Supporting Information

Molecular mechanism of silver nanoparticles in human intestinal cells

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Figure S 1: Results of the CTB assays to assess cell viabilities and of DAPI staining to assess the cell number of differentiated Caco-2 cells after incubation with surfactant control, surfactant coated silver nanoparticles, and silver ions for 4, 24 and 48 h. 10 000 Caco-2 cells per well were seeded into 96 well plates and differentiated for 21 days. They were incubated in 300 µL. Medium control was set to 100 %. The experiment was reproduced three times. Asterisks (*) mark significant differences, as compared to the medium control (Student's t test $p \leq 0.05$).

Figure S 2: Results of the CTB assays to assess cell viabilities and DAPI staining to assess the cell number of proliferating Caco-2 cells after incubation with surfactant control, surfactant coated silver nanoparticles, or silver ion for 4, 24 and 48 h. 10 000 Caco-2 cells per well were seeded into 96 well plates and cultivated for 24 h in 100 µL medium. Medium control was set to 100 %. The experiment was reproduced three times. Asterisks (*) mark significant differences compared to the medium control (Student's t test $p \leq 0.05$).

Figure S 3: (A) Transmission electron microscopic (TEM) pictures and (B) energy dispersive X-ray (EDX) analysis of differentiated Caco-2 cells. Cells were grown on transwell inserts. Fully differentiated cells were exposed to 20 μg/mL surfactant coated silver nanoparticles for 24 h. Afterwards, cells were fixed and analyzed by TEM and EDX. The EDX analysis shows a representative spectrum of sample regions where nanoparticles are located. Red square indicates the silver signal.

Figure S 4: (A) Venn diagram and (B) heat map of genes that are regulated in differentiated Caco-2 cells after 24 h treatment with silver nanoparticles and silver ions. All genes are significantly ($p < 0.05$) and at least ± 1.4 fold regulated, as compared to the medium control.

Figure S 5: The xCELLigence impedance measurements are shown for proliferating Caco-2 cells. Δ cell index is plotted as a measure of impedance-based time-dependent cell response of Caco-2 cells to the exposure to surfactant control, surfactant coated silver nanoparticles, or silver ions for up to 48 h. 6,125 Caco-2 cell per well (corresponding 10 000 cell per well of a 96 well plate) were seeded into E-plates and grown for 24 h. Then, they were incubated with medium control, surfactant control, silver ions, or silver nanoparticles (100 μL) and monitored for 48 h. The medium control and the time of incubation were set to 0 cell index. The experiment was reproduced two times.

Figure S 6: The xCELLigence impedance measurements are shown for differentiated Caco-2 cells. Δ cell index is plotted as a measure of impedance-based time-dependent cell response of Caco-2 cells to the exposure to surfactant control, surfactant coated silver nanoparticles and silver ions up to 48 h. 6 125 Caco-2 cell per well (corresponding 10 000 cell per well of a 96 well plate) were seeded into E-plates and differentiated for 21 days. Then, they were incubated with medium control, surfactant control, silver ions and silver nanoparticles (100 μL) and monitored for 48 h. The medium control and the time of incubation were set to 0 cell index. The experiment was reproduced two times.

Figure S 7: Annexin V/AAD staining of proliferating Caco-2 cells in serum free cell culture medium after incubation with silver nanoparticles. 100,000 Caco-2 cells per well were seeded into 12 well plates, cultivated for 24 h and incubated. Staurosporine (10 μ M) was used as a positive control. Afterwards, cells were harvested, stained and measured using FACS analysis analyzing 10 000 cells per treatment. The experiment was reproduced two times. The figure shows the average for all experiments.

Figure S 8: Measurements of the mitochondrial depolarization in differentiated Caco-2 cells after incubation with surfactant coated silver nanoparticles or silver ions for 24 h. 10,000 Caco-2 cells per well were seeded into 96 well plates, differentiated for 21 days and incubated. Positive control was 2.5 µM staurosporine. Measurements were done by JC-10 assay. Medium control was set to 100 %. The experiment was reproduced three times.

