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Engineered nanoparticle bio-conjugates toxicity screening: The xCELLigence cells viability impact

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Table S1. Engineered Nanoparticle (NP) bio-conjugates (ENPBC) cytotoxicity dose estimation *in vitro* XCELLigence cells viability technology

Type of ENPBC	Instrument of interest	Size of ENPBC	Cells of exposure	Toxicology dose level estimation	Outcome of dose level toxicity	Toxicity nature	Ref
Citrate stabilized gold NPs (AuNPs)	XCELLigence RTCA	14nmm and 20nm	Bronchial epithelial cell line BEAS-2B, the Chinese hamster ovary cell line CHO, and the human embryonic kidney	1nM, 2nM and 5nM	The AuNPs were relatively non-toxic. However, the cells were sensitive to 20 nm AuNPs having the highest toxicity. Uptake of both 14 nm and 20 nm AuNPs was observed in all cell lines in a time	Partially nontoxic	14

			cell line HEK 293				
Ru(II) complex (2–4) 4	xCELLigence RTCA	Not Mentioned	HeLa, A549 and multidrug-resistant (A549R) tumor cell lines	0.5 µM and 2.0 µM	With 0.5 µM cells began to die after a short while confirming the high cytotoxicity of complex 4 against A549R cells. With 2.0 µ M abolished cellular proliferation, indicating that the cells underwent irreversible senescence	Toxic	21
lauric acid/albumin hybrid iron oxide nanoparticle	xCELLigence RTCA	20 nm to 500 nm	Primary human endothelial cells	µg/mL, 10 µg/mL, and 100 µg/mL	The drug-loaded system exhibited excellent therapeutic potential *in vitro* , exceeding that of free mitoxantrone.	Nontoxic	22
Carbon-Based NPs, Diamond and Graphite	xCELLigence RTCA DP system	100nm	Human Glioblastoma and Hepatoma Cell Lines	20, 50 and 100 µg/mL	Diamond NPs were nontoxic. Graphite NPs exhibited a negative impact on glioblastoma, but not on hepatoma cells. The studied carbon NPs could be a potentially useful tool for therapeutics delivery to the brain tissue with minimal side effects on the hepatocytes.	Partially nontoxic	23
Titanium Dioxide NPs	xCELLigence RTCA DP system	Not mentioned	Human bronchial epithelial cells (16HBE14o-)	25, 50 and 100 µg/mL	TiO_2-NP induced endoplasmic reticulum (ER) stress in the cells and disrupted the mitochondria-associated endoplasmic reticulum membranes (MAMs) and calcium ion balance, thereby increasing autophagy. suggesting that TiO_2-NP	Toxic	20

					promoted toxicity via ER stress		
Antibody–drug conjugate (ADC) loaded micellar NPs	xCELLigence RTCA	Not mentioned	Human pancreatic cancer cell lines, BxPC3 (high TF expression) and SUIT2 (low TF expression), and a gastric cancer cell line, 44As3 (high TF expression).	0.05 lM in BxPC3 or 0.5 lM in SUIT2	Anti-TF-NC-6300 showed a superior antitumor activity in BxPC3 and 44As3 xenografts. The anti-TF-NC-6300 appeared to be localized to the tumor cells with high TF expression	Non toxic	24
Multi-walled carbon nanotubes (MWCNTs)	xCELLigence RTCA	Range of 200nm	Mice spermatocyte cell line (GC-2spd)	0, 0.05, 0.25, 0.5, 1, and 5 μg/mL	A maximal concentration of 0.5 μg/mL MWCNTs was found to be nonlethal to GC-2spd. However, MWCNTs accumulated in mitochondria, which caused potential mitochondrial DNA damage in spermatocyte. germ cells, mitochondrion cellular organelle accumulated MWCNTs	Partially toxic	25
Hydrogen sulfide (H_2S) mesoporous silica NPs (MSNs)= DATS-MSN	xCELLigence RTCA	20nm	Endothelial Cell lines	DATS-MSNs ranging from 3.13 μg/mL to 800 μg/mL under hypoxic conditions (95% N2/5% CO_2)	DATS-MSN could alleviate endothelial cell inflammation and enhance endothelial cell proliferation and migration	Toxic	26
CTAB layer Gold Nanorods	xCELLigence	N/A	Human leukemia cell line K562	N/A	AuNR-dPGS, a promising tool for enabling diagnosis of	Nontoxic	27

