On-Line Supporting Information (SI)

In Vivo Imaging of Transport and Biocompatibility of Single Silver Nanoparticles in Early Development of Zebrafish Embryos

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The on-line SI includes

A. One table (**Table I**) summarizes representative and rare deformities of zebrafish treated chronically with given Ag nanoparticle concentrations (0.04-0.71 nM) for 120 hpf since the cleavage (8-cell) stage, showing the high dependence of severities of deformation on nanoparticle concentration;

B. Four real-time videos:

(a) Movies 1-2 illustrate the direct observation of single Ag nanoparticles entering into chorionic space (CS) of a cleavage (64-cell) stage embryo (2-2.25 hpf) from egg water via an array of chorion pore canals (CPCs), and transporting into inner mass of embryos (IMS), respectively. Single Ag nanoparticles exhibit signature size-dependent LSPRS (colors). We characterized single nanoparticles *in situ* using color images and LSPRS via SNOMS (Figs. 1D and 5A in the main text), allowing us to distinguish them from tissue residues present inside embryos, which appear white.

(b) Movies 3-4 show the diffusion trajectories of single nanoparticles in a cleavage (64-cell) stage embryo: near or in chorion pore canals of chorion layers, and in chorion pore (either near the chorion layer or near the surface of inner mass of embryos), respectively.

The preparation and treatment of the embryos in Movies 1-4 and Table I are described in Figs. 3-5 and Methods of the main text.

