Supporting Information

Removal of toxic *Microcystis aeruginosa* using discharge plasma: mechanism and potential environmental risks

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2 Texts

2 Tables

7 Figures

Text S1. Chlorination experiments and analytical methods of DBPs

After the discharge plasma treatment, DOM samples with different treatment times were chlorinated with NaClO according to the DOC concentration (CDOC: CCl_2=1:3) [1], with the addition of 0.8 mL of phosphate buffer solution (pH=7.0). After 6 d of chlorination reaction in the dark, L-ascorbic acid was immediately added into the mixtures to prevent the residual chlorine reaction. Afterwards, each sample of 20 mL solution was used for DBP extraction and analysis. For THMs, HANs, HKs, HALs, and HNMs extraction, 4 mL methyl tert-butyl ether (MTBE) containing 8.0 g anhydrous sodium sulfate was added to the samples, and they were put under vortex movement for 2 min to complete the extraction. After 30 min of settling, 1 mL solution from the organic phase was drawn out and analyzed on an Agilent 6890 GC equipped with a DB-5 column (J&WScientific 30 m × 0.25 mm × 0.001 mm), and an electron capture detector (ECD). The GC temperature program: initial temperature at 35℃ for 9 min, rise to 40 ℃ at 2 ℃/min and hold for 1 min, rise to 80 ℃ at 20℃/min, rise to 160℃ at 40℃/min and hold for 4 min. 2 μL injection volumes were used in splitless mode. The carrier and make-up gases were ultra-high purity (UHP) hydrogen and UHP nitrogen, respectively. The total run time was 30 min. The injector and detector temperatures were set at 230 and 260 ℃, respectively. The minimum reporting limit for DBP measurements was 0.5 $\mu g \cdot L^{-1}$.

For HAAs extraction, 20 mL of HA samples was mixed with 1.0 mL H_2SO_4 and 8.0 g anhydrous sodium sulfate uniformly, and shaken by vortex until Na_2SO_4 was dissolved completely. 4 mL of MTBE was then added to the solution and put under vortex movement for 2 min to extract entirely. After 30 min of settling, 2 mL supernatant was drawn out and thoroughly mixed with 2 mL acidified methanol (10% H_2SO_4) in a glass bottle, and heated in water bath at 50 ℃ for 2 h to complete the derivatization of HAAs. 5 mL Na_2SO_4 (150 $g \cdot L^{-1}$) was added to the above cooled sample, and the solution was settled until stratified absolutely. The next step was to remove lower layer solution. 1 mL saturated $NaHCO_3$ solution was added into the glass bottle, and the solution was shaken vigorously to release the CO_2. After 5 min of settling, 1 mL solution from the MTBE phase was drawn out, and analyzed on an Agilent 7890 GC [2]. The GC temperature program: initial temperature at 40 ℃ for 10 min, rise to 65 ℃ at 2.5 ℃/min, rise to 85 ℃ at10 ℃/min, rise to 205 ℃ at 20 ℃/min and hold for 5 min. 2 μL injection volumes was used in splitless mode. The carrier and make-up gases were ultra-high purity (UHP) hydrogen and UHP nitrogen, respectively. The total run time was 24.5 min. The injector and detector temperatures were set at 200 and 300 ℃, respectively. The minimum reporting limit for each HAA species measurements was 0.5 $\mu g \cdot L^{-1}$.

Text S2. Cytotoxicity test

The CHO-k1 cell line purchased from the American Type Culture Collection was used to perform the toxicity assay. The culture medium DMEM/F12 (1:1) was added with streptomycin (0.1 $mg \cdot mL^{-1}$), penicillin G (100 $unit \cdot mL^{-1}$), and fetal bovine serum (10%). Cells culture dishes were placed in an incubator with saturated humidity and 5% CO_2 gas at 37 ℃. Cells undergoing 2-5 passages were used for the toxicity assay. Cell passaging was conducted every 48 h.