					inflammatory diseases through volumetric molecular imaging using the example of rheumatoid arthritis		
Surface coating of Fe3O4 magnetic NPs (MNPs) with a polymer poly(lactic-co-glycolic acid) (PLGA)	xCELLigance RTCA	Ranged from 9.6nm to 53.2 nm with average hydrodynamic diameter of MNP and PLGA-MNP was 54.3 ± 8.7 nm and 293.4 ± 31.9 nm	Human lung carcinoma cell line A549	MNP or PLGA-MNP exposed to 50 µg/ml	No cytotoxicity was observed when up to 100 µg/ml PLGA-MNP when applied to the cultured human lung epithelial cells	Nontoxic	28
Superparamagnetic iron oxide NPs (SPIONs)	xCELLigence RTCA	Ranged from 3.0 nm to 6.6 nm and had a mean value of 4.6±0.8 nm for SEONDEX 2.0 and 4.3±0.9 nm for SEONDEX 4.5	Jurkat cell line of nonadherent human T-cell leukemia cells and human prostate cancer cell line, PC-3 (CRL-1435)	Concentration 0.2µg/mL to 26.7 µg/mL	The oxide NPs was biocompatible to cells. The drug induced apoptosis in a dose-dependent manner, and necrosis after prolonged incubation with cancer cells. The drug nanoconjugates were biocompatible with cells and showed no cytotoxic effects. Particles with incorporated cisplatin induced apoptosis in a dose-dependent manner, with secondary necrosis after prolonged incubation	Nontoxic	29
Poly(methyl vinyl ether-co-maleic anhydride) (PVM/MA) copolymer shelled selol nanocapsules	xCELLigence real-time cell analyzer (RTCA)	207.9 ± 80.9 nm	Human lung adenocarcinoma (A549) and promyelocytic leukemia (HL60) cell lines	Concentrations ranged from 50, 100 and 150 µg/mL	Selol promotes G2/M arrest in cancerous cells.	Nontoxic	30

Fumed silica NPs (SiO_2 NPs)	xCELLigence RTCA	160 ± 90 nm	Human alveolar epithelial cells. A549	0.1, 1.0, 1.5, 3.0, and 6.0 μg/cm^2)	A hierarchical, dose-dependent cellular response was significant to silica NPs. At 1.5μg/cm^2, the Rho signaling cascade, actin cytoskeleton remodeling, and clathrin-mediated endocytosis were induced. At 3.0 μg/cm^2, many inflammatory mediators were upregulated and the coagulation system pathway was triggered. Lastly, at 6.0 μg/cm^2, oxidative stress was initiated.	Partially nontoxic	19
Zinc oxide and aluminum-doped zinc oxide NPs(ZnONPs or Al-ZnONPs,)	xCELLigence RTCA	ZnONPs, including $ZnONP_20$ (average diameter of 20 nm), ZnONP90 (average diameter of 90–210 nm), and Al-$ZnONP_20$ (average diameter of 20–40 nm),	Human alveolar basal epithelial A549 cells	0, 20, 50, 150, 300, and 500 μg/mL ZnONPs or Al-ZnONPs	The size and surface char-acteristics of ZnONPs play important roles in the regulation of cell viability, which was inferred from proteomic analyses.	Nontoxic	31
Polystyrene nanobeads: functionalized (PS-NF), carboxylated (PS-COOH) and aminated (PS-NH_2) nanobeads	xCELLigence system.	100nm	THP-1 differentiated cells as a model for lung macrophages and Calu-3 cells were used as model for lung epithelium	Concentration s ranging from 1 to 100 μg/ml corresponding to 0.3 to 32.3 μg/cm^2	Aminated nanobeads significantly increased DNA damages in association with a strong depletion of reduced glutathione in both cell lines. Also aminated polystyrene nanobeads were more cytotoxic and genotoxic than unmodified	Toxic	32

