Electronic Supplementary Information

Functional silver-based nanomaterials affecting zebrafish development: the adverse outcomes in relation to the nanoparticle physical and chemical structure

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This Electronic Supplementary Information document (ESI) contains one text section, N° 7 figures and N°2 tables

Materials and Methods

S1: *Chemicals*

All analytical-grade reagents, Tricaine (MS-222), reagents for histology and scanning electron microscopy analysis, salts for FET solution, except Instant Ocean (Aquarium systems, Sarrebourg, France) were purchased from Sigma-Aldrich S.r.l., Italy.

S2: *Animals*

A breeding colony of Wild-type zebrafish (AB strain) was raised in a facility at University of Milano-Bicocca, Dept. of Environmental and Earth Sciences (Italy), according to the Italian laws, rules and regulations (Legislative Decree no. 116/92; authorization n. 0020984-12/02/2018). In detail, adults were maintained at 28 °C with a 14/10 h light/dark cycle in a closed flow-through system (Tecniplast s.p.a., Buguggiate Italy). Water supplied to the system was filtered by reverse osmosis (pH 7.5–8), and Instant Ocean® salt was added to the water to raise the conductivity to ~500 μS/cm (system water).

Zebrafish embryos were collected after a natural spawning of adult zebrafish pairs, located in breeding tanks and separated by sex (3:2 male to female ratio) with a barrier overnight (ON). The next morning at the onset of light, the barrier was removed and adults were allowed to mate. Fertilized eggs were collected in a strainer within 30 min after mating and sorted to eliminate faeces and unfertilized eggs, then selected and developmentally staged by hours post fertilization (hpf) under a stereomicroscope (Zeiss, Germany).
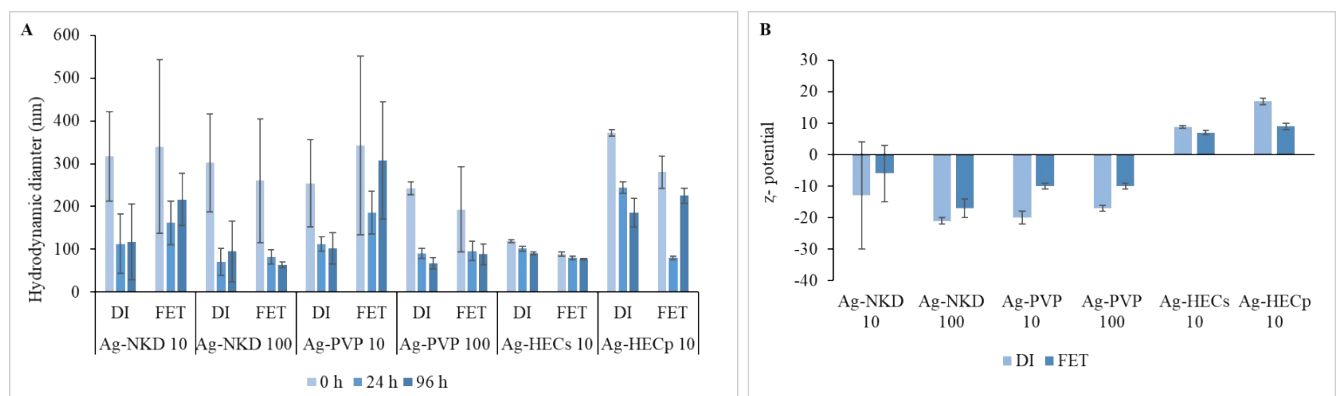
Results

