Supporting Information for:

"One Low-Dose Exposure of Gold Nanoparticles Induces Long-Term Changes in Human Cells"

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Material and Methods

Synthesis and Preparation of Gold Nanoparticles

20 nm spherical gold NPs were prepared by a scaled-up Turkevich synthesis method.[1] 25 mL of 0.01 aqueous mM gold (III) chloride (trihydrate, $HAuCl_4{\bullet}3H_2O$, ≥99.9%, Aldrich) was added to 975 mL of ultrapure deionized water (18.2 MΩ, Barnstead NANOpure II) and the solution was brought quickly to a rolling boil with rapid stirring. 20 mL of 5% (w/w) sodium citrate (tribasic dehydrate, $C_6H_5Na_3O_7{\bullet}2H_2O$, ≥99%, Sigma-Aldrich) was added and the heat was lowered to keep solution at a gentle boil. After 30 min, another 5 mL of 5% sodium citrate was added and the solution was stirred for 10 more minutes. The heat was then turned off, and the solution was allowed to cool to room temperature slowly on the hotplate. The particles were cleaned by centrifugation at 8,000 rcf for 20 min and resuspended in minimal water. These as-made NPs were used in exposure experiments as "citrate spheres".

To make poly(acrylic acid)-coated nanospheres ("PAA spheres") the as-made citrate NPs were wrapped first with poly(allylamine hydrochloride) (PAH, M.W. 17,500 g/mol, Aldrich) polyelectrolyte followed by a PAA (sodium salt, M.W. 15,000 g/mol, Aldrich) layer following typical layer-by-layer procedures.[2] A portion of citrate Au spheres were diluted back to their as-made concentration in water. 25 mL of 0.01 M NaCl and 50 mL of 10 mg/mL PAH (with 1 mM NaCl) were combined, then slowly added to 1 L of NPs while stirring vigorously. The solution was allowed to stir for 3 h, then centrifuged at 8,000 rcf for 20 min and resuspended in 1 L of water. The procedure was repeated a second time with 10 mg/mL PAA solution (with 1 mM NaCl), and after centrifugation the solution was resuspended in minimal water to get "PAA spheres".

Gold nanorods were produced *via* a scaled up seed-mediated silver-assisted method.[3] Gold seeds were first made by combining 0.25 mL of 0.01 M $HAuCl_4$ with 9.75 mL of 0.1 M hexadecyltrimethylammonium bromide (CTAB, for molecular biology, Sigma-Aldrich) and stirring vigorously. 0.6 mL of freshly made, ice-cold 0.01 M sodium borohydride ($NaBH_4$, Sigma-Aldrich) was injected into the solution, resulting in a brown solution. The seed solution was stirred for 10 minutes and allowed to sit for 1 hour before use. Gold nanorods were synthesized in 2 L batches by first mixing 16 mL of freshly made 0.01 M silver nitrate ($AgNO_3$, 99.0%, Sigma-Aldrich) with 1900 mL of 0.1 M CTAB solution. Then 100 mL of 0.01 M $HAuCl_4$ was added, turning the solution orange. 11 mL of freshly made 0.1 M L-ascorbic acid (BioXtra, ≥99.0%, crystalline, Sigma) was then added, and the solution reverted to colorless. Finally, 2.4 mL of the gold seed solution was added, and the nanorod solution was stirred slowly overnight. The next day, the solution was centrifuged at 13,500 rcf for 20 min two times and finally resuspended to 40 mL in water.

The as-made gold nanorods contained a CTAB bilayer on the surface. Two techniques were used to make these into either "PAA rods" or "PEG rods". Firstly, nanorods were modified by sequential layer-by-layer polyelectrolyte three times (PAA/PAH/PAA). 20 mL of the nanorod solution was diluted 2X in a centrifuge tube. 4

mL of 0.01 M NaCl and 8 mL of 10 mg/mL PAA (with 1 mM NaCl) were added. The solution was put on a shaker for 2 hours, then centrifuged at 8,000 rcf for 20 min. This procedure was followed twice more, once with PAH and another with PAA, respectively. After the third and final coating of PAA, the "PAA rods" were redispersed in minimal water. To make "PEG rods" the CTAB layer was displaced by thiolated methoxy-PEG (mPEG-SH, M.W. 5,000 g/mol, Nanocs, stored at -20°C). 20 mL of as-made nanorods were diluted to 40 mL in water and 20 mL of 0.5 mM mPEG-SH was added. The solution was left on a shaker overnight, and centrifuged twice at 8,000 rcf for 20 min before being redispersed in minimal water.

Nanosphere solutions were characterized by UV-Vis spectroscopy (Cary 500 Scan UV-vis-NIR spectrophotometer, Varian), dynamic light scattering (DLS), zeta potential measurements (ZetaPALS DLS/zeta potential analyzer, Brookhaven Instruments) and transmission electron microscopy (TEM, 2100 JEOL Cryo TEM). Nanorod solutions were characterized by UV-Vis, DLS, zeta potential measurements and TEM. The absence of endotoxin contamination of Au NP suspensions was confirmed using the Pierce™ LAL Chromogenic Endotoxin Quantitation Kit.

Cell Culture and NP Exposure Protocol

Human dermal fibroblast (HDF) cells were cultured for at least two passages before beginning the long-term (20 passages, 5 months) cultures. These primary cells are obtained from the dermis of normal human neonatal foreskin or adult skin, being cryopreserved at the end of primary culture. According to the manufacture (Sigma-Aldrich), HDF cells can be cultured and propagated at least 16 population doublings. All cell culture and experiments involving cells were completed in a sterile environment with autoclaved supplies. Cell media contained Dulbecco's modified eagle medium (DMEM, without phenol red), nonessential amino acids and 1% penicillin-streptomycin solution from Mediatech, along with 10% fetal bovine serum (FBS) from Gemini Bio-Products. Cells were plated in triplicate for controls (cells never exposed to NPs) and per sample type (CIT spheres, PAA spheres, PAA rods and PEG rods; chronic and "non-chronic") into 6-well plates at 100,000 cells/well with 1.2 mL media. At each passage, all cells were plated with NP-free media for 24 h to ensure that NP sedimentation did not interfere with cell adhesion to the surface. During the first week of exposure, the media in all samples (other than controls, which was just changed with NP-free media) was changed to media containing 0.1 nM NPs 24 h after plating cells. After another 24 h, the NP-containing media on the non-chronic samples was removed and replaced with NP-free media. The non-chronic samples only received NP doses (0.1 nM) for this 24-hour period and never again throughout the study. The media on all samples was replaced again four days after plating, with new 0.1 nM NP/media solutions being added to chronic samples. After one week, cells in each sample were trypsinized and counted with a hemocytometer before being plated again in new 6-well plates at 100,000 cells/1.2 mL media/well. Only chronic samples were given NP-containing media after 24 h (all other samples received new media without NPs). This cycle continued for 20 weeks. In order to have enough cells to study at the end of the 20 weeks, we began

plating extra cells from passage 17-19 to increase the total number of cells in each sample type. These extra wells were treated the same, based on sample type.