			junctions		and carboxylated ones on both cell lines.		
Carbon Nanotubes	xCELLigence RTCA)	SCNT(2nm), SCNTc(2nm), MCNT8(4.7 ± 0.48),MCNT8c(4.2 ± 0.8),MCNT20(18.9 ± 0.9), MCNT20c(15.3 ±2.5), MCNT50(62.8 ± 5.7), MCNT50c(63.6 ± 11.3)	DMBM-2 mouse macrophages, murine L929 and V79 Chinese hamster lung fibroblasts, endothelial EAhy926 cells and human MRC-5 (ATCC) fibroblasts	Concentrations of 100 μg/mL, 200 μg/mL, 300 μg/mL polystyrene particles	All systems identified thin (<8 nm) CNTs as more cytotoxic than thick (>20 nm) CNTs, but detection by xCELLigence system was less sensitive to CNT-induced cytotoxicity.	Partially nontoxic	33
Superparamagnetic iron oxide NPs UL-D	xCELLigence DP analysis system	3 nm and 5 nm	Carcinoma of the tonsilla (UT-SCC-60A) and the corresponding metastasis (UT-SCC-60B)	Concentrations (0.2 mM, 0.5 mM, 0.9 mM, 1.8 mM Fe) of UL-D and Resovist®-labeled tumor cells. Cells were incubated with either UL-D or Resovist in concentrations of 0.2 mM, 0.5 mM, 0.9 mM, and 1.8 mM Iron oxide	Results from cell viability of UL-D-labeled cells showed that cells were not altered suggesting that the particles did not cause oxidative stress. Tumor necrosis factor alpha (TNF-α) and interleukins IL-6, IL-8, and IL-1β were measured to distinguish inflammatory responses. Only the primary tumor cell line labeled with 0.5 mM showed a significant increase in IL-1β secretion. Data suggest that UL-D SPIONs are a promising tracer material for use in innovative tumor cell	Nontoxic	34

Eupafolin NPs delivery system (ENDS)	xCELLigence Real-Time Cell Analyzer (RTCA)	70.7±5.2 nm	Fresh pig skin, HaCaT keratinocyte cell line and primary native human epidermal keratinocytes, Human dermal fibroblasts, human pulmonary alveolar epithelial cells and human fibroblast-like synoviocytes	10 µM, 40 µM, and 100 µM)	ENDS can suppress PM-induced ROS production, COX-2 expression through downregulation of mediated NF-κB and c-Fos signaling pathways in HaCaT keratinocytes. ENDS can be potentially used as a medicinal drug and/or cosmeceutical product to prevent PM-induced skin oxidative stress and inflammation in the future	Nontoxic	35
Carbon fluoroxide NPs	xCelligence system.	Range of 4–6 nm.	Mouse 3T3-L1 cells	Varying concentrations 0.25 to 0.5 mg	NPs provoke cell destruction after application of an ultrasound treatment. No significant toxic effect with 0.5 mg ml^{-1} without applying ultrasound treatment	Nontoxic	36
Silica-coated cobalt zinc ferrite NPs (CZF) and poly-l-lysine-coated iron oxide superparamagnetic NPs (PLL-coated γ-Fe2O3)	xCELLigence RTCA	N/A	Human iPSC line was derived from female (IMR90) human fetal lung fibroblasts	5, 10 and 15 µg Fe/mL	Cell proliferation was not affected by PLL-coated γ-Fe_2O_3 PLL-coated γ-Fe_2O_3 are suitable for MR detection, did not affect the cells	Nontoxic	37
50-nm diameter halloysite nanotube carriers.	xCELLigence Real-Time Cell Analyzer	15 nm lumen and 50 nm	A549 - human lung carcinoma epithelial cells; and Hep3b -	Concentration ranged from 0, 25, 50 and 100 µ g	Drug-loaded nanotubes penetrate through the cellular membranes and elimination of human lung carcinoma cells	Nontoxic	38