FIGURES

Fig. S1 Physical-chemical properties of the Ag-NPs. Hydrodynamic diameter (A) and ζ potential (B) by Dynamic and Electrophoretic Light Scattering. The average size and standard deviation were calculated using the maximum peak values for each hydrodynamic and ζ-potential measurements from triplicate trials for each suspension. Data are represented as mean ± SD. FET: embryo rearing solution; DI: distilled water.
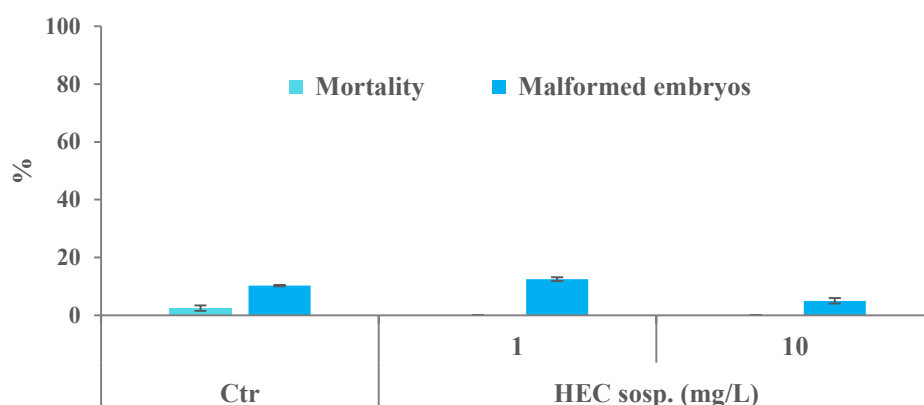