Viability and Proliferation Assays

A standard trypan blue assay, combined with automated cell quantification, was used to test the viability and proliferation rates of cells exposed to NPs for up to 14 days. Samples were plated in triplicate (including controls) for each time point (72 hours, 7 days and 14 days) into 24-well plates at 40,000 cells/well with 0.5 mL of media. The cells were allowed to adhere to the plate overnight before the media was changed and replaced with 0.5 mL of 0.1 nM NPs in all but controls. After 24 h, the media in non-chronic samples was replaced with NP-free media. 72 h after adding NPs, the samples for the 72-hour time-point were trypsinized, centrifuged and resuspended in 300 µL of media. 10 µL of cell suspension were combined with 10 µL of trypan blue solution (0.4%, Invitrogen) and the cells were counted using a Countess Automated Cell Counter (Thermo Fisher). This instrument counts cells in a 10 µL sample and differentiates between live and dead (based on trypan blue uptake).

In order to give the 7- and 14-day samples enough room to keep growing, the cells were passaged and re-plated into larger wells. Four days after plating the cells, the 7-day samples were trypsinized, centrifuged and re-plated into 6-well plates with 1.2 mL of NP-free media. The next day, the media was again replaced by 0.1 nM NP/media in the chronic samples (and new media was given to all other samples). These cells were counted 7 days after adding NPs. The 14-day samples were re-plated into 60 mm dishes with 2.4 mL of media (with NPs being added to chronic samples again the next day). Media was changed again for the 14-day samples a few days later before counting those cells 14 days after originally adding NPs.

Confocal Fluorescence Microscopy

To visualize any changes in cell morphology during chronic and non-chronic NP exposure, confocal fluorescence microscopy was done on cells exposed to NPs for 24 hours, 48 hours and long-term (after 20 weeks). For the 24-hour and 48-hour samples, fresh cells were plated into 35 mm tissue culture dishes containing 14 mm glass-bottomed wells coated in poly-d-lysine (MatTek Corporation). In order to keep cells at low enough density to see individual morphologies, cells were plated at 4,000 cells/14 mm well. 72-hour samples were attempted, but this cell type is difficult to grow for 72 hours at a low enough cell density to avoid near-complete monolayer formation, which rendered morphological examination impossible. To keep the NP/cell concentration in these samples equal to the main 6-well exposure experiments, NPs were added at a concentration of 0.024 nM in 200 µL media. Cells were plated in NP-free media for 24 h before adding NP-containing media. Non-chronic 48-hour samples only received NP-laden media for 24 h. For the long-term samples, chronic and non-chronic cells at passage 19 of exposure were plated the same way into MatTek dishes. Only chronic

long-term samples were given NP-containing media after 24 h. Long-term samples were imaged 24 h after adding NPs (48 h after plating).

To prepare the samples for imaging, they were first fixed at the appropriate time point (24 hours, 48 hours or 20 weeks) by adding 200 μL of pre-warmed 4% paraformaldehyde (in PBS) for 15 min. All steps were completed at room temperature. The fixative was washed away by three 2-5 min washes with PBS. The samples were then permeabilized with 0.5% Triton X-100 (Sigma, BioUltra) in PBS for 15 min, followed by three 2-5 min PBS washes. To prevent non-specific binding, 3% bovine serum albumin (BSA, ChemCruz) in DPBS was added for 1 hour. A working solution of 5 units/mL (~0.17 μM) fluorescein phalloidin (F-actin stain, Molecular Probes) in PBS was added to each dish for 90 min in the dark, followed by three 2-5 min PBS washes. Finally, samples were incubated in a 300 nM working solution of 4',6-diamidino-2-phenylidole dihydrochloride in PBS (DAPI, nuclei stain, Molecular Probes) for 30 min. Samples were wash three times with PBS and stored in PBS at 4°C until imaging. Samples were imaged with a Zeiss LSM 710 multiphoton confocal microscope with the laser power, gain, magnification, and all other parameters held constant. Ten to twenty images were collected across two samples for each sample type. A threshold was applied to images in ImageJ software (NIH) to select only the cells and the area was measured to get average cell areas. Threshold values were held constant for all images within each time point, but were adjusted between time points. Cell area was calibrated to the number of cells per image by counting the nuclei.

Nanoparticle Uptake Measurements

The uptake of NPs into the cells was measured after 72 hours and long-term chronic exposure to NPs by inductively-coupled plasma optical emission spectrometry (ICP-OES; PerkinElmer 2000DV ICP-OES). For the 72-hour time-point, fresh cells were plated at 100,000 cells/well in a 6-well plate with 1.2 mL media. To ensure that the concentration of NPs was high enough to be detected by ICP-OES, nine wells were plated per sample type to be combined into three ICP-OES samples per sample type. After 24 h, media was replaced with media containing 0.1 nM NPs (except for controls, which received NP-free media again). After another 48 h (72 in total), wells were washed three times with PBS (to remove excess NPs not endocytosed by cells) and trypsinized. Three wells were combined into each 15 mL centrifuge tube for ICP-OES samples to total three ICP-OES samples per NP type. Cells in each sample were centrifuged, resuspended in PBS and counted with a hemocytometer. Samples were then centrifuged again and redispersed in 1 mL of lysis buffer (2% Triton X-100 in PBS). 1 mL of 30% hydrogen peroxide and 3 mL of 70% nitric acid were added to each tube to dissolve cell debris; tubes were left with caps loose inside acid hood overnight (CAUTION: be careful not to mix these chemicals at higher concentration of nitric acid, as the solution may become unstable; must allow pressure release and handle inside hood with proper personal protective equipment (PPE)). The next day, 4 mL of fresh aqua regia (3:1 $HCl:HNO_3$) was added very carefully to each sample to dissolve gold (CAUTION: aqua regia is extremely corrosive and should be handled with proper PPE inside acid hood. Gas release from samples will occur, so allow pressure release by

leaving tubes uncapped). These were left for three hours with loose caps in the acid hood before being diluted to 10 mL with ultrapure deionized water. These samples were analyzed by ICP-OES for gold content. Controls with cells and without cells (treated the same after the point of lysis buffer addition) were made in triplicate. To minimize gold contamination from the laboratory, new bottles of reagents were opened and sterile serological pipettes were used in place of micropipettes. The same procedure was used for the long-term samples, except cells from passage 19 long-term exposure were plated in the first step.

Transmission electron microscopy (TEM) was also used to visualize the uptake of NPs into the cells after 20 weeks. Three wells of cells from each sample type (chronic and non-chronic, all four NP types) and controls were washed with PBS three times, trypsinized and centrifuged in microcentrifuge tubes. The cell pellets were then re-suspended in a few drops of pre-warmed, sterile-filtered Karnovsky's fixative, followed by microwave fixation and washing in Sorenson's buffer. A secondary 2% osmium tetroxide fixative was then added, followed by the addition of 3% potassium ferricyanide for 30 minutes. Samples were then washed with water and stained with saturated uranyl acetate and dehydrate with a series of increasing concentrations of ethanol. Samples then were transferred into epoxy using acetonitrile. These epoxy sample blocks were polymerized at 90°C overnight, trimmed and ultrathin sectioned with diamond knives. Sections were then loaded onto TEM grids, stained again with uranyl acetate and lead citrate, and imaged using a Hitachi H600 TEM at 75kV.