			hepatocellular carcinoma cells	respectively per 105 cells	(A549) as compared with hepatoma cells (Hep3b). Dextrin-coated halloysite nanotubes is a promising platform for anticancer treatment.		
Germanium nanowires and water dispersible Germanium nanowires (WDW)	xCELLigence system (ACEA)	5nm, 50nm 100nm	MCF-7 cells (human epithelial breast adenocarcinoma	MCF-7 and L929 cells were grown in the presence of increasing concentrations of 2 mM, 4 mM, and 7 mM of WDWs	Treated germanium nanowires promote cell adhesion and cell proliferation which we believe is as a result of the presence of an etched surface giving rise to a collagen like structure and an oxide layer.	Nontoxic	39
Maghemite NPs (SAMNs) versus commercial Resovist	xCELLigence impedance system (Roche)	SAMN = 10–20 nm and **Resovist =** 45–60 nm	Rat bone marrow stromal cells (Mesenchymal stromal cell MSC)	Wide range of 10–200 µg/mL of SAMN and Resovist	SAMNs do not affect MSC viability <100 µg ferumoxide/mL, and this concentration does not alter their cell phenotype and long-term proliferation profile.	Nontoxic	40

Table 2. *In vivo* analysis and dose concentration of engineered NPs bio-conjugates (ENPBC) using animals models

Type of ENPBC used	Instrument of interest used characterizing ENPBC	Size of ENPBC	ENPBC animal organ of exposure	ENPBC animal of exposure	ENPBC toxicology levels	Effect of ENPBC on animals	Toxicity nature	Ref
Antibody–drug conjugate (ADC) loaded micellar NPs	High-performance liquid chromatography (HPLC)	Not mentioned	Human pancreatic cancer cell lines, BxPC3 (high TF expression) and SUIT2 (low TF expression), and a gastric cancer cell line, 44As3 (high TF expression).	Female BALB mice	Subcutaneously Inoculation of 59, 105 in 44As3 cells (high TF expression), 1 9, 107 in BxPC3 cells (high TF expression) or 39, 106 SUIT2 cells (low TF expression) in the flank region.	The therapeutic effect of anti-TF-NC-6300 was significantly greater than that of NC-6300 and conventional epirubicin in both the 44As3 and the BxPC3 xenografts. However, no severe body weight loss or toxicity-related death was observed in the anti-TF-NC-6300 and NC-6300 groups	Nontoxic	24
Hydrogen sulfide	Transmission	20nm	Aortic allografts.	Balb/c	DATS-MSNs	DATS-MSN demonstrated	Toxic	26

(H2S) mesoporous silica NPs (MSNs)= DATS-MSN	Electronic Microscope (TEM)			mice aged 4–6 weeks	ranging from 3.13 µg/mL to 800 µg/mL under hypoxic conditions (95% N_2/5% CO_2)	the apoptosis of graft endothelium cells. The results indicated that DATS-MSN, releasing H_2S in a controlled release fashion, could serve as an ideal H_2S donor.		
CTAB layer Gold Nanorods	UV-VIS absorption spectra from. TEM	N/A	left knee and ankle	Mice	Injection of 5mg/kg of AuNR intravenously in mice	CTAB layer Gold Nanorods were found suitable for biological applications as well as a low-cost, actively targeted, and high contrast imaging agent for the diagnosis of rheumatoid arthritis	Nontoxic	27
Surface coating of Fe3O4 magnetic NPs (MNPs) with a polymer poly(lactic-co-glycolic acid) (PLGA)	dynamic light scattering (DLS), TEM	Ranged from 9.6nm to 53.2 nm with average hydrodynamic diameter of MNP and PLGA-MNP was 54.3 ± 8.7 nm and 293.4 ± 31.9 nm	Measurement of IL-6 levels in BAL fluid from mice treated with a single intranasal delivery at 1 days, 4 and 7 days	Mouse model.	Mice were treated intranasally with 50 µl solution containing 1 mg/ml of MNP preparations	PLGA-MNP preparation was well-tolerated *in vivo* in mice when applied intranasally as measured by glutathione and IL-6 secretion assays after 1, 4,or 7 days post-treatment. The data demonstrate inhibition of lung adenocarcinoma growth by aerosol delivery of quercetin loaded in the PLGA-MNPs.	Nontoxic	28
Biodegradable polymeric NPs: polymer structure,	TEM	138 ± 4 nm	Rats were evaluated daily for signs of pain	Female F344 rats, weighing	Injected by bolus (manual injection) or	The treated animals showed a significant benefit in survival (p =	Nontoxic	46

