min, and then held for 7 min for column back-conditioning. 7meG was detected by multiple reaction monitoring (MRM) using mass transition (precursor ions→ product ions, m/z 166.0 → m/z 149.0) for quantification, (precursor ions→ product ions, m/z 166.0 → m/z 124.0) for confirmation. The ion source parameters were as follows: gas temperature, 350 °C; gas flow, 8 L/min; nebulizer, 25 psi. 8oxodG was detected by multiple reaction monitoring (MRM) using mass transition (precursor ions→ product ions, m/z 284.0 → m/z 168.0) for quantification, (precursor ions→ product ions, m/z 284.0 → m/z 140.0) for confirmation.

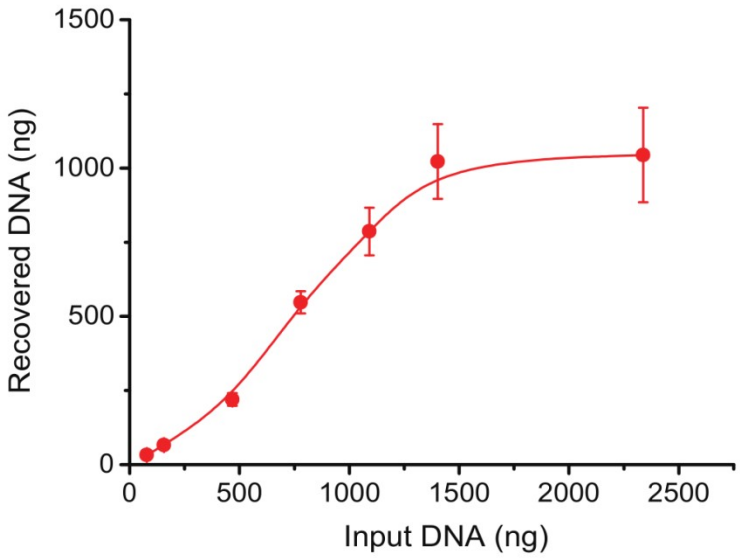

Figure S1. Characterization of DNA binding capacity of Whatman filter paper (Grade 1) using RT- PCR.

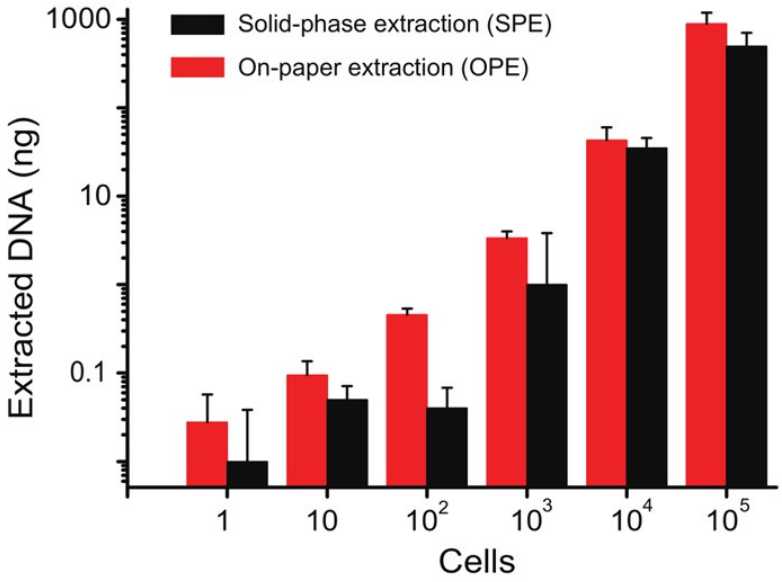

Figure S2. Comparison of DNA extraction yields between on-paper DNA extraction method and traditional solid-phase extraction method.