To perform cytotoxicity assay, 1×10^4 cells per well was seeded in a sterile 96S5 well plate (3599, Corning, USA), and cultured for 12 h before the cells were exposed to organic byproducts. The dried organic extracts were firstly dissolved in 1mL DMEM/F12 containing 0.5% DMSO (V:V). Then 100 μL of cell cultures were exposed to 100 μL of the solution for 72 h. DMEM/F12 containing 0.5% DMSO was used as the negative control, while phenol dissolved in DMEM/F12 containing 0.5% DMSO was used as the reference compound. All the 96-well plates for toxicity assay were covered with the sterilized seal films to avoid the cross contamination. After 72 h exposure, the culture medium was discarded. Cells were washed with 100 μL phosphate buffered solution (PBS). Then 100 μL of the reagent in Cell Counting Kit-8 (CCK-8) dissolved in DMEM/F12 was added into each well of the 96-well plates. Cells were then cultured in the incubator (37℃) for another 2 h, then the

absorbance of each well at 450 nm was measured using the microplate reader SpectraMax i3 (Molecular Devices, USA). Each test was performed in 6-12 replicates.

The cell viability (CV) for a sample or reference compound was calculated according to the following formula [3]:

$$CV = (A_S - A_B)/(A_N - A_N)$$

where CV is the cell viability for a sample or phenol against the negative control. The A_S is the absorbance of the sample or phenol at 450 nm. A_B is the absorbance of the blank control (only added the Cell Counting Kit-8 reagent dissolved in DMEM/F12) at 450 nm. The A_N is the absorbance of the negative control at 450 nm.

Table S1. Molecular weight and LC_{50} values of different DBPs in this study

DBPs	MW (g mol^{-1})	LC_{50} (M)	Reference
Trichloromethane (TCM)	119.38	9.62E-03	[4]
Trichloroacetic acid (TCAA)	163.38	2.40E-03	[5]
Dichloroacetic acid (DCAA)	128.94	7.30E-03	[4]
1,1,1-Trichloropropanone (1,1,1-TCP)	147.42	NA	NA
1,1-Dichloro-2-Propanone (1,1-DCP)	126.96	NA	NA
Chloral hydrate (CH)	165.40	1.16E-03	[6]
Dichloroacetonitrile (DCAN)	109.94	5.73E-05	[6]
Trichloroacetonitrile (TCAN)	144.38	1.60E-04	[4]
Trichloronitromethane (TCNM)	164.38	5.36E-04	[7]

Table S2. UV-Vis characteristic value of DOM with different treatment times

Treatment time (min)	E2/E3	E3/E4	$SUVA_{254}$
0	6.15	4.06	0.28
1	5.96	6.77	0.62
2	6.88	6.28	0.69
3	8.58	7.48	0.63
5	10.86	10.39	0.53
10	12.19	9.52	0.36
15	14.58	9.78	0.38
20	16.74	12.24	0.35
30	23.14	13.29	0.44
40	28.41	13.81	0.51

E2/E3: UVA_{250}/UVA_{365}; E3/E4: UVA_{300}/UVA_{400}; $SUVA_{254}$: $UV_{254}\times100/DOC$, $L\cdot(mg\cdot m)^{-1}$.