Figure S 9: Oxidative stress measurements in differentiated Caco-2 cells after 24 h of incubation with surfactant coated silver nanoparticles and silver ions. 10,000 Caco-2 cells per well were seeded into 96 well plates, differentiated for 21 days and incubated. Positive control was 100 µg/mL iron ions (FeSO₄). Prior to incubation, cells were treated with 2',7'-dichlorodihydrofluorescein diacetate in serum free media for 1 h and washed with PBS. Results are expressed as % oxidative stress and medium control was set to 100 %. The experiment was reproduced four times.

Figure S 10: Results of preincubation with the antioxidants N-acetylcysteine and vitamin C and cell viability measurements with CTB assays and DAPI staining of proliferating Caco-2 cells after incubation with surfactant control, surfactant coated silver nanoparticles and silver ion in serum free media. 10,000 Caco-2 cells per well were seeded into 96 well plates and cultivated for 24 h. They were preincubated with antioxidants for 1 h and incubated in 100 µL for 24 h. Medium control was set to 100 %. The experiment was reproduced three times. * marks significant differences compared to preincubated cells (student's t test $p \leq 0.05$).

Table S 1: Comparative list of gene expression results measured by real-time RT-PCR analysis. Caco-2 cells were differentiated for 21 days and incubated with surfactant coated silver nanoparticles for 24 h in serum free and serum containing cell culture medium. The color red indicates an upregulation and green a downregulation, as compared to the medium control

category	gene	+ FCS		- FCS	
		silver nanoparticles 5 µg/mL	silver nanoparticles 10 µg/mL	silver nanoparticles 5 µg/mL	silver nanoparticles 10 µg/mL
stress and inflammation	ALB	-2.1			-2.9
	HSPA6	238.0	358.2	23.5	466.4
	HMOX1	51.3	105.2	4.8	77.2
cellular morphology and cellular movement	FN1				
	OCLN	-2.1	-3.1		
	CLDN3		-2.2		
	TJP3		-2.4		
	ACTB	2.2	2.9		2.1
	FOS	4.0	8.7		6.3
	ITGA2	3.1	3.3		
	TUBB3	29.8	44.1	2.6	20.0
calcium homeostasis	CXCR4	2.4	4.8	2.4	5.1
	CAMK2D		-2.5		

Table S 2: Comparative list of gene expression results measured by microarray and real time RT PCR analysis. Caco-2 cells were differentiated in 75 cm² cell culture flask for 21 days and incubated with surfactant coated silver nanoparticles or silver ions for 24 h in serum free medium. The microarray and the PCR analysis no. (1) were performed with the same mRNA samples, whereas the PCR analysis no. (2) was done with samples of a biological replicate. The color red indicates an upregulation and green a downregulation, as compared to the medium control (*fold change* $\leq 1,4$ and *Student's* t-Test $p < 0.05$).