Fig. S2 FET test on hydroxyethyl cellulose suspension (HECs). Mortality and malformation rates in 96 hpf zebrafish embryos after exposure to 1 and 10 mg/L of HECs.
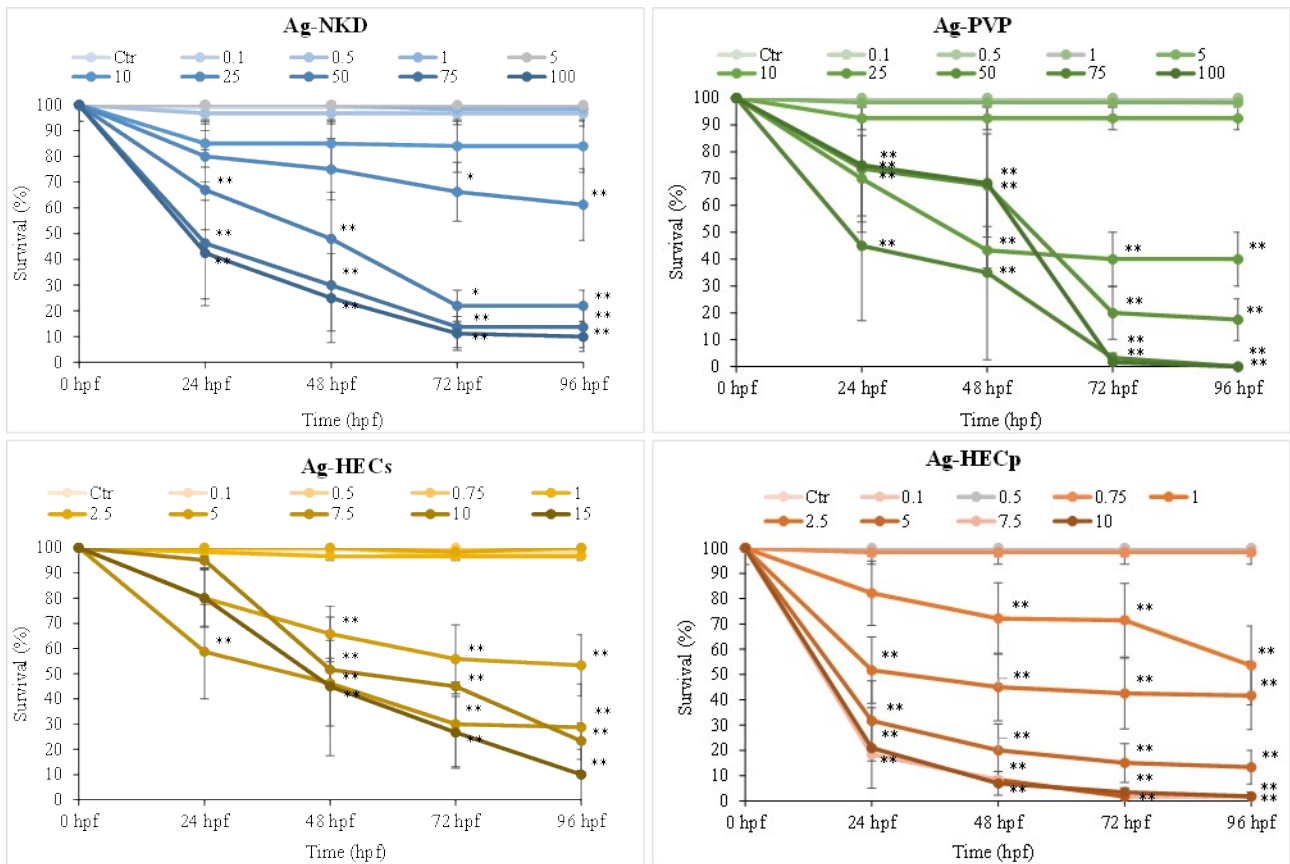
Fig. S3 Cumulative daily survival rate in zebrafish embryos exposed to Ag-NPs during FET. All values in the graphs are given as mean ± SE of at least three independent assays. *p < 0.05, **p < 0.01 indicate statistical difference from control (ANOVA + post-hoc Bonferroni).
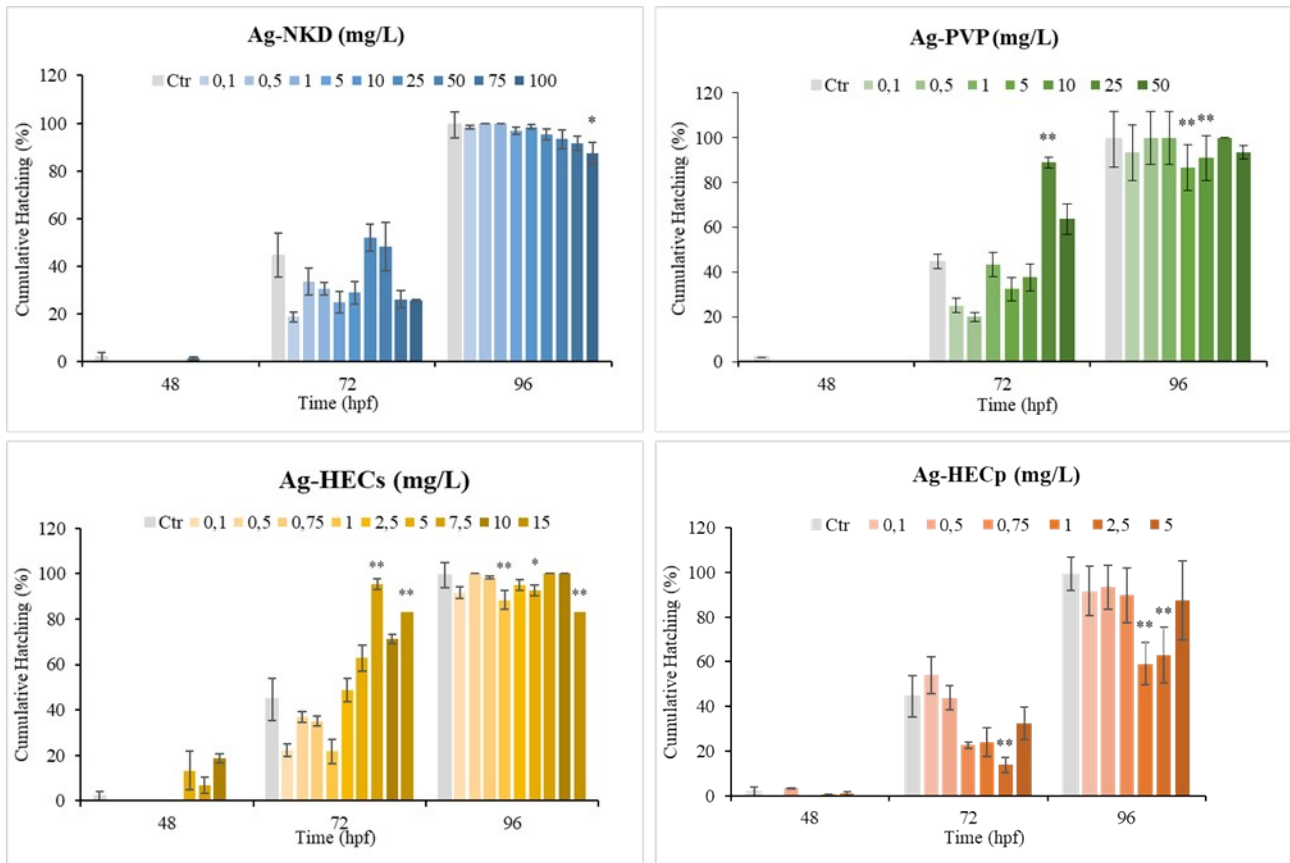
Fig. S4 Cumulative percentage of hatched embryos in control and Ag-NPs exposed zebrafish during FET. All values in