RNA Extraction and Purification

RNA extraction and purification was performed in triplicate for long-term (20 weeks) samples and controls (unexposed cells cultured for 20 weeks) using the AllPrep® DNA/RNA/miRNA universal kit from Qiagen, according to the manufacturer's instructions. Both genomic DNA and total RNA were collected, but only total RNA was used in this study. Briefly, after 20 weeks of culture under both chronic and non-chronic exposure conditions, cells from the samples in 6-well plates were trypsinized, centrifuged, then lysed and homogenized with the lysis buffer provided with the kit. Lysate was transferred to a DNA Mini spin column in a collection tube and centrifuged. The RNAs were contained in the flow-through, to which Proteinase K and ethanol were added for digestion. These samples were transferred to an RNeasy Mini spin column and centrifuged, leaving total RNA bound to the column. This was followed by a washing step, a DNase I digestion step, and various other washing steps to remove contaminants, according to the manufacturer's instructions. Total RNA was eluted into RNase-free water and stored at -80°C. Samples were analyzed for concentration and purity by Nanodrop 1000 (Thermo Scientific) quantification. RNA integrity was verified by Agilent 2100 Bioanalyzer, and all samples have two distinct 28S and 18S peaks (28S:18S ~ 2.0) and RNA quality numbers greater than 8.

Two-Step Real-Time Polymerase Chain Reaction (qPCR)

For mRNA expression analysis, the cDNA was first synthesized from 0.5 μg of total RNA using the RT^2 First Strand Kit (Qiagen). The quantitative gene expression analysis was performed on cDNA using 2x RT^2 SYBR® Green/ROX qPCR Mastermix (Qiagen) in PCR array plates (RT^2 Profiler™ PCR Array Human Stress & Toxicity PathwayFinder, PAHS-003Z, Qiagen) containing specific primers for 84 genes related to oxidative stress, inflammatory response, osmotic stress, hypoxia, cell death (apoptosis, necrosis and autophagy) heat shock proteins and DNA damage. The plates also contained ACTB, B2M, GAPDH, HPRT1, RPLP0 and HGDC primers as housekeeping genes, three reverse transcription controls and three positive PCR controls. Experiments were performed on LightCycler® 480 (Roche) instrument with cycle conditions comprised of one 10 min cycle at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Melting curve analysis was performed for all samples.

Gene Expression Analysis

Three housekeeping genes (ACTB, B2M and RPLP0) with stable expression levels across all samples were used to normalize the gene expression levels of all samples *via* geometric averaging. Relative fold expression differences and accompanying statistics were calculated by the $\Delta\Delta C_t$ method and web-based RT^2 Profiler™ PCR Array Data Analysis software (SABiosciences, http://www.SABiosciences/pcrarraydataanalysis.php). Relative fold changes (compared to measured levels in control samples) are expressed in $\log_2$ format so that down-regulation will be represented by a negative number and up-regulation by a positive number (with zero change relative to control being equal to a fold change (FC) of 1.00).

The STRING database version 10 was used to map functional interactions among genes differently expressed based on different sources, including experimental repositories, computational prediction methods, and public text collections.[4] Individual networks were generated for each specific type of Au NP according to the exposure (chronic or non-chronic).

Statistical Analysis

All experiments in this study were made at least in triplicate. Data are shown as mean ± standard deviation (SD). Student's t-tests were performed to examine the differences among variables. P-values <0.05 were considered to be statistically significant.

For the gene expression analysis, fold changes [FC] ≥ 1.5 or ≤ -1.5 with a p-value < 0.05 were considered statistically significant.

References

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3. Sau TK, Murphy CJ (2004) Seeded High Yield Synthesis of Short Au Nanorods in Aqueous Solution. *Langmuir* 20:6414–6420.

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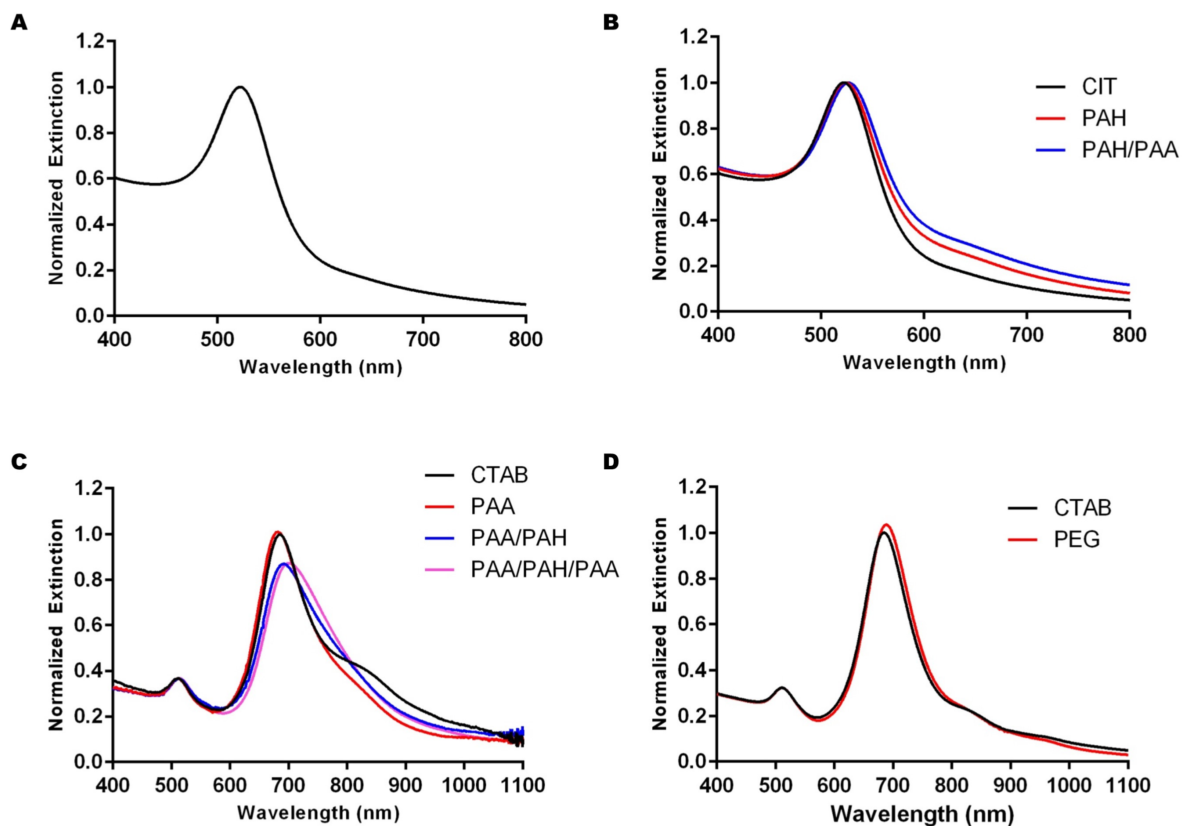

Fig. S1. UV-visible spectra of all four nanoparticle types used in water. Spectra for each step of the coating procedure shown. a) CIT spheres, b) PAA spheres (layer one = CIT, layer two = PAH, layer three = PAA), c) PAA rods (layer one = CTAB, layer two = PAA, layer three = PAH, layer four = PAA) and PEG rods (original CTAB coating replaced by PEG). All spectra within the same plot are normalized to the transverse peaks.