poly(1,4-butanediol diacrylate-co-4-amino-1-butanol) endmodified with 1-(3-aminopropyl)-4-methylpiperazine, is a poly(β-amino ester) (PBAE) and formed NPs with HSVtk DNA			and distress, including ruffled fur, dehydration, hunched position, weakness, lethargy, immobility, lack of coordination, labored respiration, or cyanosis	125175 g	Infusion. PBAE 447/GFP DNA NPs (26 μg DNA/780 μg polymer) were infused in a volume of 25 μL using CED in six wild type healthy rats and three 9L tumor-bearing rats for a total number of 9 rats	0.0012 vs control). This study highlights a nanomedicine approach that is highly promising for the treatment of malignant glioma		
chitosan-coated poly (d,l-lactide-co-glycolide) (CS-PLGA)	dynamic light scattering (DLS),	274.6 ± 4.1 nm	NPs showed high plasma concentrations	Wistar male rats, weighing 200–230 g	oral delivery or administration exendin-4 solution (500 μg/kg)	Unmodified PLGA and chitosan-coated NPs showed only slight cytotoxicity. The study demonstrates that chitosan-coated NPs have a higher transport potential over both free drug and unmodified NPs, providing support for their potential development as a candidate oral delivery agent for exendin-4.	Partially Nontoxic	47
Silver NPs (AgNPs) coated with either citrate (CT) or polyvinylpyrrolidone (PVP)	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)	10 nm, 40 nm, 100 nm),	The highest silver concentrations were found in the spleen and	Male CD-1(ICR) mice	Intravenously injection of AgNPs of different sizes (10 nm, 40 nm,	Toxic effects (midzonal hepatocellular necrosis, gall bladder hemorrhage) were found in mice treated with 10 nm	Partially nontoxic	45

			liver, followed by lung, kidney, and brain. Silver concentrations were significantly higher in the spleen, lung, kidney, brain, and blood of mice treated with 10 nm AgNPs than those treated with larger particles		100 nm), Citrate or polyvinylpyrrolidone-coated, at a single dose of 10 mg/kg body weight.	AgNPs, while in mice treated with 40 nm and 100 nm. 40nm AgNPs lesions were milder and significantly lower in the spleen and lung, and higher in the kidney than in mice treated with 10 nm AgNPs, and a different target organ of toxicity was identified (kidney).		
Polymeric NPs based on hyaluronic acid-poly(butyl cyanoacrylate) and D-alpha-tocopheryl polyethylene glycol 1000 succinate: MH-PNs and MH-MNs	Dynamic light scattering (DLS), TEM	Size less than 200 nm,	Measurement of Tumor sizes	S180 murine sarcoma model in nude mice	Injected intravenously via the tail vein with 1) saline; 2) blank PNs; 3) blank MNs; 4) MH drug solution (15 mg/kg); 5) MH-PNs (15 mg/kg); and 6) MH-MNs (15 mg/kg)	The CD44 receptor competitive inhibition and the internalization pathway showed NPs mediating the CD44 receptors through a clathrin-dependent endocytic pathway. The MH-MNs exhibited a higher *in vivo* antitumor potency and induced more tumor cell apoptosis than did MH-PNs, following intravenous administration to S180 tumor-bearing mice.	Partially nontoxic	48

						Finally, NPs are promising vehicles for the targeted delivery of lipophilic anticancer drugs.		
Felodipine loaded PLGA	FTIR and Dynamic laser scattering (DLS), Differential scanning calorimetry (DSC), SEM, Atomic force microscopy (AFM)	307 ± 4.51, 226 ± 5.39 and 273 ± 6.28nm	pathological examinations of different organs of wistar albino mice	Wistar albino mice	Oral gavaged in a single dose of 60, 120, 240 and 480 mg/kg BW of felodipine in distilled water	The results showed no noticeable change in biochemical parameters and histopathology of organs by Felodipine loaded PLGA.	Nontoxic	49