the graphs are given as mean ± SE of at least three independent assays. *$p < 0.05$, **$p < 0.01$ indicate statistical difference from control (ANOVA + post-hoc Bonferroni)
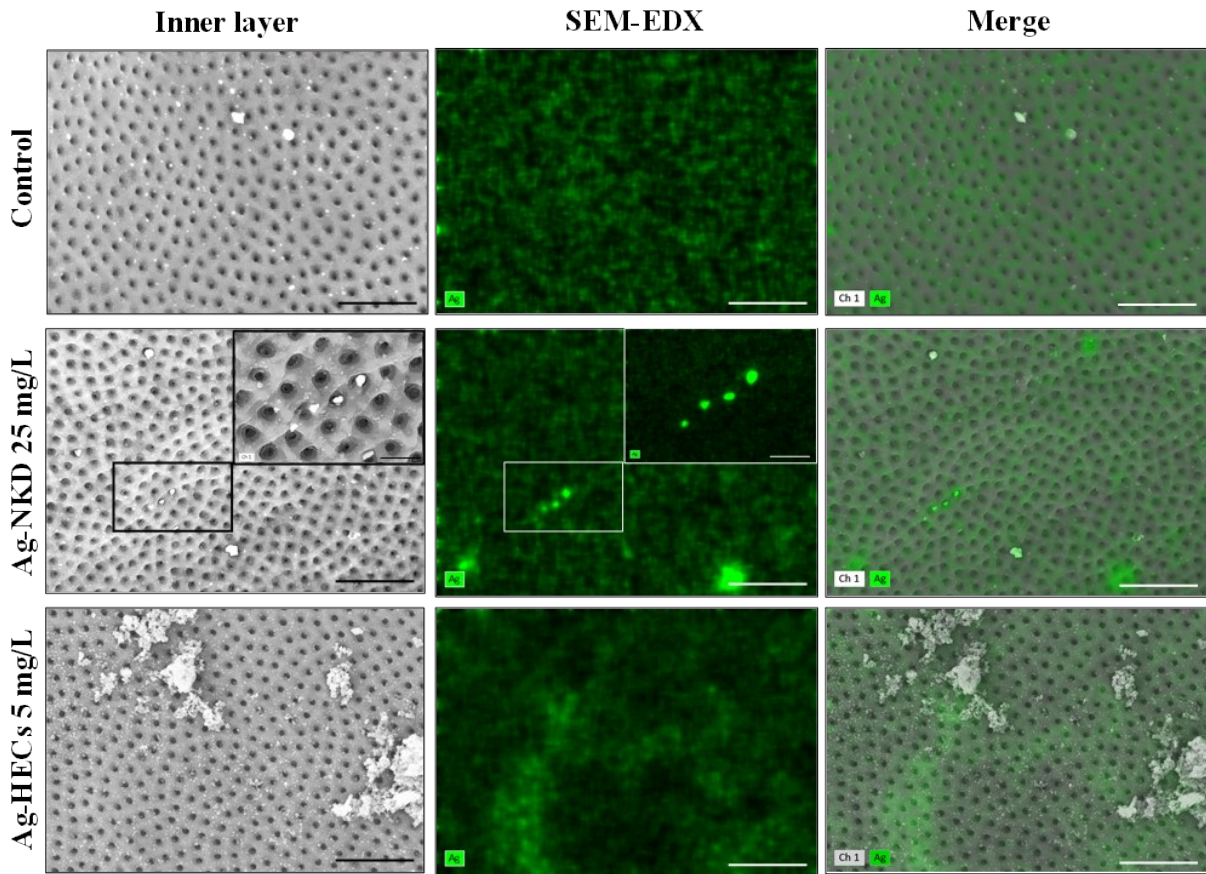
Fig. S5 Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDX) investigations of the 48 hpf embryos' chorion inner surface. Representative images of chorion inner layer from Control, Ag-NKD and Ag-HECs (column 1); Ag is detected by SEM-EDX microanalysis. EDX map of Ag (column 2); chorion surface overlay with Ag EDX map (column 3). The inset is providing a magnification of Ag detection. Scale bar = 8 µm; magnification scale bar = 2 µm