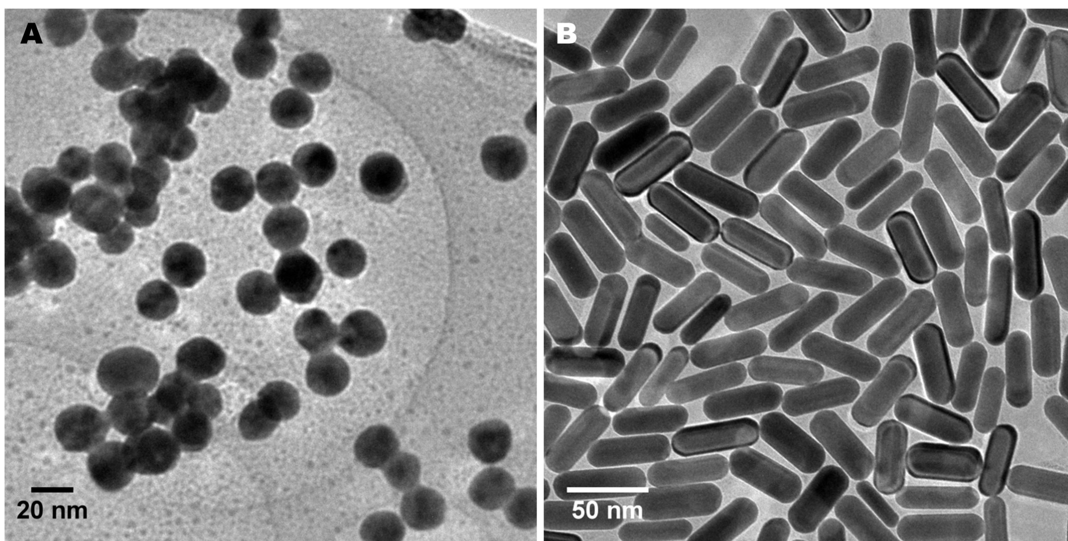

Fig. S2. Representative transmission electron microscopy images of gold nanoparticles. a) Gold nanospheres (18.4 nm ± 2.0 nm) and b) nanorods (46.0 nm ± 4.1 nm; 15.9 nm ± 2.6 nm)

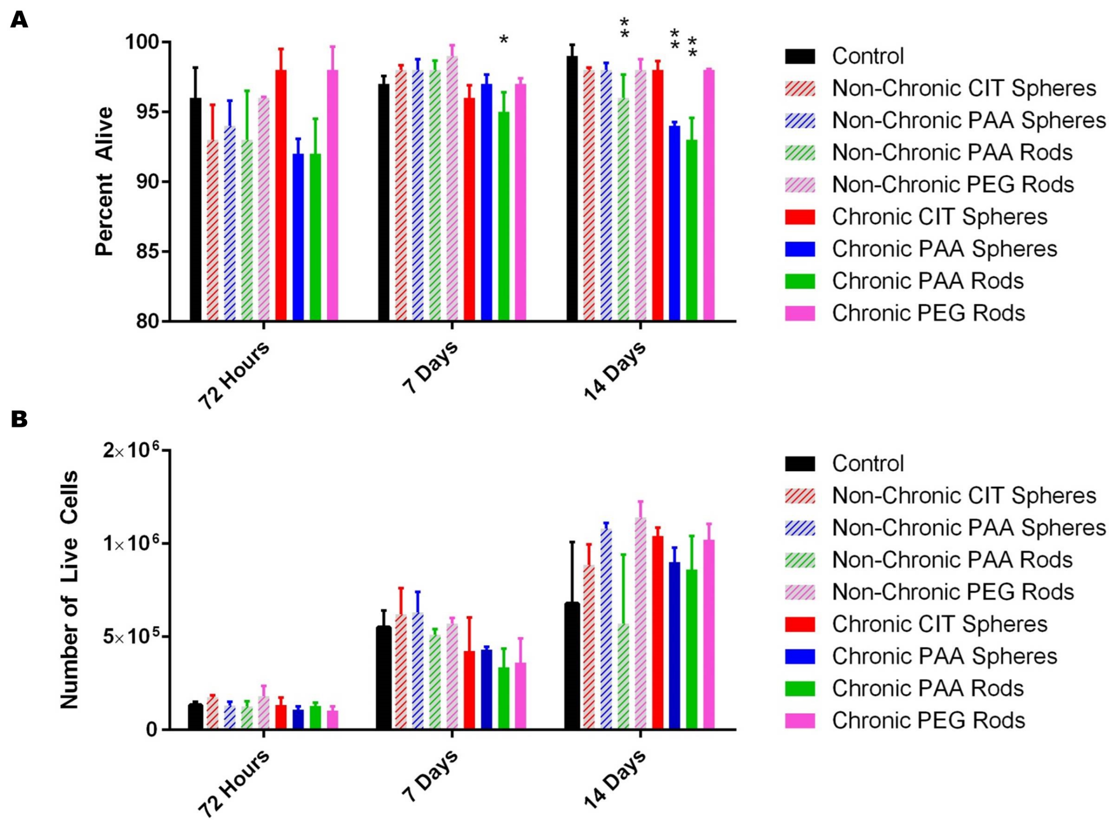

Fig. S3. Viability and proliferation results. a) Viability and b) proliferation results for HDF cells exposed to 0.1 nM CIT spheres, PAA spheres, PAA rods and PEG rods both chronically and non-chronically at 3, 7 and 14 days. $^{*}p < 0.05$, $^{**}p < 0.01$ versus control.