	stress and inflammation	cellular morphology and cellular movement	cell death and apoptosis	cell cycle and proliferation	cell-cell communication and cellular signaling	silver nanoparticles								silver ions	
						microarray	PCR array (1) 2.5 µg/mL	PCR array (2)	PCR array (2) 5 µg/mL	PCR array (2) 10 µg/mL	microarray	PCR array (1) 25 µg/mL	PCR array (2)	microarray	PCR array (1) 0.5 µg/mL
ACTB	X	X	X	X	X				3.7				5.0		
ACTG2		X									2.7	2.1	-2.1		
ACTN2		X			X					145.0	3.0	156.3	60.9		
ACTN4	X	X	X	X					11.7		2.1	4.4	15.5		
ALB	X	X	X	X	X						-4.7	-4.7		-1.5	
ASAP1		X			X				9.9		1.8	5.9	14.2		
ATF3	X	X	X	X	X			1.9		13.3	3.4	3.5	2.0		
B2M	X	X	X	X	X			1.6		1.7	-1.6	-5.8			
BAG3	X	X	X						1.8	16.7	3.9	7.8	7.3		
BCL10		X	X	X	X				-2.7	1.0	-2.8		-2.5		
BCL2L11	X	X	X	X	X				-2.8		-2.6	-3.2		-1.6	
BDNF	X	X	X	X	X						2.3	6.5	3.3		
BRAF		X	X	X	X				2.4		-4.4				
CAPN10			X						17.8		-2.1	4.0	11.9		
CAPN3		X	X						6.0		-2.2		3.1		
CAT	X	X	X	X	X				4.8		-3.0		1.6		
CCT5	X		X	X							2.3				
CDKN1A	X	X	X	X	X					7.1	2.0	2.3			
COL5A2									2.6		-5.1				
CTNND1		X		X	X				10.6		-2.0	2.2	9.7		
CTTN	X	X	X	X					44.5		-1.3	8.7	49.2		
CUX1		X	X	X		1.5			4.7			2.7	4.9		
CYFIP2			X				1.9				-2.6				
CYR61	X	X	X	X	X				14.0		3.5	16.3	62.8		
DAPK1			X	X					-2.1	-5.2	-2.1	-3.0	-5.3		
DIDO1		X	X	X		1.6			11.9			2.5	9.4		
DNAJB6	X	X	X	X					4.3	11.6	2.6	5.8	7.1		
DOCK1		X	X						2.8		-2.0	1.7	1.6		
DUSP1	X	X	X	X	X				6.4	142.7	3.7	16.4	66.1		
DUSP2	X		X	X				93.3	74.4	619.0	3.9	11.4	403.8		
DUSP4			X	X					13.1		3.7	23.1	69.5		
DUSP6		X	X	X					12.4		4.7	14.8	31.6		
DUSP9			X	X					2.3		-2.9	-5.1			
EGR1		X	X	X	X						4.4	4.9			
EPHX2	X		X	X					2.7		-4.3	11.1			
ETS1	X	X	X	X	X						2.7	31.3			
EXT1						1.6			-2.5		2.0		-2.6		
FAM129A	X					1.7			8.5		2.2	9.6	6.2		
FN1	X	X	X	X	X				6.0	2.3	2.0	3.0	9.1		
FOS	X	X	X	X	X				12.7		6.0	21.9	118.1		
FOSB	X		X	X					8.7		2.6	23.5	27.6		
FOSL1		X	X	X	X				3.4	433.4	7.8	99.8	106.8		