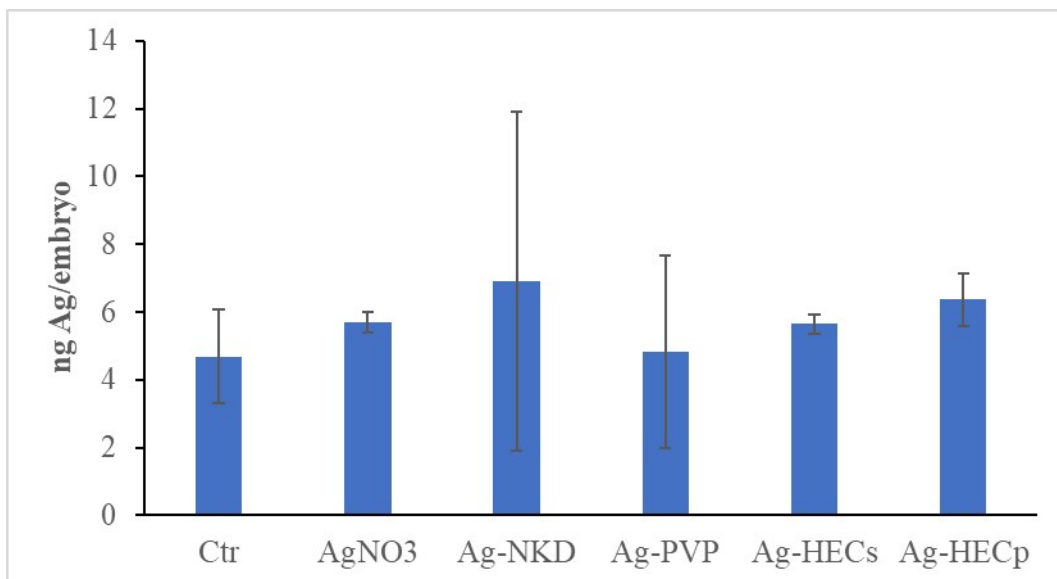
Fig. S6 Silver uptake in zebrafish larvae at the end of FET test. Total silver ion concentrations were measured by ICP-OES in 96 hpf zebrafish exposed to 10 mg/L of Ag-NKD and Ag-PVP, 1 mg/L of Ag-HECs and Ag-HECp, 0.01 mg/L AgNO$_3$. Data are presented as mean ± standard error of three independent pools of animals. No statistical difference from control and among different NPs (ANOVA + post-hoc Bonferroni).
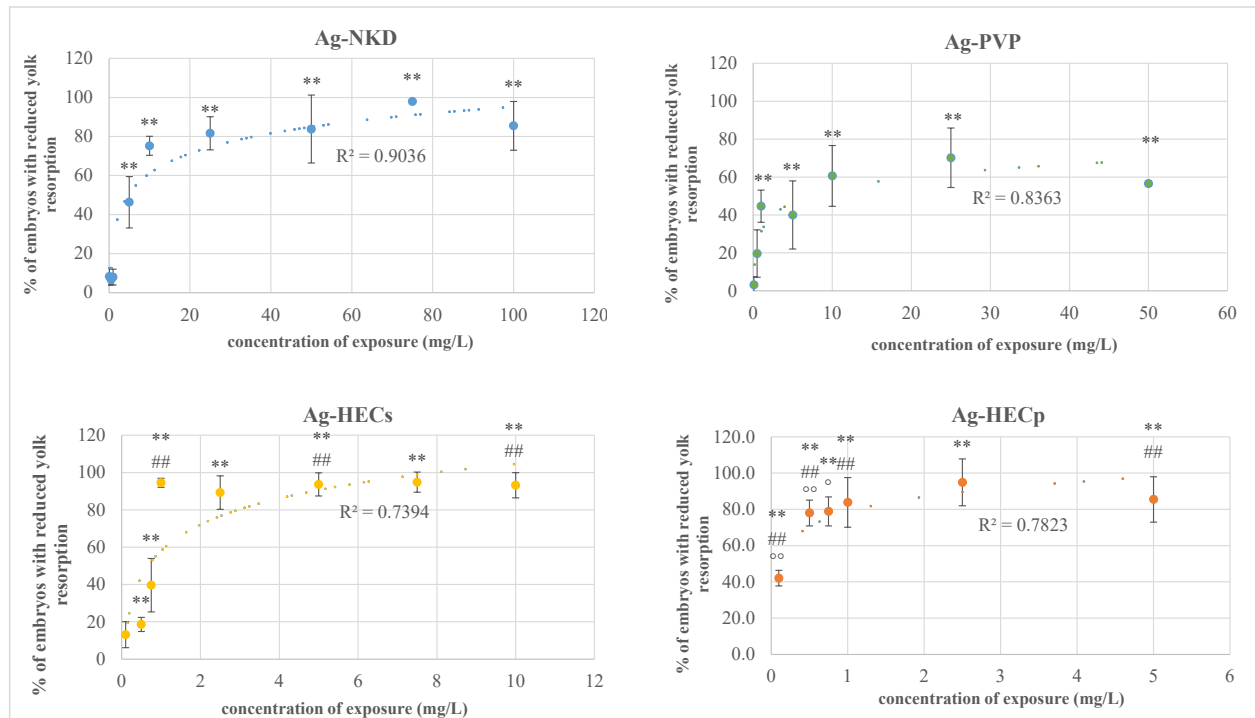
Fig. S7 Percentage of 96 hpf zebrafish embryos exposed to AgNPs with reduced yolk resorption. All values in the graphs are given as mean ± SD of at least three independent assays. The dotted lines represent a logarithmic interpolation of the data with the relative R^2. After log transformation of the concentrations, the straight line equations are: y = 34.215x + 26.795 for Ag-NKD, y = 22.295x + 30.982 for Ag-PVP, y = 46.532x + 58.114 for Ag-HECs, y = 27.411x + 78.672 for Ag-HECp. *p < 0.05, **p < 0.01 indicate statistical difference from control; #p < 0.05, ##p < 0.01 indicate statistical difference between Ag-HEC NPs and reference NPs; °p < 0.05, °°p < 0.01 indicate statistical difference between Ag-HECs and Ag-HECp NPs (ANOVA + Post-hoc Bonferroni).

