Journal of Pharmaceutical Research International

18(6): 1-8, 2017; Article no.JPRI.36154
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Cell-specific Reduction in Viability of Two Breast Cancer Cell Lines after Exposure to Gold Nanoparticles

Olivia G. Waszczuk[1] and Jennifer R. Schroeder[1*]

[1]Department of Biology, Millikin University, Decatur, Illinois, USA.

Authors' contributions

This work was carried out in collaboration between both authors. Authors OGW and JRS together designed the study. Author OGW performed the experimentation and wrote the first draft of the manuscript. Author JRS performed the statistical analysis, managed the literature searches and analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2017/36154
Editor(s):
(1) Sami Nazzal, College of Pharmacy, University of Louisiana at Monroe, USA.
Reviewers:
(1) Pall Emoke, University of Agricultural Sciences and Veterinary Medicine, Romania.
(2) Farag M. Abo Mosalam, National Center for Radiation Research and Technology (NCRRT), Egypt.
(3) Asita Elengoe, Lincoln University College, Malaysia.
(4) Michel Mansur Machado, Universidade Federal do Pampa, Brasil.
(5) Moisés Armides Franco Molina, University Autonomous of Nuevo Leon, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/21354

Original Research Article

Received 16th August 2017
Accepted 7th October 2017
Published 12th October 2017

ABSTRACT

Aims: Nanoparticle composites are a recent research hotspot, with the potential to be drug-delivery vehicles for more efficient treatment of malignant cancerous tumors. However, as this is a relatively new field, the safety of these nanoparticles is of concern. In this study, we assess whether two preparations of gold nanoparticles, HPN1 and HGN2, affect cell viability using a metabolic assay.
Study Design: We treated two breast cancer cell lines, MCF-7 and MDA-MB-231, with two different nanoparticle preparations for five days. Following treatment, we assessed changes in cellular metabolic activity using an MTT assay.
Methodology: HPN1 are 32 nm diameter colloidal gold nanoparticles, which reflect a purple hue, while HGN2 are 10 nm diameter and reflect a yellow-orange color. We plated MDA-MB-231 or MCF-7 cells into a 6-well plate at 60% confluence. After 24 hours, we treated cells with fresh media containing 5-10% of HPN1 or HGN2 nanoparticles or PBS control. After 120 hours, we assessed

**Corresponding author: E-mail: jrschroeder@millikin.edu;*

the metabolic activity of live cells using a standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, performed in triplicate. Univariate analysis of variance (ANOVA) was performed using SPSS, with *P*=0.05 indicating significant variation from the controls.
Results: Interestingly, we show little change in cell activity after exposure of human MDA-MB-231 breast cancer cells to fresh and aged nanoparticles for five days; however, MCF-7 breast cancer cells responded to the two nanoparticles differently. After nanoparticles had been stored for several months, treatment with HPN1 led to a loss of viability; in contrast, HGN2 increased proliferation.
Conclusion: We must be cautious moving forward in the development of new chemotherapeutic techniques, since acute tests may not be indicative of the true toxicity of these compounds.

Keywords: Nanoparticle; breast cancer; cytotoxicity; chemotherapy.

1. INTRODUCTION

From detection of pathogens to treatment of cancers, the use of nanoparticles as a component of new technology is evident [1-7]. Unfortunately, the American Cancer Society reports that there will be more than 1.6 million new cancer diagnoses in the United States of America in 2017 (http://www.cancer.org). While breast cancer mortality has decreased over the past few decades, the statistics show more work is needed to increase early detection and maximize efficient treatment of malignant tumor cells. While there are several specific and non-specific chemotherapeutics available, most of these are non-selective, thus damaging healthy tissue such as the linings of the gastrointestinal tract and bladder as well as destroying tumor cells [8-12].