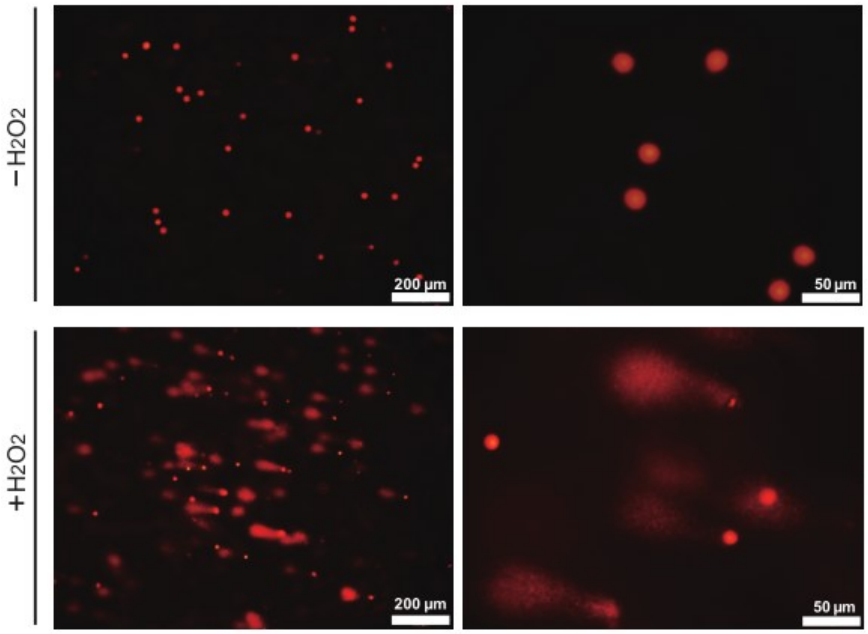

Figure S3. Traditional comet assay for examining DNA damage in ZFL cells. ZFL cells were first treated with or without 50 mM H_2O_2 at RT for 30 min.

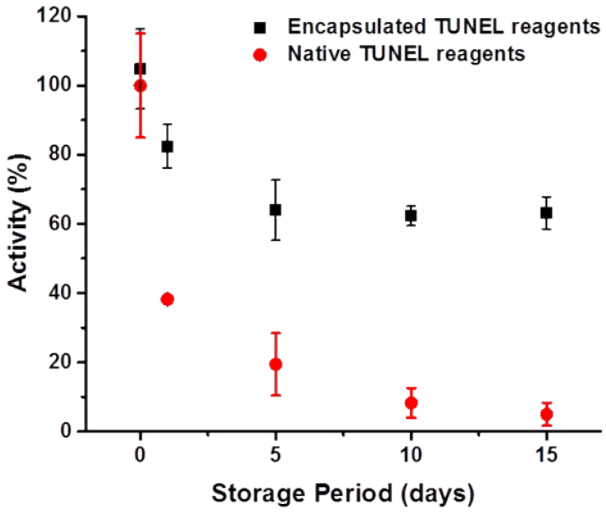

Figure S4. TdT activity loss during room-temperature storage with and without pullulan encapsulation.

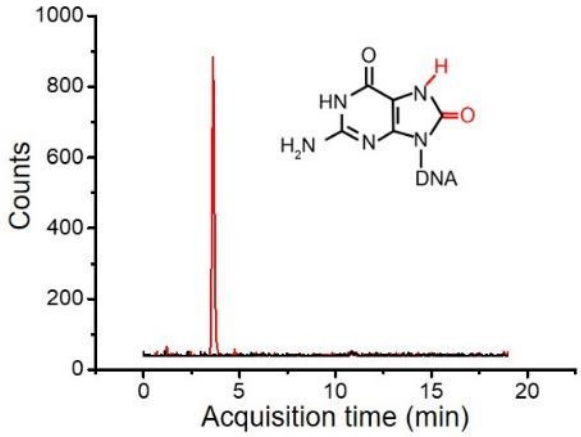

Figure S5. Multiple reaction monitoring (MRM) chromatogram of 8oxodG in ZFL cells pre-treated with 5 mM H_2O_2 and 1 mM Fe^{2+} at 37 °C for 2 h (red line). Non-treated ZFL cells were used as control (black line).

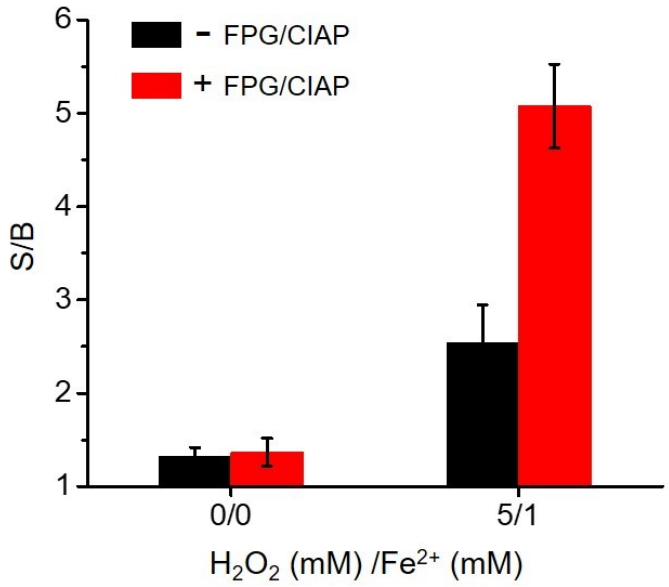

Figure S6. Fluorescence intensity of paper chips for H_2O_2/Fe^{2+}-treated and non-treated ZFL cells following TUNEL or FPG/CIAP-mediated TUNEL.