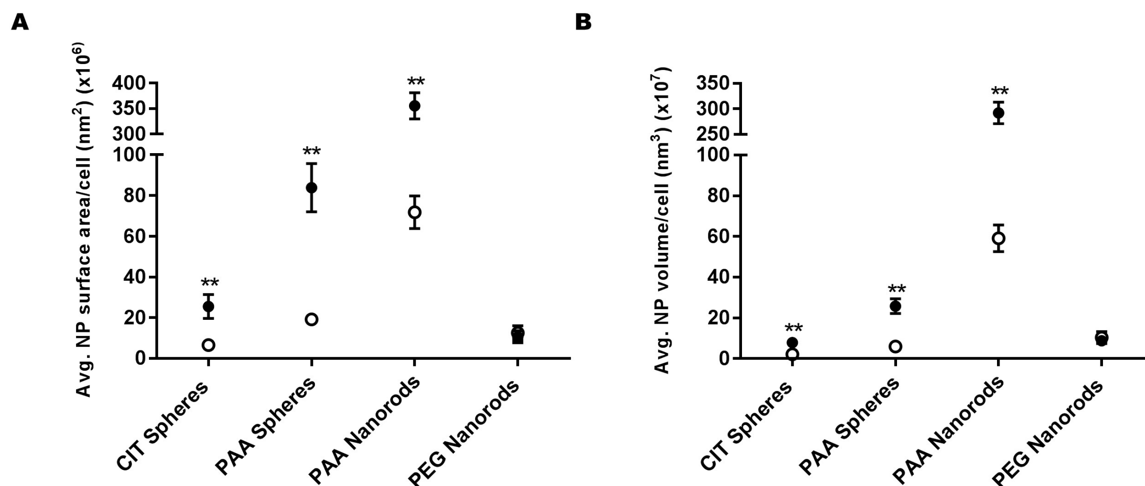

Fig. S4. Correlation of average total surface area and volume of Au NPs uptaken into cells. Average number of NPs per cell measured by ICP-OES analysis was measured and the dimensions of the NP cores were used to calculate the average total surface area (A) and volume (B) of gold NP surface per cell at 72 hours (open circles) and 20 weeks (solid circles). Stars indicate significant different between short- and long-term samples of the same NP type. $*p < 0.05$, $**p < 0.01$. Error bars represent one standard deviation from the mean of three measurements.

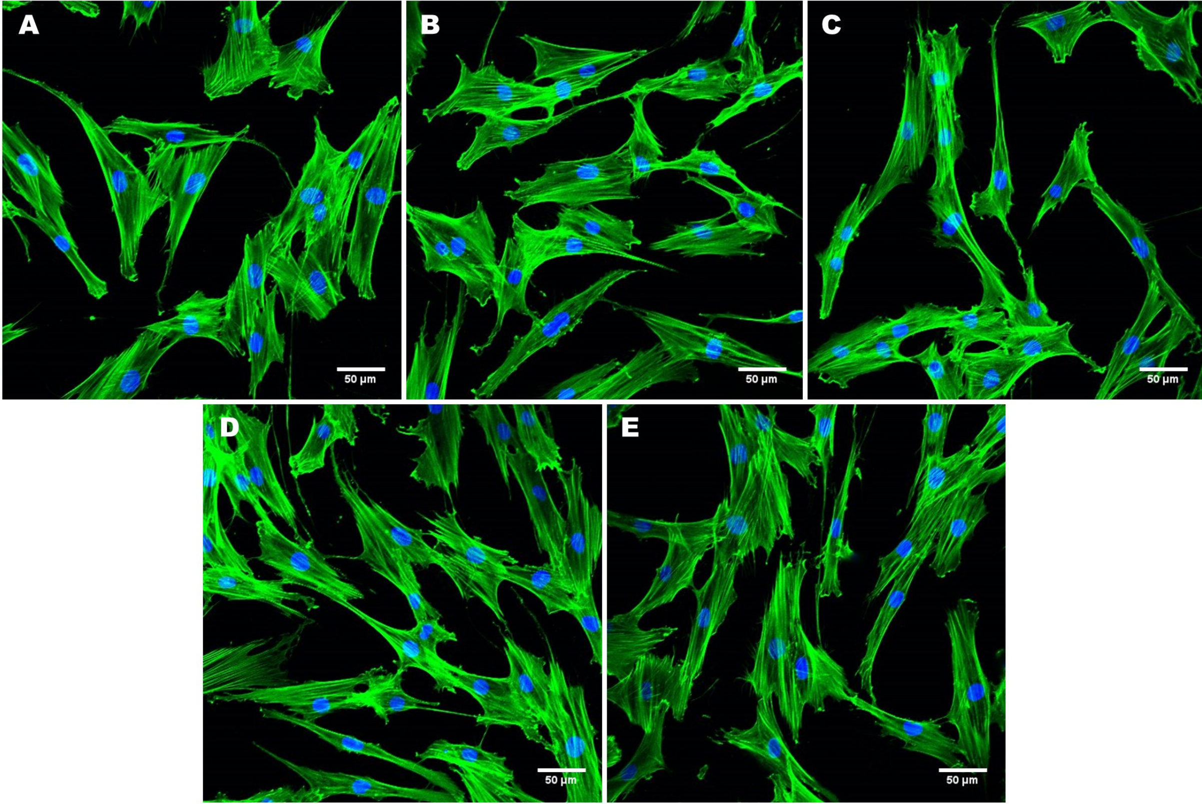

Fig.S5. Confocal fluorescence micrographs of HDF cells at 24 hours. Cells exposed to a) no NPs (control), b) citrate spheres, c) PAA spheres, d) PAA rods and e) PEG rods for 24 hours. Green signal = phalloidin stain for actin, blue signal = DAPI stain for nuclei. All scale bars are 50 µm.