One of our goals is to develop novel mechanisms for detecting and treating cancers using nanoparticles. First, nanoparticles can detect cancerous cells early in development. For example, gold nanoparticles preferentially bioaccumulate near cancerous lung cells compared to normal cells [13,14]. Second, nanoparticle composites may become drug-delivery vehicles for efficient treatment of malignant cancerous tumors. Most cancers initially respond to chemotherapy; however, even with potent chemotherapeutics such as paclitaxel, tumors develop resistance beyond the reported negative side effects [15,16]. Synthesized nanoparticle-based anti-cancer composites, where the chemotherapeutic is encapsulated in either a transition metal nanoshell or within a liposome tethered to metal nanospheres, have brought new light to this area of medicine (reviewed in Jin, et. al. amongst others [1,7,17,18]). While this method proposes to be relatively non-invasive, cost-effective, and more accurate for cancer treatment, concern exists regarding the safety of components of these new composites, especially upon degradation within the body.

Previous studies in both murine and porcine cells, as well as breast cancer cells, indicate that although silver nanoparticles may be toxic, gold nanoparticles are much safer [19-21]. For example, gold and silver nanoparticles show no negative effect on porcine oocytes and murine blastomere development [20,21]. Additionally, real-time PCR analysis of six genes involved in apoptosis or development did not show an influence in gene expression in the presence of nanoparticles [20]. However, a problem arises with these studies: to treat a human with these methods requires a larger volume of composites. Given that only an estimated 0.7% of dosed nanoparticles reach their target, we must perform more research to assess toxicity and the ability of a nanoparticle to damage DNA structure [22].

As we work to synthesize a composite for treatment of cancers, we must examine in detail the toxicity of individual components before taking our composite to a mammalian model. In order to assess changes in metabolism with these nanoparticles, we performed MTT assays in two well-studied breast cancer cell lines (MDA-MB-231 and MCF-7). We exposed cells to varying amounts of either relatively fresh nanoparticles, or nanoparticles that had been synthesized several months earlier. We assessed a five-day treatment instead of the traditional forty-eight hours to ensure that changes in metabolic activity were not missed due to short treatment times.

2. MATERIALS AND METHODS

2.1 Synthesis of HGN/Liposome Components

Synthesis of hollow gold nanoparticles used a modified version of the galvanic replacement

reaction method first described by Prevo et al. [23], completing the reaction at ambient room temperature (~25°C) instead of 60°C. Nanoparticles with 10 nm diameters were prepared by combining a 100 mM nanoparticle solution of $AgNO_3$ with 180 µL of 25 mM $HAuCl_4$. The $HAuCl_4$ was added incrementally over an 8 min period (10 µL added every 45 seconds) while stirring rapidly. The reaction mixture stirred for an additional two hours after addition of the final $HAuCl_4$ aliquot prior to overnight refrigeration. Hollow gold nanoparticles with 32 nm diameters were produced by growing the silver nanoparticles in 100 mM $AgNO_3$ followed by addition of 800 µL 25 mM $HAuCl_4$. The sizes of the HGN samples were confirmed by UV-visible spectrophotometry.

2.2 Cell Culture and Maintenance

We obtained MCF-7 and MDA-MB-231 human breast cancer cells from ATCC (HTB-22 and HTB-26, Manassas, VA). We maintained cells in a humidified incubator at 37°C with 5% CO_2. Growth media for MCF-7 cells was MEM-EBSS (HyClone, Logan, UT, USA) supplemented with 5% calf serum, non-essential amino acids, HEPES, NEAA, and antibiotics (penicillin/streptomycin and gentamycin). Growth media for MDA-MB-231 cells was DMEM/F12 media (Gibco/Life Technologies, Grand Island, NY, USA) supplemented with 10% newborn calf serum, HEPES, and antibiotics (penicillin/streptomycin and gentamycin). Cells were subcultured upon 80% confluence.