	stress and inflammation	cellular morphology and cellular movement	cell death and apoptosis	cell cycle and proliferation	cell-cell communication and cellular signaling	silver nanoparticles								silver ions			
						microarray	2.5 µg/mL		5 µg/mL		10 µg/mL		microarray	25 µg/mL		microarray	0.5 µg/mL
							PCR array (1)	PCR array (2)	PCR array (2)	PCR array (2)	PCR array (1)	PCR array (2)					
GADD45B	X		X	X					-2.2	7.7	3.6	4.6	2.4				
GSTA1	X		X								-2.4						
GSTA4	X										-2.0		-2.2				
HMOX1	X	X	X	X	X			84.2	22.0	649.1	17.2	45.7	277.9				
HSPA1A	X	X	X	X	X			2.6		18.6	5.6	20.7	6.3				
HSPA6	X							14.0		240.3	106.8	296.0	42.7				
HSPH1			X		X				-2.0	7.6	3.3	5.2	2.2				
IGFBP7	X	X		X	X						-1.9						
ITGA2		X	X	X	X				14.6		2.9	15.3	47.7				
JMJD1C									4.6			2.5	5.6				
JUN	X	X	X	X	X				49.2		4.2	31.3	265.9				
MAP1A								1.9		8.9	3.2	4.1	3.4				
MCL1	X	X	X	X						1.3	3.0						
MMP1	X	X	X	X	X						7.6	11.3	6.4				
MMP10	X	X		X					2.7		5.3	17.5	17.7				
MMP11		X	X	X							-2.0	-5.7					
MMP7		X	X	X	X					12.0	2.8	8.4	2.0				
MPHOSPH8						0.4	1.5		5.1	2.2	-1.2	4.2					
MT1F	X	X	X			-1.9		16.1		53.2	12.2	18.3	19.6				
MT1G	X					-1.7		29.9		299.9	11.9	191.5	248.5				
MT1H	X	X	X	X		-1.8	-2.4	30.0		148.6	14.2	96.6	88.7				
MT1M	X							110.7		536.0	43.6	523.4	209.3				
MT1X	X		X	X		-2.3	-2.2	83.0		414.0	9.5	51.6	273.2	-1.6	-2.8		
MT2A	X	X	X	X		-2.1	-2.3	8.0		36.5	7.7	22.5	17.8				
MYL12A	X									1.8							
MYLK	X	X	X	X					-2.3		-2.3						
PAK1		X	X	X	X						-2.1		-2.2				
PPARA	X	X	X	X	X				6.4	3.7	-1.9		2.6	-1.5			
PPARG	X	X	X	X	X						-2.1						
PPP1R14A											-2.9						
PPP1R15A		X	X	X	X				26.8		3.0	21.5	125.1				
PPP1R16A									14.0		-2.8	2.0	10.3				
PPP2R5C		X	X	X					1.5		-2.5						
PRDX2	X	X	X	X				23.1	24.7	18.3	2.0		16.7				
PTK2		X	X	X	X				5.4		-3.3	2.8	4.6				
RXRA	X	X	X	X	X						-3.0	-2.4					
SOS1		X		X					3.2	2.3	2.0	2.5	1.8				
SPARC	X	X	X	X	X			4.6	5.5	2.9	-2.2		4.9				
SRXN1	X	X	X						24.3		6.0	42.7	106.6				
TCF7L2	X	X	X	X							1.5						
TGFBI		X	X	X	X				2.3		-2.5						
THBS1	X	X	X	X	X				2.7	5.9	2.2	8.0	3.3				
TUBB3	X	X	X	X					41.6		2.2	79.0	349.7				
TUBGCP3									6.5	8.5	-1.2	5.5	5.4				
TXNRD1	X		X	X	X					4.7	3.5	3.6					
VCL	X	X	X	X	X					2.5	2.3	2.3					
ZYX	X	X	X	X	X				7.0			8.4	14.8

Figure S 11: Network of genes that belong to the group “stress and inflammation” and were analyzed in the microarray as well as in the real time RT-PCR analysis. The network was created by Ingenuity Pathway Analysis (IPA) software using the PCR verified data. The color red indicates an upregulation and green a downregulation, as compared to the medium control.

Figure S 12: Network of genes that belong to the group “cellular morphology and cellular movement” and were analyzed in the microarray as well as in the real time RT-PCR analysis. The network was created by Ingenuity Pathway Analysis (IPA) software using the PCR verified data. The color red indicates an upregulation and green a downregulation, as compared to the medium control.

Figure S 13: Network of genes that belong to the group “cell death and apoptosis” and were analyzed in the microarray as well as in the real time RT-PCR analysis. The network was created by Ingenuity Pathway Analysis (IPA) software using the PCR verified data. The color red indicates an upregulation and green a downregulation, as compared to the medium control.

Figure S 14: Network of genes that belong to the group “cell cycle and proliferation” and were analyzed in the microarray as well as in the real time RT-PCR analysis. The network was created by Ingenuity Pathway Analysis (IPA) software using the PCR verified data. The color red indicates an upregulation and green a downregulation, as compared to the medium control.

Figure S 15: Network of genes that belong to the group “cell-cell communication and cellular signaling” and were analyzed in the microarray as well as in the real time RT-PCR analysis. The network was created by Ingenuity Pathway Analysis (IPA) software using the PCR verified data. The color red indicates an upregulation and green a downregulation, as compared to the medium control.