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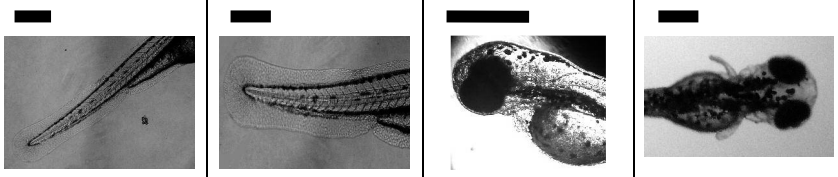
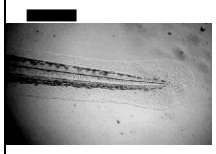
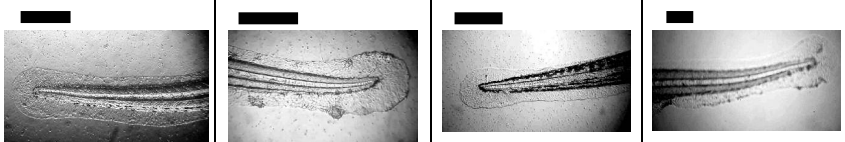
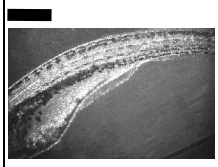
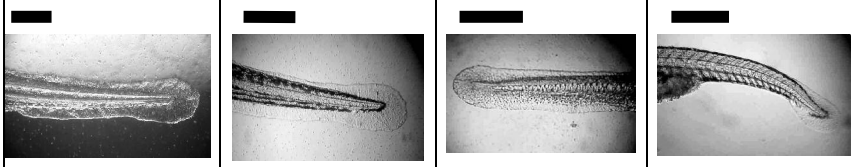
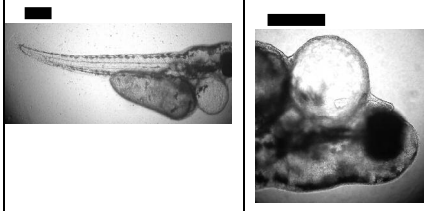
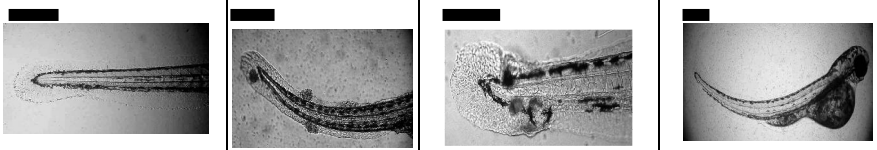
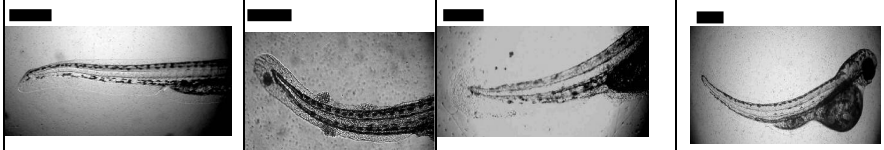
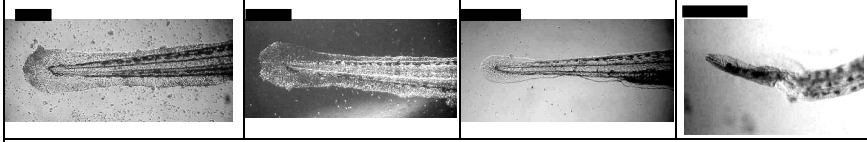
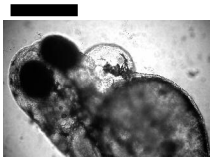
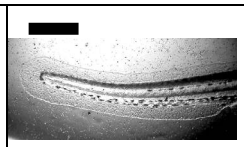
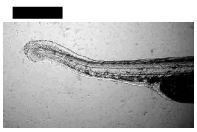
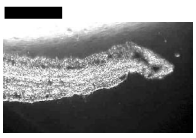
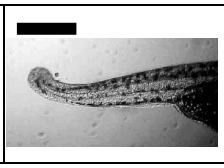
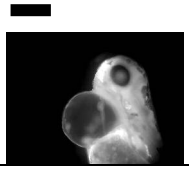
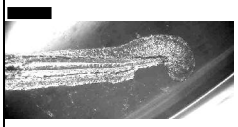
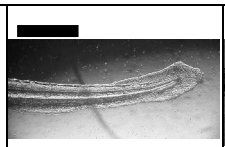
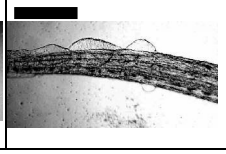
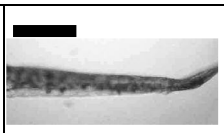
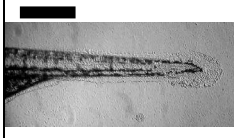
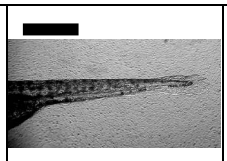
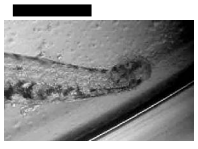
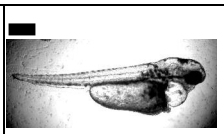
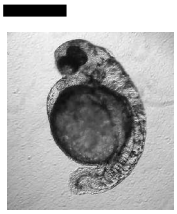
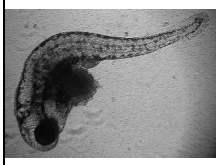
Table I: Representative and rare deformities of zebrafish treated chronically with given Ag nanoparticle concentrations for 120 hpf since the cleavage (8-cell) stage, showing the high dependence of severities of deformation on nanoparticle concentration.

C (nM)	Images	Number of Zebrafish with Severity Scale*				
		0	1	2	3	4
0	Control (Normal Development)	68				
						
0.04	Finfold Abnormality	28	1			
						
	Tail/Spinal Cord Flexure and Truncation	28		1		
						
0.06	Finfold Abnormality	22	7	1		
						
	Tail/Spinal Cord Flexure and Truncation	22		1		
						
0.07	Finfold Abnormality	23	11	2		
						

	Tail/Spinal Cord Flexure and Truncation				23	2			
									
	Cardiac Malformation[♣] and Yolk Sac Edema[♣]				23				
									
0.08	Finfold Abnormality[♣]				11	7	3	1	1
									
	Tail/Spinal Cord Flexure and Truncation[♣]				11	2	3		
									
	Cardiac Malformation[♣,≠] and Yolk Sac Edema[♣,≠]				11				
									
0.19	Finfold Abnormality					8	10	2	
									
	Cardiac Malformation[≠]								
									