2.3 MTT Assay

We assessed changes in metabolic activity using a standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. We subcultured cells into a 6-well plate at 60% confluence; after 24 hours, we treated cells with fresh media containing 5-10% of HPN1 or HGN2 nanoparticles or PBS control. After 120 hours, we added 200 ul of 5 mg/ml MTT to each well, incubated for two hours at 37°C, and resuspended precipitated formazan crystals in 1 ml DMSO. Absorbance readings of the dissolved formazan crystals were taken at 570 nm in a Thermo Scientific Genesys 10S UV-Vis spectrophotometer. The assay was performed in triplicate for initial studies (during separate weeks), and again in triplicate after six months. Univariate analysis of variance (ANOVA) was performed using SPSS (IBM SPSS Statistics 21, IBM Corp., Aramonk, NY, USA), with P=0.05 indicating significant variation from the controls.

3. RESULTS AND DISCUSSION

To identify whether nanoparticles had any effect upon eukaryotic cells, we assessed changes in cellular metabolic activity using two human breast cancer cell lines, MDA-MB-231 and MCF-7. We felt it important to utilize more than one cell line, as while both cells are epithelial-derived adenocarcinomas, they have a very different cell milieu. MCF-7 cells are a non-metastatic cell line, while MDA-MB-231 cells will metastasize [24-26]. Additionally, MCF-7 cells express both the estrogen and progesterone receptors and are hormone-dependent, while MDA-MB-231 cells are hormone-resistant. Toxicity experiments continued for five days, since although toxicity may easily be determined in a 24-48 hour period, any increased viability would require adequate time for cell replication. Experimentation continued over three weeks, with results compiled and presented in Fig. 1. As expected, we observed no statistically significant change in viability when MDA-MB-231 cells were exposed to the nanoparticles (Fig. 1, P>0.23 for all treatments). This correlates with published work indicating that gold nanoparticles are not cytotoxic [27,28]. However, we did observe several changes in MTT conversion in the MCF-7 cell line when compared to the control. At the 10% concentration of both nanoparticles, the relative absorbance readings almost doubled (Fig. 1, P=0.002), indicating an increase in growth and metabolism in MCF-7 cells. This is in strong contrast to the 5% HPN1 dosage, which resulted in a 30% reduction (P=0.037).

Our results contrast with published work in several mammalian systems, where gold and silver nanoparticle exposure reduced viability of cancer cells [19,29]. One possible explanation for this difference is that our studies were longer than previous examinations of nanoparticle toxicity, and thus draw concern, although we recognize that our treatment amounts were much larger than would be given medically. Another potential difference is due to the sizes of the nanoparticles utilized. Research by Sonavane et al indicates that smaller nanoparticles may bioaccumulate and cross the blood/brain barrier [30]. Our nanoparticles are within the range that did accumulate in Sonavane's study (less than 50 nm diameter). Lastly, there have been reports of silver nanoparticles having both proliferative and apoptotic effects that we dependent upon dosage [31]. In human embryonic neural precursor cells, treatment with 20 nm silver nanoparticles increased cell proliferation by

almost thirty percent, while simultaneously also increasing TUNEL staining.

During initial review of our data, and due to the strong differences observed between the 5% and 10% HPN1 treatment within the MCF-7 cells, we had some questions regarding the stability of the nanoparticles. Thus, we allowed the nanoparticles to sit at 4C for six months in PBS prior to repeating the assays. As observed previously, we saw no statistically significant changes in MTT conversion when MDA-MB-231 cells were exposed to either nanoparticle for five days (Fig. 2, P>0.37 for all treatments); thus, we feel confident that the nanoparticles did not alter cell growth and metabolism in that cell line.