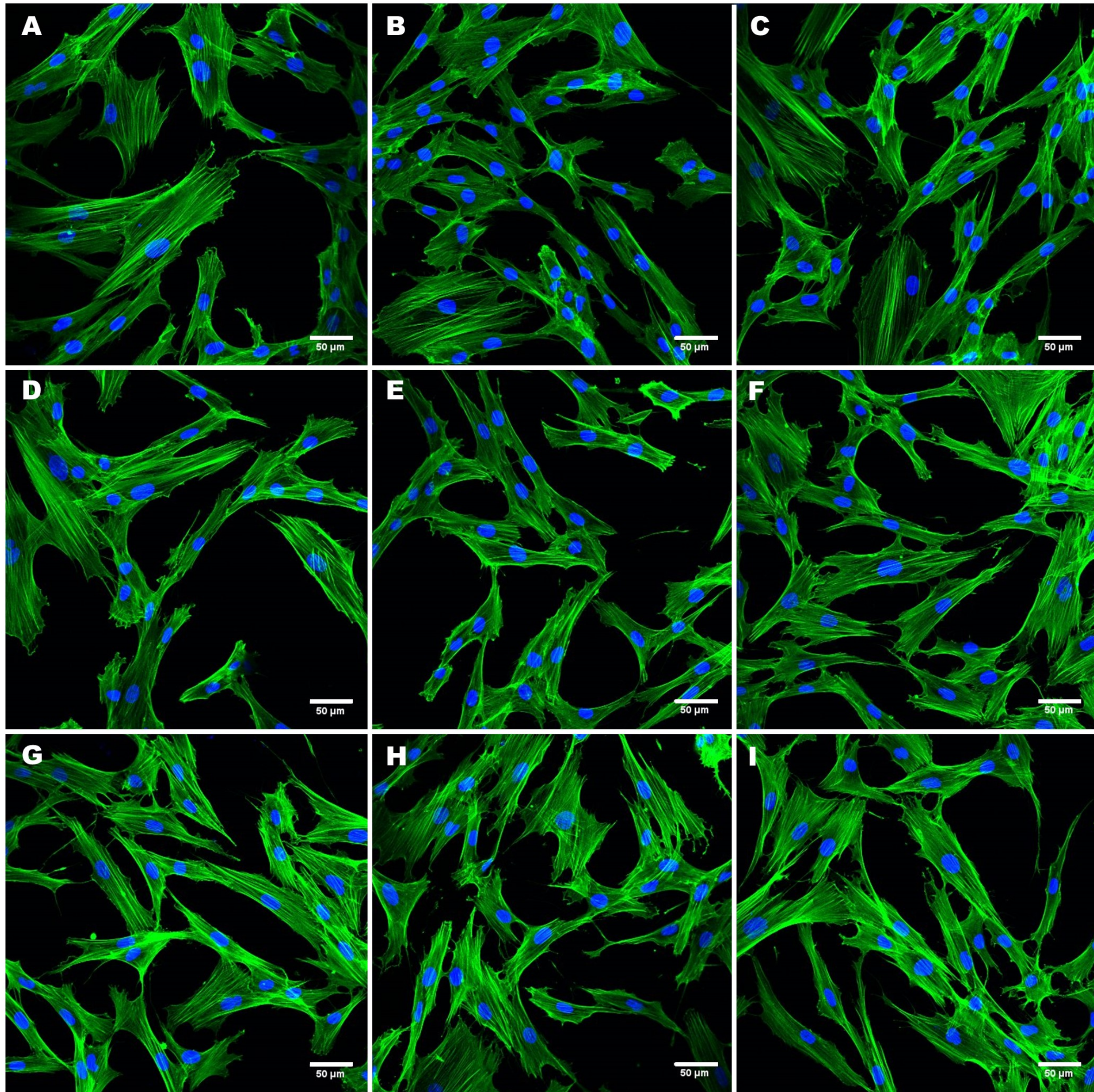

Fig.S6. Confocal fluorescence micrographs of HDF cells at 48 hours. Cells exposed to a) no NPs (control), b) non-chronic citrate spheres, c) non-chronic PAA spheres, d) non-chronic PAA rods, e) non-chronic PEG rods, f) chronic citrate spheres, g) chronic PAA spheres, h) chronic PAA rods and i) chronic PEG rods for 48 hours. Non-chronic samples exposed to NPs for 24 hours only, then media for 24 hours. Green signal = phalloidin stain for actin, blue signal = DAPI stain for nuclei. All scale bars are 50 µm.

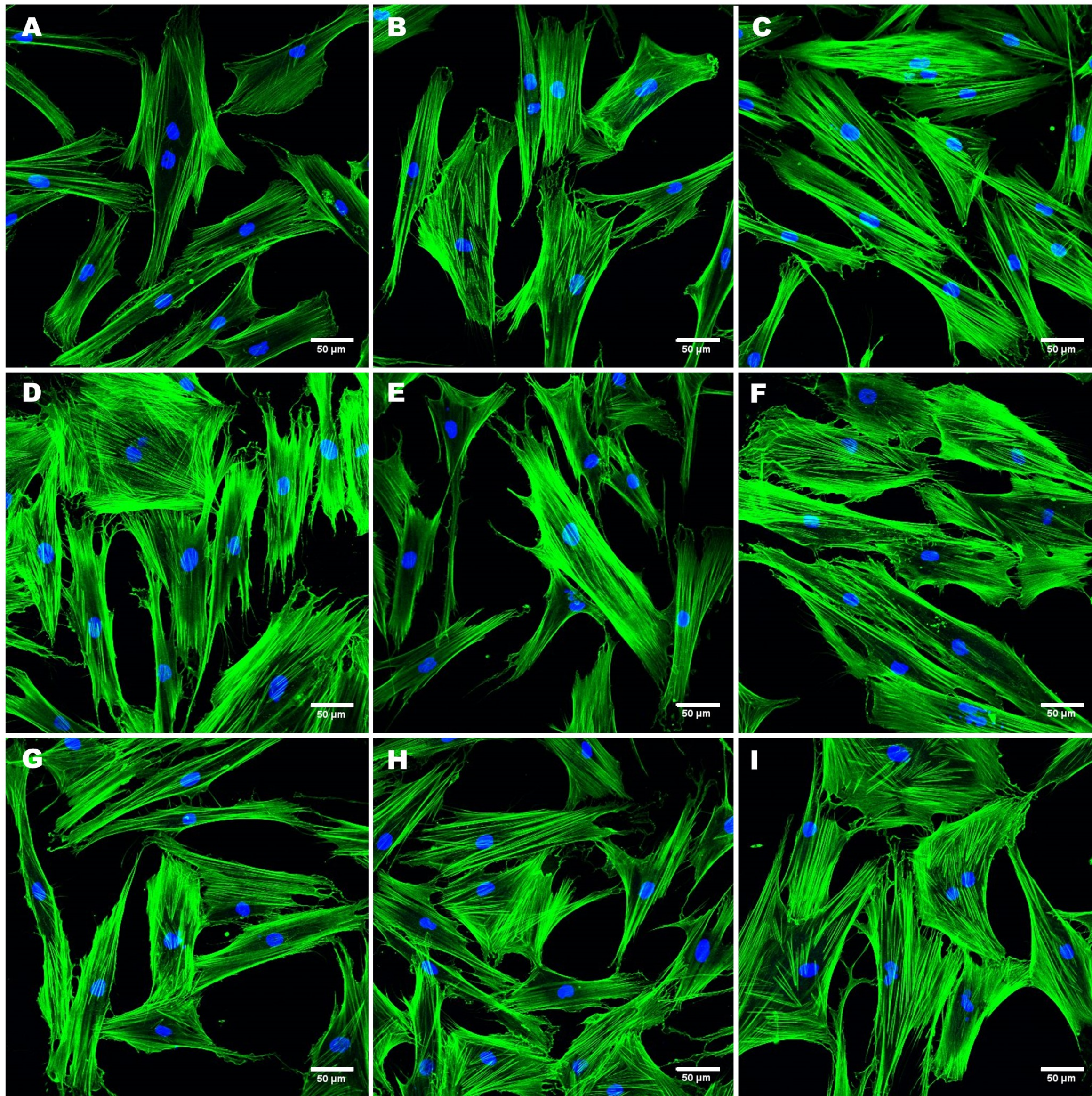

Fig.S7. Confocal fluorescence micrographs of HDF cells at 20 weeks. Cells exposed to a) no NPs (control), b) non-chronic citrate spheres, c) non-chronic PAA spheres, d) non-chronic PAA rods, e) non-chronic PEG rods, f) chronic citrate spheres, g) chronic PAA spheres, h) chronic PAA rods, and i) chronic PEG rods over 20 weeks. Cells plated into glass-bottomed lysine-coated dishes for imaging; NPs added to chronic samples after 24 hours; cells allowed to adjust for 48 hours total before fixation. Green signal = phalloidin stain for actin, blue signal = DAPI stain for nuclei. All scale bars are 50 µm.