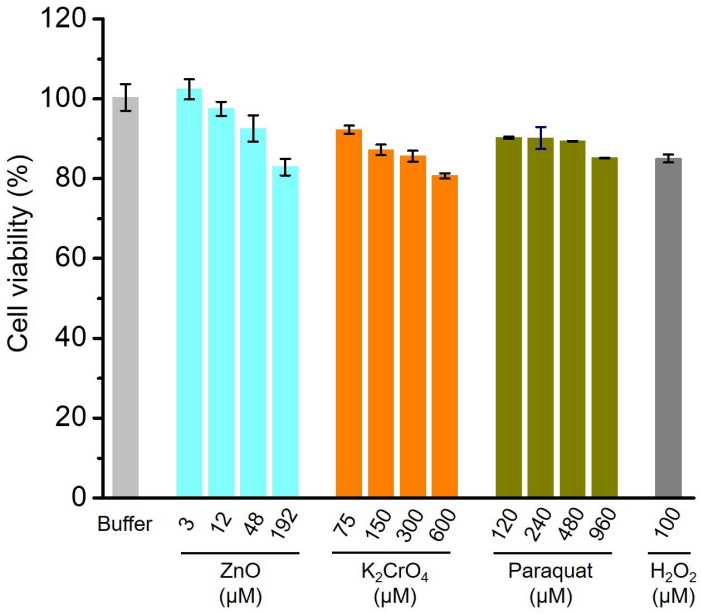

Figure S7. Cellular viability assessment of ZFL cells exposed to different concentrations of ZnO, K_2CrO_4, and paraquat.

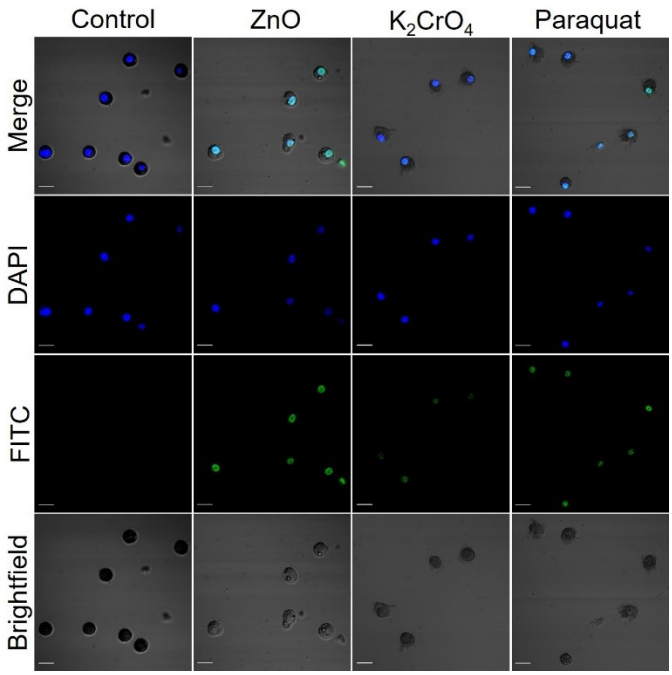

Figure S8. CLSM images of TUNEL reaction in ZFL cells after being treated with ZnO (48 μM), K_2CrO_4 (300 μM), and paraquat (480 μM). Scale bar: 10 μm.

Table S1. Comparison of Comet assay, traditional TUNEL and paper-based TUNEL assay for measuring DNA damages.

Comet Assay	**Time**	**Traditional TUNEL**	**Time**	**Paper-based TUNEL**	**Time**
Cell lysis	90 min	Cell fixation	20 min	Cell lysis	5 min
Washing	2 min	Digestion	10 min	Washing	2 min
Electrophoresis	20 min	TUNEL	60 min	TUNEL	20 min
Washing	2 min	Washing	2 min	Washing	2 min
Neutralization	15 min	DNA Staining	15 min	Imaging	1 min
DNA Staining	60 min	Washing	2 min		
Imaging	1 min	Imaging	1 min		