Although we observed little change with MDA-MB-231 cells, our results in the MCF-7 cells were more dramatic than in our initial trials. Even at only 5% treatment, the HPN1 (larger) nanoparticles caused over 75 percent death (Fig. 2, P= 0.009), compared to the 30% death initially observed (Fig. 1). In stark contrast to the growth seen in our initial trials, at the 10% treatment, an average of 90% cell death occurred, with 100% death in one trial (Fig. 2, P=0.005). However, the HGN2 nanoparticles acted similarly to our earlier results. HGN2 nanoparticle treatment caused a statistically significant increase in viability compared to wells treated with the PBS control. An average 30% increase in MTT activity was observed with the 5% HGN2 treatment and 60% increase in the 10% HGN2 treatment (Fig. 2, P=0.04 for both treatments).

Such dramatic differences in proliferation were unexpected, and drew much concern about the stability of the nanoparticles we had utilized; additionally we note the importance of monitoring safety in multiple cell lines. We know that gold nanoparticles are naturally drawn to highly proliferating cells such as those found in cancerous tumors, and some nanoparticles are able to utilize receptor-mediated endocytosis to enter into the cells [32,33]. This may in part explain the differences in responsiveness between our two tested cell lines. While MCF-7 cells responded with either death or enhanced proliferation, the nanoparticle treatment had almost no effect on MDA-MB-231 cells. However, this is not the first time we have observed differences in responsiveness between these two cell lines. In fact, in previous studies within our lab, we have found that MDA-MB-231 cells are much more resistant to treatment with toxic substances than MCF-7 cells (discussed in [34,35] and unpublished work). We are now examining the stability of several different nanoparticles developed by our colleagues to identify preparations that are more stable in solutions such as PBS as well as within cell medias that are more complex. This is a critical step within preparation of any potential pharmaceutical, as a limited shelf life would prohibit the usefulness of this a medication. The WHO recommendation for stable shelf life of any pharmaceuticals is twenty-four months at room temperature, or with extensive real-time studies to support variations from this recommendation [36].

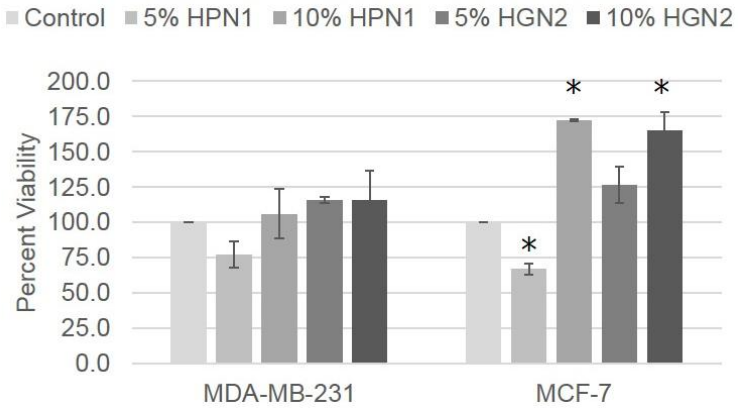

Fig. 1. Changes in metabolic activity in breast cancer cells after nanoparticle exposure

We applied HPN1 or HGN2 gold nanoparticles (5 or 10% v/v) to MDA-MB-231 or MCF-7 human breast cancer cells for 120 hours. PBS-treated controls were also included. Following treatment, an MTT assay was performed. Viable cells with active metabolism converted the MTT to insoluble formazan crystals, which we resuspended in DMSO and quantified by absorbance spectroscopy at 570 nm. We standardized the results by normalizing control cells to 100%. Results represent three independent experiments and indicate cell viability ± standard error of the mean. Statistically significant variation from the PBS-treated controls is indicated (, P<0.05)*