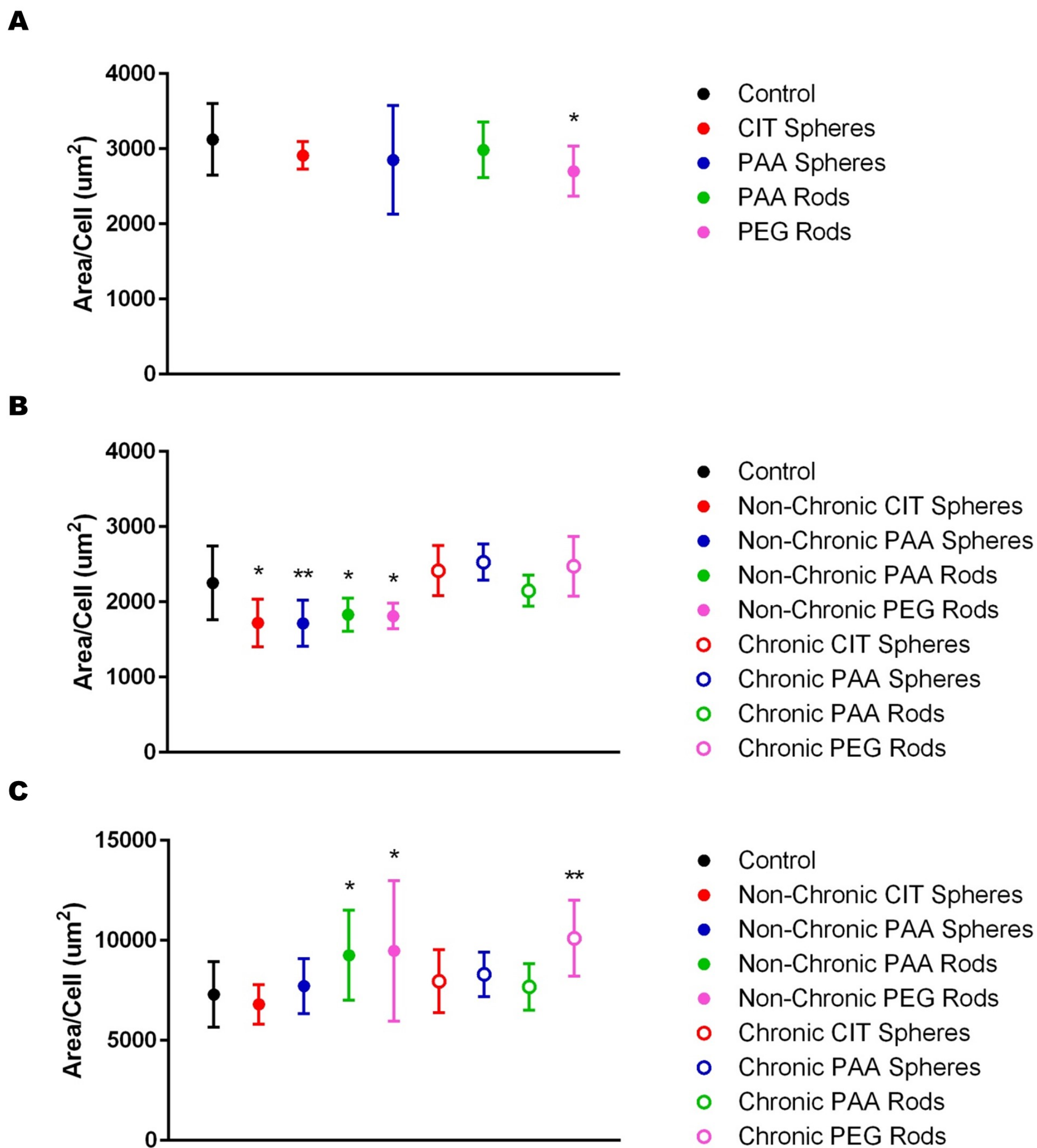

Fig.S8. Average area (μm^2) per cell at three time points of NP exposure. a) 24 hours, b) 48 hours, and c) 20 weeks. *$p < 0.05$, **$p < 0.01$ relative to control. Error bars represent one standard deviation from the mean.

Table S1. Characterization Results for CIT–Au Spheres and PAA-Au Spheres

NP Type	Coating	Solvent	UV-Vis peak (nm)	DLS (nm)	TEM (nm)	Zeta potential (mV)
CIT Spheres	CIT	Water	522	31.2 ± 0.2	18.4 ± 2.0	-31.7 ± 1.5
	CIT	Media	527	65.4 ± 0.5	18.4 ± 2.0	-0.1 ± 6.1
PAA Spheres	PAA	Water	526	54.6 ± 0.2	18.4 ± 2.0	-33.2 ± 1.1
	PAA	Media	528	56.1 ± 0.3	18.4 ± 2.0	-14.1 ± 3.3

Table S2. Characterization Results for PAA-Au Rods and PEG-Au Rods

NP Type	Coating	Solvent	UV-Vis peak (nm)	TEM length (nm)	TEM width (nm)	Zeta potential (mV)
PAA Rods	PAA	Water	700	49.2 ± 5.0	17.0 ± 3.0	-53.8 ± 1.2
	PAA	Media	702	49.2 ± 5.0	17.0 ± 3.0	-18.0 ± 2.1
PEG Rods	PEG	Water	689	46.0 ± 4.1	15.9 ± 2.6	-7.2 ± 0.8
	PEG	Media	680	46.0 ± 4.1	15.9 ± 2.6	4.7 ± 8.3

Table S3. NP Uptake Levels at 72 Hours and 20 Weeks

	Avg. NP/Cell ($x10^4$)		Standard Deviation	
NP Type	72 Hours	20 Weeks	72 Hours	20 Weeks
CIT Spheres	0.6	2.4	0.15	0.55
PAA Spheres	1.8	7.9	0.21	1.1
PAA Rods	7.3	36.2	0.82	2.6
PEG Rods	1.3	1.1	0.36	0.30