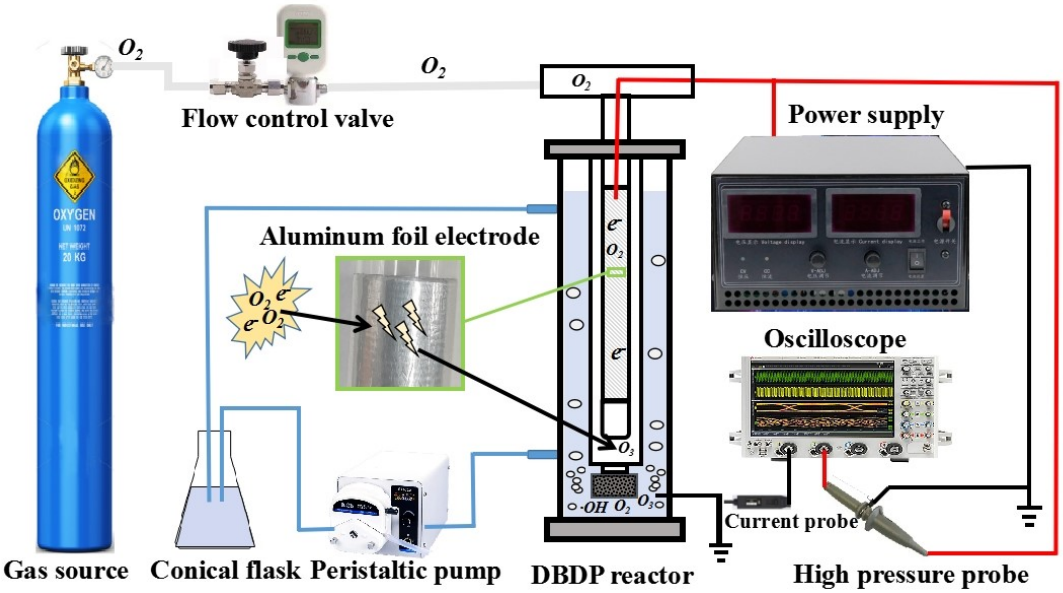

Fig. S1. Experimental system

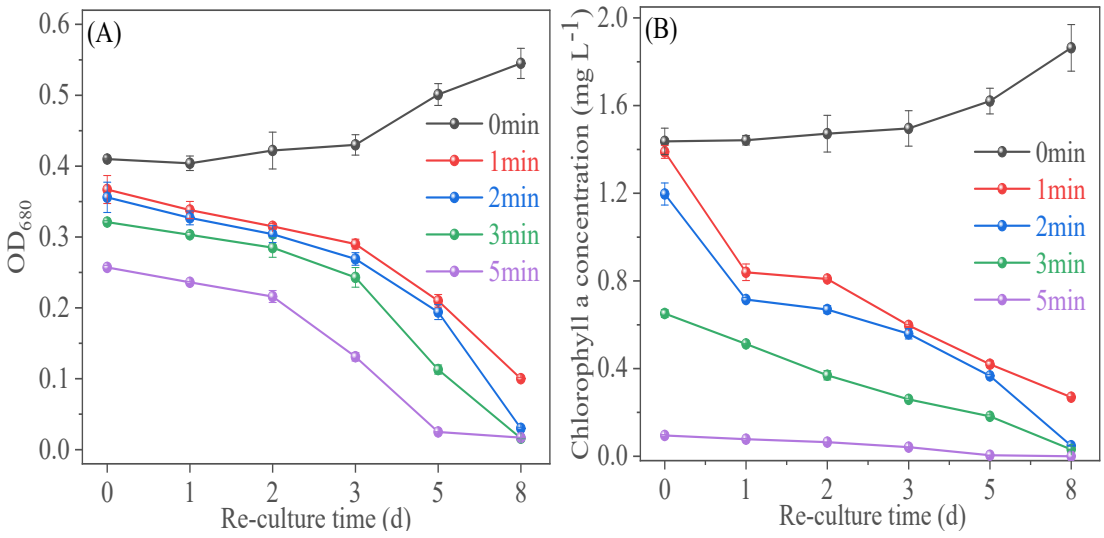

Fig. S2. The chlorophyll a and OD_{680} of re-cultured *M. aeruginosa* cells after discharge plasma treatment