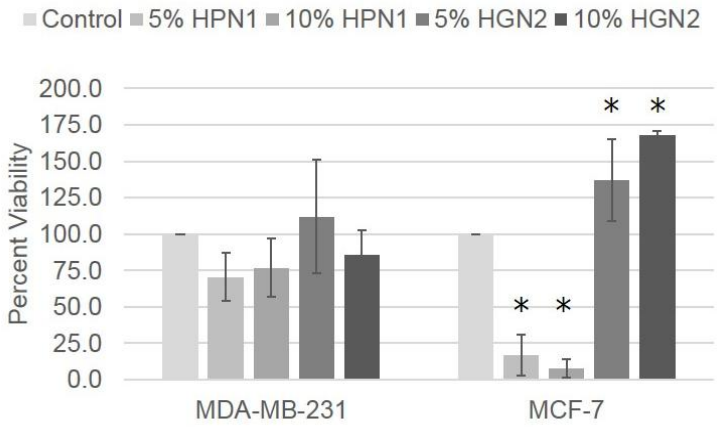

Fig. 2. Changes in metabolic activity in breast cancer cells after aged nanoparticle exposure

HPN1 or HGN2 nanoparticles were stored at 4C for six months. We applied these aged HPN1 or HGN2 gold nanoparticles (5 or 10% v/v) to MDA-MB-231 or MCF-7 human breast cancer cells for 120 hours. PBS-treated controls were also included. Following treatment, an MTT assay was performed. Viable cells with active metabolism converted the MTT to insoluble formazan crystals, which we resuspended in DMSO and quantified by absorbance spectroscopy at 570 nm. We standardized the results by normalizing control cells to 100%. Results represent three independent experiments and indicate cell viability ± standard error of the mean. Statistically significant variation from the PBS-treated controls is indicated (, P<0.05)*

As we proceed with further testing and development of novel drug delivery models, we must continue to ensure the safety of the patient while maximizing the efficacy of the delivery mechanism. One current focus of our research is expanding the time of our toxicity studies. Conflicting reports of gold nanoparticle toxicity have complicated our understanding of these compounds (reviewed in [37]). One concern we have as we move forward in the synthesis of new drug delivery models is that many of the tests currently done are acute [38,39]. Additionally, these studies are often performed in the presence of either bacteria [40] or monolayer cultures [41-43], where the nanoparticles may come in direct contact with individual cells; however, this does not necessarily mimic a tumor environment and thus may not be indicative of the true toxicity of these compounds. Further studies in our laboratory will examine the mechanisms through which any changes in viability occur, such as through the assessment of repair pathways in mammalian cells, i.e. both base excision repair and nucleotide excision repair pathways, which have a variety of enzymes that could potentially be activated [44,45].

4. CONCLUSION

We observed differences in the toxicity of two nanoparticle preparations in a cell- and time-dependent manner. This indicates that changes in the nanoparticles may have occurred during the lag period between experimentation, and bodes caution towards selecting gold nanoparticles in the development of drug delivery mechanisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