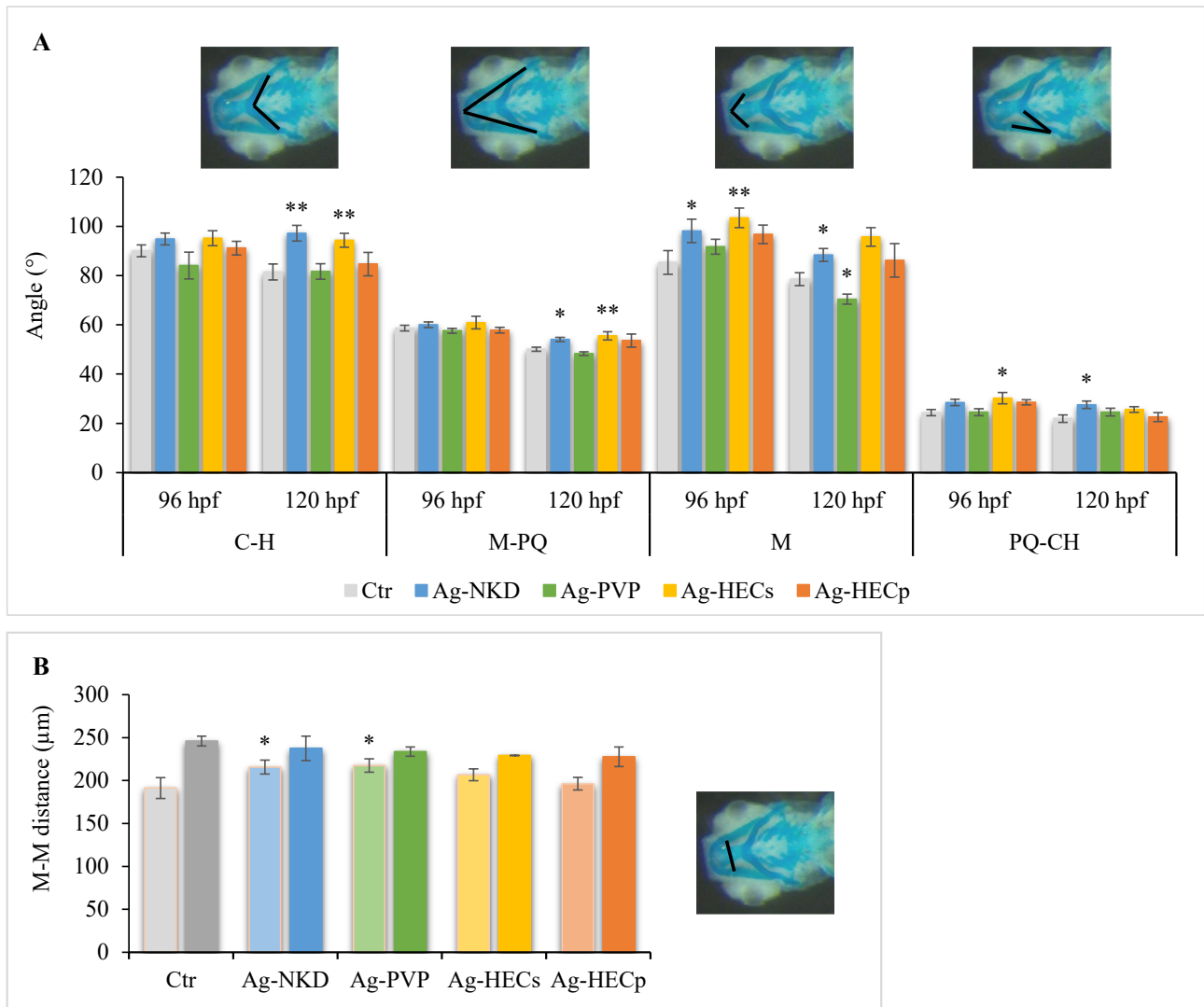Fig. S8 Craniofacial cartilages angles in 96 hpf (A) and 120 hpf (B) zebrafish embryos exposed until 96 hpf to Ag-NKD (10 mg/L), Ag-PVP (10 mg/L), Ag-HECs (5 mg/L) and Ag-HECp (1 mg/L) NPs. M = Meckel's cartilage; CH = ceratohyal cartilage; PQ = palatoquadrate cartilage