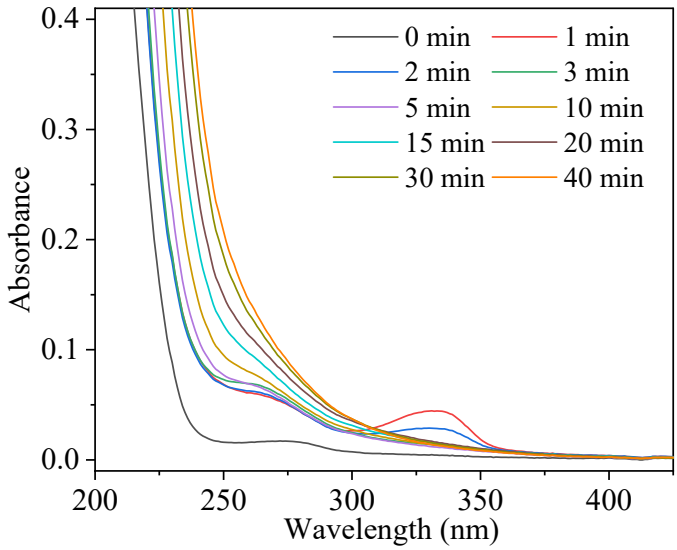

Fig. S3. UV-Vis spectra of DOM with different treatment times

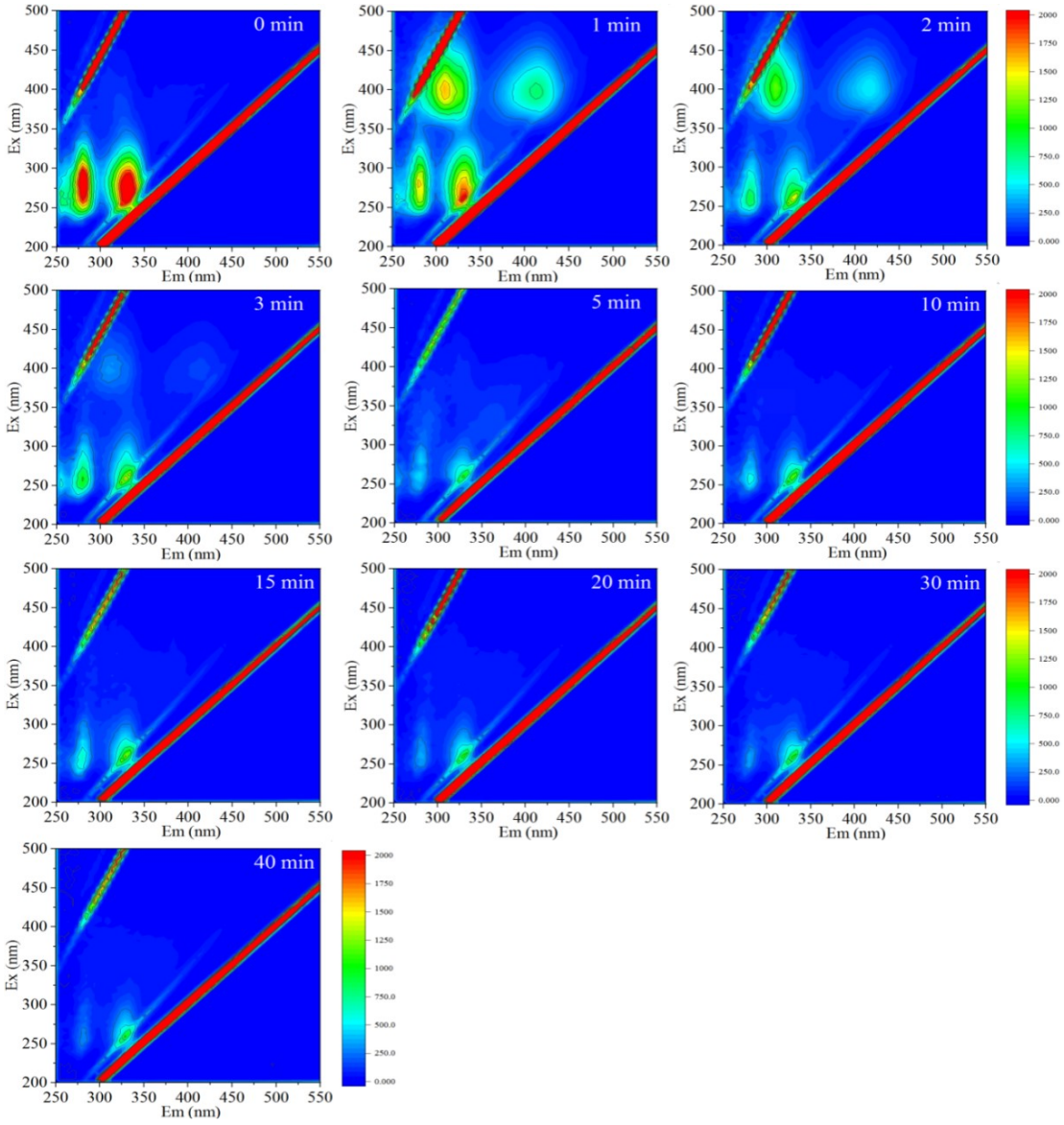

Fig. S4. EEM contour maps of DOM during discharge plasma treatment process