0.38	Finfold Abnormality[♣]					4	6	3	4

									
	Tail/Spinal Cord Flexure and Truncation♣					2	5	2	
									
	Cardiac Malformation♣,≠								
									
	Yolk Sac Edema♣,≠ and Head Edema♣,≠								
									
0.57	Finfold Abnormality					1	3	2	3
									
	Tail/Spinal Cord Flexure and Truncation♣,≠						2		
									
	Cardiac Malformation♣,≠ , Yolk Sac Edema♣,≠ and Head Edema♣,≠								
									
0.66	Finfold Abnormality					1	4	1	2
									
	Tail/Spinal Cord Flexure and Truncation♣,≠							2	1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	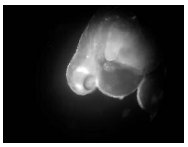	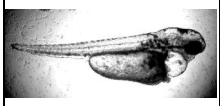	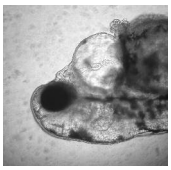						
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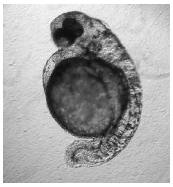
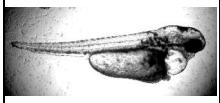
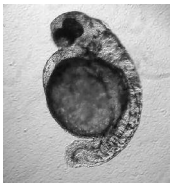
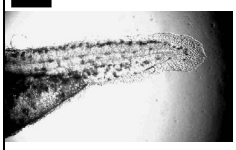
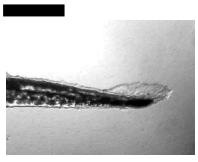
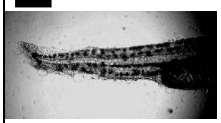
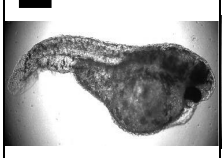
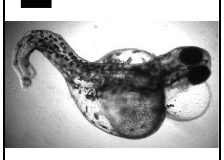
									
	Head Edema[♣,≠]								
									
	Eye Abnormality[♣,≠]								
									
0.71	Finfold Abnormality						2	4	1
									
	Tail/Spinal Cord Flexure and Truncation					1		1	2
									

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	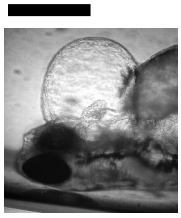	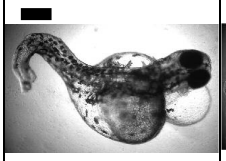	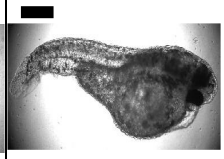						
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		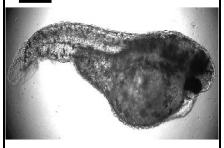							
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	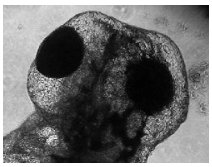	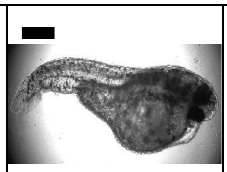							
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	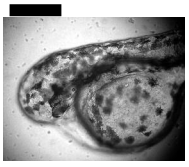	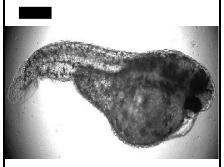							

* Scale of severity from 0 to 4 represents the normal development to the most severe deformity.

♣ Multiple types of deformities are observed in one zebrafish, which is repeatedly listed in the corresponding category.

≠ Rare type of deformities makes the rating of deformation severity impossible.

The scale bar = 500 μm