TABLES

Table S1 ICP-OES analysis for the determination of Ag-NPs dissolution in FET solution

	Exposure time (h)	Nominal concentration (mg/L)	Ag total concentration (mg/L)		Ag released (mg/L)		Percentage Ag released (%)	
			mean	standard deviation	mean	standard deviation	mean	standard deviation
Ag NKD	0	1	0.000	0.000	0.000	0.000	0.000	0.000
		10	2.567	0.350	0.000	0.000	0.000	0.000
		100	38.050	27.724	0.000	0.000	0.000	0.000
	24	1	0.000	0.000	0.000	0.000	0.000	0.000
		10	2.450	0.592	0.000	0.000	0.000	0.000
		100	36.283	13.176	0.117	0.041	0.322	0.117
	96	1	0.000	0.000	0.000	0.000	0.000	0.000
		10	2.667	1.140	0.000	0.000	0.000	0.000
		100	36.983	2.282	0.200	0.000	0.541	0.033
Ag PVP	0	1	0.217	0.117	0.000	0.000	0.000	0.000
		10	2.800	0.363	0.010	0.009	0.357	0.046
		100	28.217	19.867	0.010	0.006	0.035	0.025
	24	1	0.433	0.103	0.000	0.000	0.000	0.000
		10	3.100	0.141	0.000	0.000	0.000	0.000
		100	14.200	6.275	0.007	0.005	0.047	0.021
	96	1	0.583	0.611	0.002	0.004	0.286	0.299
		10	2.450	0.058	0.007	0.005	0.272	0.006
		100	15.067	0.821	0.018	0.004	0.122	0.007
Ag HECs	0	1	0.300	0.167	0.000	0.000	0.000	0.000
		10	2.250	0.327	0.033	0.052	1.481	0.215
		100	27.933	4.196	0.067	0.052	0.239	0.036
	24	1	0.267	0.103	0.000	0.000	0.000	0.000
		10	2.717	0.098	0.000	0.000	0.000	0.000
		100	58.750	8.385	0.067	0.052	0.113	0.016
	96	1	0.117	0.183	0.000	0.000	0.000	0.000
		10	1.817	0.679	0.050	0.055	2.752	1.029
		100	41.083	8.403	0.225	0.050	0.548	0.112
Ag-HECp	0	1	0.233	0.082	0.000	0.000	0.000	0.000
		10	2.250	0.208	0.000	0.000	0.000	0.000
		100	50.817	21.146	0.002	0.004	0.003	0.001
	24	1	0.317	0.041	0.000	0.000	0.000	0.000
		10	2.367	0.163	0.000	0.000	0.000	0.000
		100	43.350	9.218	0.002	0.004	0.004	0.001
	96	1	0.333	0.151	0.003	0.005	1.000	0.452
		10	2.817	0.098	0.007	0.010	0.237	0.008
		100	72.250	5.236	0.002	0.004	0.002	0.000

Table S2 96 hpf-EC$_{50}$ values for each abnormal phenotype in 96 hpf larvae exposed to Ag-NPs

	96 hpf EC$_{50}$									
	CD	**OT**	**EM**	**HE**	**AD**	**YD/E**	**GR**	**ST**	**IF**	**RYR**
Ag-NKD	91.3	143.9	115.6	85.1	41.3	114.3	160.3	112.4	60.7	17.9
Ag-PVP	54.9	525.3	107.8	36.5	52.8	38.6	nd	126.8	449.8	18.6
Ag-HECs	8.4	11.5	10.7	7.1	6.5	7.7	8.6	11.6	10.7	1.7
Ag-HECp	4.2	8.6	5.6	3.9	3.0	4.8	10.9	10.9	8.0	1.3

CD = craniofacial defects, OT = Otoliths defects, EM = eye malformations, HE = hearth edema, AD = axial defects, YD/E= yolk deformation/edema, RYR= reduced yolk resorption, GR = general growth retardation