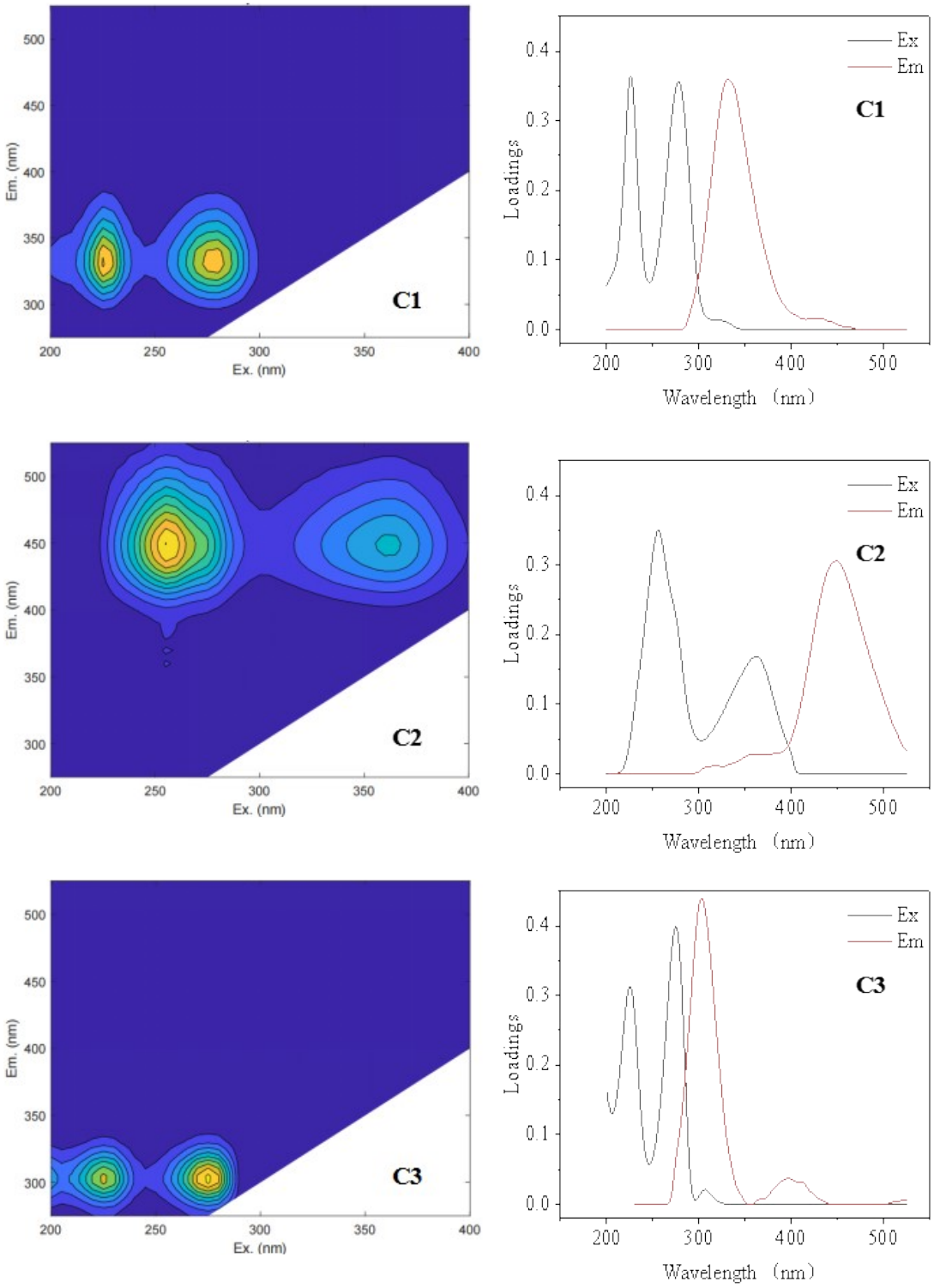

Fig. S5. Three PARAFAC components (C1, C2 and C3) and their corresponding wavelength loadings identified by EEM-PARAFAC analysis