Table S4. Gene expression data for non-chronic HDF exposure to Au NPs*

Gene Symbol	Entrez ID	Functional Pathway	Citrate Spheres		PAA Spheres		PAA Rods		PEG Rods	
			Fold Change	p value	Fold Change	p value	Fold Change	p value	Fold Change	p value
IL6	3569	Inflammatory Response	2.76	0.0670	6.38	0.1580	5.02	0.1464	12.24	$4.35x10^{-4}$
VEGFA	7422	Hypoxia	1.37	0.1370	2.77	0.1290	3.55	0.0985	4.04	$2.20x10^{-5}$
CCL2	6347	Inflammatory Response	1.15	0.4500	1.64	0.3160	1.84	0.2113	2.58	0.0023
PRDX1	5052	Oxidative Stress	1.64	0.0401	1.52	0.0036	1.44	0.0296	1.74	0.0047
FTH1	2495	Oxidative Stress	1.52	0.0031	1.57	0.0303	1.55	0.1081	1.73	0.0018
MCL1	4170	Cell death (apoptosis)	1.56	0.0400	1.70	0.0023	1.62	0.0460	1.69	0.0041
GCLC	2729	Oxidative Stress	1.04	0.8730	-1.12	0.7020	1.08	0.6967	1.69	0.0234
EDN1	1906	Osmotic Stress	1.05	0.7640	1.40	0.1830	1.51	0.1470	1.62	0.0370
DDIT3	1649	Unfolded Protein Response/DNA Damage Signaling	1.43	0.0665	1.47	0.0204	1.41	0.2950	1.61	0.0104
NQO1	1728	Oxidative Stress	1.41	0.0328	1.44	0.0266	1.36	0.1569	1.53	0.0326
SLC2A1	6513	Hypoxia	-1.10	0.4810	1.26	0.4660	1.53	0.2683	1.38	0.0358
NFAT5	10725	Osmotic Stress	-1.07	0.0281	-1.01	0.9110	-1.14	0.5297	-1.17	0.0767
GADD45G	10912	DNA Damage Signaling	-1.07	0.7880	-1.08	0.7420	-1.81	0.0872	-1.23	0.3270
TNFRSF10A	8797	Cell death (necrosis/apoptosis)	-1.19	0.0456	-1.38	0.2430	-1.35	0.2300	-1.35	0.0451
TP53	7157	DNA Damage Signaling	-1.32	0.0270	-1.11	0.1370	-1.32	0.0270	-1.35	0.0150
BBC3	27113	Unfolded Protein Response	-1.05	0.8400	-1.02	0.9360	-1.21	0.2522	-1.40	0.0466
RAD9A	5883	DNA Damage Signaling	-1.09	0.2280	-1.16	0.4560	-1.49	0.0330	-1.50	0.0072
ATG7	10533	Cell death (autophagy)	-1.32	0.0142	-1.05	0.5590	-1.24	0.0906	-1.55	0.0232
CHEK2	11200	DNA Damage Signaling	-1.03	0.8020	-1.24	0.3870	-1.55	0.0321	-1.61	0.0405
ATF6	22926	Unfolded Protein Response	-1.37	0.0673	-1.35	0.2000	-1.14	0.3310	-1.62	0.0268
MRE11A	4361	DNA Damage Signaling	-6.18	0.2360	-1.16	0.1220	-1.36	0.0802	-1.72	0.0232
RIPK1	8737	Cell death (necrosis)	-1.41	0.1170	-1.35	0.1430	-1.29	0.1590	-1.81	0.0297
TLR4	7099	Inflammatory Response	-1.37	0.0033	-1.09	0.5010	-1.05	0.7010	-2.01	0.0033
HSPA5	3309	Unfolded Protein Response	1.75	0.0145	1.67	0.0120	1.81	0.0103	-2.18	0.7240
AQP1	358	Osmotic Stress	1.03	0.8380	-2.39	0.0095	-2.63	0.0147	-3.10	0.0035

*Genes included have an average FC ≥ 1.5 or ≤ -1.5 and a p-value < 0.05 in at least one sample type in the chronic and/or non-chronic exposures. Entries that are significant in both aspects are highlighted in red (up-regulated) or blue (down-regulated).

Table S5. Gene expression data for chronic HDF exposure to Au NPs*

Gene Symbol	Entrez ID	Functional Pathway	Citrate Spheres		PAA Spheres		PAA Rods		PEG Rods	
			Fold Change	p value	Fold Change	p value	Fold Change	p value	Fold Change	p value
IL6	3569	Inflammatory Response	1.48	0.0639	-1.28	0.2060	-1.23	0.1520	1.24	0.0633
VEGFA	7422	Hypoxia	-1.08	0.7840	-1.60	0.0101	1.39	0.0013	-1.74	0.0089
CCL2	6347	Inflammatory Response	-1.01	0.9640	-1.62	0.0660	-1.89	0.0375	-1.61	0.1410
PRDX1	5052	Oxidative Stress	1.02	0.8770	1.07	0.6070	1.34	0.0279	1.50	0.0141
FTH1	2495	Oxidative Stress	1.16	0.2900	1.06	0.5290	1.09	0.3340	1.33	0.0144
MCL1	4170	Cell death (apoptosis)	-1.03	0.7760	-1.08	0.3950	1.38	0.0353	1.08	0.5930
GCLC	2729	Oxidative Stress	-1.24	0.2620	1.09	0.6710	1.04	0.8640	1.30	0.2300
EDN1	1906	Osmotic Stress	1.41	0.0326	2.01	0.0078	1.07	0.6920	2.77	0.0011
DDIT3	1649	Unfolded Protein Response/DNA Damage Signaling	1.21	0.1740	-1.17	0.2700	-1.20	0.1580	1.08	0.5610
NQO1	1728	Oxidative Stress	1.33	0.0661	-1.08	0.4530	1.19	0.2230	1.92	0.0078
SLC2A1	6513	Hypoxia	1.25	0.1330	-1.30	0.1240	-2.03	0.0031	-1.20	0.1810
NFAT5	10725	Osmotic Stress	-1.07	0.3950	-1.53	0.0454	-1.36	0.0155	-1.03	0.6640
GADD45G	10912	DNA Damage Signaling	1.05	0.8570	-2.16	0.0964	-2.71	0.0160	-1.51	0.1100
TNFRSF10A	8797	Cell death(necrosis/apoptosis)	1.07	0.2530	-1.46	0.0020	-1.99	0.0081	-1.74	0.0027
TP53	7157	DNA Damage Signaling	-1.04	0.3840	-1.22	0.2420	-1.50	0.0137	-1.40	0.0106
BBC3	27113	Unfolded Protein Response	-1.17	0.3330	-1.39	0.0597	-1.47	0.0299	-1.56	0.0421
RAD9A	5883	DNA Damage Signaling	-1.01	0.9110	-1.65	0.1770	-1.33	0.1870	-1.17	0.1490
ATG7	10533	Cell death (autophagy)	1.09	0.2390	-1.42	0.0176	-1.25	0.1570	-1.18	0.0771
CHEK2	11200	DNA Damage Signaling	-1.14	0.3080	-1.49	0.0446	-1.38	0.0865	-1.37	0.1800
ATF6	22926	Unfolded Protein Response	1.22	0.0778	1.10	0.4460	-1.16	0.1370	-1.01	0.8600
MRE11A	4361	DNA Damage Signaling	-1.07	0.4620	-1.20	0.2300	-1.44	0.0331	-1.42	0.0562
RIPK1	8737	Cell death (necrosis)	1.16	0.2970	-1.15	0.5210	-1.14	0.4120	-1.03	0.7900
TLR4	7099	Inflammatory Response	1.11	0.1240	1.01	0.8500	-1.47	0.0577	-1.30	0.0395
HSPA5	3309	Unfolded Protein Response	-1.09	0.0685	1.20	0.0885	1.65	0.0158	1.39	0.0251
AQP1	358	Osmotic Stress	-1.25	0.6570	-1.06	0.8810	1.04	0.7360	-1.46	0.0771

*Genes included have an average FC ≥ 1.5 or ≤ -1.5 and a p-value < 0.05 in at least one sample type in the chronic and/or non-chronic exposures. Entries that are significant in both aspects are highlighted in red (up-regulated) or blue (down-regulated).