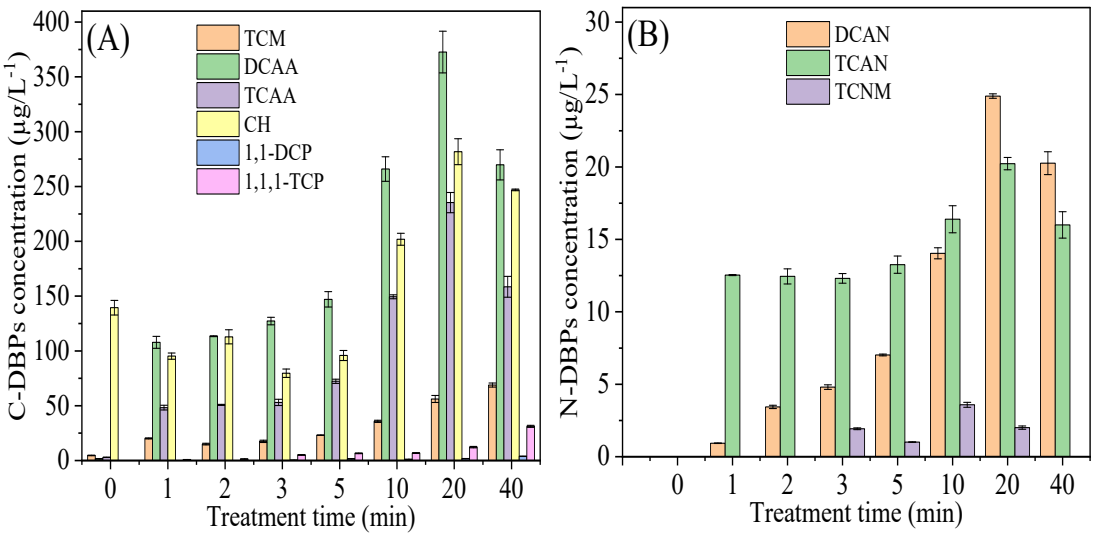

Fig. S6. Concentrations of DBPs: (A) concentrations of C-DBPs, (B) concentrations of N-DPBs

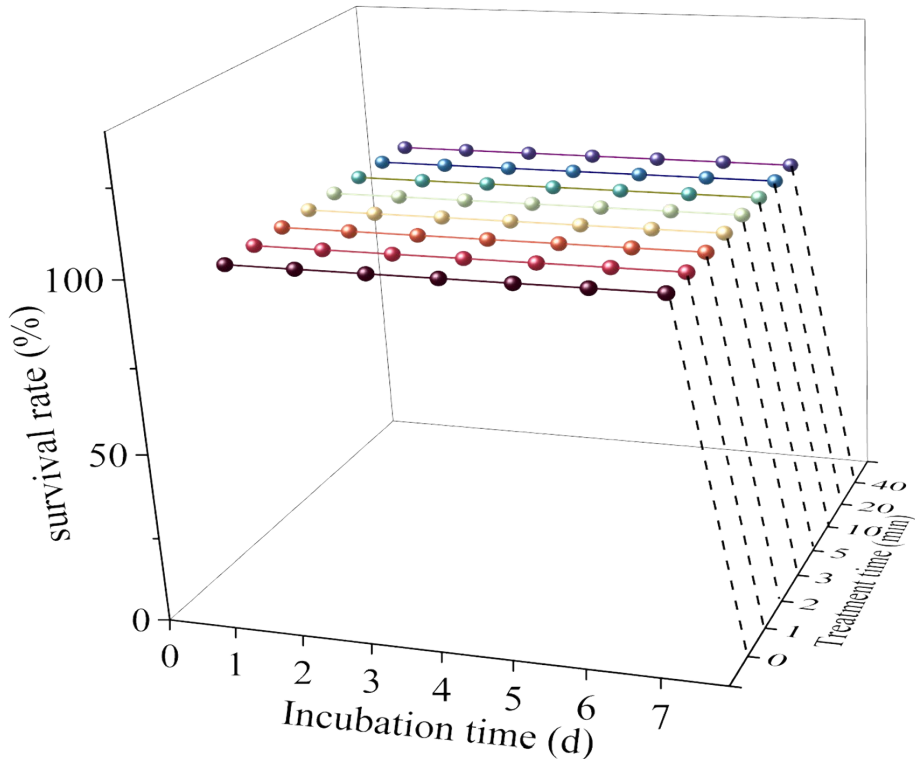

Fig. S7. The survival rate of zebrafish cultured in DOM sample solution with different plasma treatment time for 7 days

References

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