We are indebted to Millikin University colleagues Peter Piers, Dr. Paris Barnes and Dr. Anne Rammelsberg (Department of Chemistry) for synthesis of nanoparticles, as well as Dr. Travis Wilcoxen (Department of Biology) for support of statistical analysis. Funded by the Leighty Tabor Science Scholars Program (to OGW), the Howard L. Gravett Endowed Chair (to JRS), and the Millikin University Department of Biology.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alexiou C, Schmid RJ, Jurgons R, et al. Targeting cancer cells: Magnetic nanoparticles as drug carriers. Euro Biophys J. 2006;35(5):446-50.
2. Chen Y, Xianyu Y, Wang Y, et al. One-step detection of pathogens and viruses: Combining magnetic relaxation switching and magnetic separation. ACS Nano. 2015;9(3):3184-91.
3. Han W, Wang S, Liang R, et al. Non-ionic surfactant vesicles simultaneously enhance antitumor activity and reduce the toxicity of cantharidin. Int J Nanomedicine. 2013;8:2187-96.
4. Joshi P, Chakraborti S, Ramirez-Vick JE, et al. The anticancer activity of chloroquine-gold nanoparticles against MCF-7 breast cancer cells. Colloids Surf B Biointerfaces. 2012;95:195-200.
5. Li YH, Shi QS, Du J, et al. Targeted delivery of biodegradable nanoparticles with ultrasound-targeted microbubble destruction-mediated hVEGF-siRNA transfection in human PC-3 cells *in vitro*. Int J Mol Med. 2013;31(1):163-71.
6. Shagholani H, Ghoreishi SM, Mousazadeh M. Improvement of interaction between PVA and chitosan via magnetite nanoparticles for drug delivery application. Int J Biol Macromol. 2015;2015:128-32.
7. Wei T, Chen C, Liu J, et al. Anticancer drug nanomicelles formed by self-assembling amphiphilic dendrimer to combat cancer drug resistance. Proc Natl Acad Sci USA. 2015;112(10):2978-83.
8. Cella D, Wang M, Wagner L, Miller K. Survival-adjusted health-related quality of life (HRQL) among patients with metastatic breast cancer receiving paclitaxel plus bevacizumab versus paclitaxel alone: Results from Eastern Cooperative Oncology Group Study 2100 (E2100). Breast Cancer Res Treat. 2011;130(3): 855-61.
9. Evans WE, McLeod HL. Pharmacogenomics--drug disposition, drug targets, and side effects. New England Journal of Medicine. 2003;348(6):538-49.
10. Iwamoto T. Clinical application of drug delivery systems in cancer chemotherapy: Review of the efficacy and side effects of approved drugs. Biol Pharm Bull. 2013;36(5):715-8.
11. Shapiro CL, Recht A. Side effects of adjuvant treatment of breast cancer. New England Journal of Medicine. 2001;344(26):1997-2008.
12. Wu CH, Yang CH, Lee JN, Hsu SC, Tsai EM. Weekly and monthly regimens of paclitaxel and carboplatin in the management of advanced ovarian cancer. A preliminary report on side effects. International Journal of Gynecological Cancer. 2001;11(4):295-9.
13. Peng G, Tisch U, Adams O, et al. Diagnosing lung cancer in exhaled breath using gold nanoparticles. Nat Nanotechnol. 2009;4(10):669-73.
14. Shipp G. Ultrasensitive measurement of protein and nucleic acid biomarkers for earlier disease detection and more effective therapies. Biotechnol Healthc. 2006;3(2):35-40.
15. Andre F, Hatzis C, Anderson K, et al. Microtubule-associated protein-tau is a bifunctional predictor of endocrine sensitivity and chemotherapy resistance in estrogen receptor-positive breast cancer. Clinical Cancer Research. 2007;13(7): 2061-7.
16. Yu D, Liu B, Jing T, et al. Overexpression of both p185c-erbB2 and p170mdr-1 renders breast cancer cells highly resistant to taxol. Oncogene. 1998;16(16):2087-94.
17. Jin SE, Jin HE, Hong SS. Targeted delivery system of nanobiomaterials in anticancer therapy: From cells to clinics. Biomed Res Int. 2014;2014:814208.
18. Yu MK, Jeong YY, Park J, et al. Drug-loaded superparamagnetic iron oxide nanoparticles for combined cancer imaging and therapy *in vivo*. Angew Chem Int Ed Engl. 2008;47(29):5362-5.
19. Gurunathan S, Han JW, Eppakayala V, Jeyaraj M, Kim JH. Cytotoxicity of biologically synthesized silver nano-particles in MDA-MB-231 human breast cancer cells. Biomed Res Int. 2013;2013:535796.
20. Taylor U, Garrels W, Barchanski A, et al. Injection of ligand-free gold and silver nanoparticles into murine embryos does not impact pre-implantation development. Beilstein J Nanotechnol. 2014;5:677-88.
21. Tiedemann D, Taylor U, Rehbock C, et al. Reprotoxicity of gold, silver, and gold-silver

alloy nanoparticles on mammalian gametes. Analyst. 2014;139(5):931-42.

22. Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of nanoparticle delivery to tumours. Nature Reviews Materials. 2016;1:16014.
23. Prevo BG, Esakoff SA, Mikhailovsky A, Zasadzinski JA. Scalable routes to gold nanoshells with tunable sizes and response to near-infrared pulsed-laser irradiation. Small. 2008;4(8):1183-95.
24. Berthois Y, Katzenellenbogen J, Katzenellenbogen B. Phenol red in tissue culture media is a weak estrogen: Implications concerning the study of estrogen-responsive cells in culture. Proceedings of the National Academy of Sciences of the United States of America. 1986;83:2496-500.
25. Gupta PB, Kuperwasser C. Contributions of estrogen to ER-negative breast tumor growth. J Steroid Biochem Mol Biol. 2006;102(1-5):71-8.
26. Harrell JC, Dye WW, Allred DC, et al. Estrogen receptor positive breast cancer metastasis: Altered hormonal sensitivity and tumor aggressiveness in lymphatic vessels and lymph nodes. Cancer Res. 2006;66(18):9308-15.
27. Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small. 2005;1(3):325-7.
28. Shukla R, Bansal V, Chaudhary M, Basu A, Bhonde RR, Sastry M. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. Langmuir. 2005; 21(23):10644-54.
29. Selim ME, Hendi AA. Gold nanoparticles induce apoptosis in MCF-7 human breast cancer cells. Asian Pac J Cancer Prev. 2012;13(4):1617-20.
30. Sonavane G, Tomoda K, Makino K. Biodistribution of colloidal gold nanoparticles after intravenous administration: Effect of particle size. Colloids Surf B Biointerfaces. 2008;66(2): 274-80.
31. Soderstjerna E, Johansson F, Klefbohm B, Englund Johansson U. Gold- and silver nanoparticles affect the growth characteristics of human embryonic neural precursor cells. PLoS One. 2013;8(3): e58211.
32. Chithrani DB. Intracellular uptake, transport, and processing of gold nanostructures. Mol Membr Biol. 2010;7:299-311.
33. Chithrani DB, Dunne M, Stewart J, Allen C, Jaffray DA. Cellular uptake and transport of gold nanoparticles incorporated in a liposomal carrier. Nanomedicine. 2010; 6(1):161-9.
34. Florian CP, Mansfield SR, Schroeder JR. Differences in GPR30 regulation by chlorotriazine herbicides in human breast cells. Biochem Res International. 2016;2016:1-7.
35. Kern KM, Schroeder JR. Comparison of cantharidin toxicity in breast cancer cells to two common chemotherapeutics. Int J Breast Cancer. 2014;2014:423059.
36. WHO. Quality assurance of pharmaceuticals: A compendium of guidelines and related materials (v. 1). Geneva: World Health Organisation. 1997;1:248.
37. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. Science. 2006;311(5761):622-7.
38. Kroll A, Pillukat MH, Hahn D, Schnekenburger J. Interference of engineered nanoparticles with *in vitro* toxicity assays. Arch Toxicol. 2012;86(7): 1123-36.
39. Shim KH, Hulme J, Maeng EH, Kim MK, An SS. Analysis of SiO2 nanoparticles binding proteins in rat blood and brain homogenate. Int J Nanomedicine. 2014;9(Suppl 2):207-15.
40. Bhattarai SR, Bahadur R, Aryal S, et al. Hydrophobically modified chitosan/ gold nanoparticles for DNA delivery. J Nanoparticle Res. 2008;10(1):151-62.
41. Song L, Connolly M, Fernandez-Cruz ML, et al. Species-specific toxicity of copper nanoparticles among mammalian and piscine cell lines. Nanotoxicology. 2014;8(4):383-93.
42. Unak G, Ozkaya F, Medine EI, et al. Gold nanoparticle probes: Design and *in vitro* applications in cancer cell culture. Colloids Surf B Biointerfaces. 2012;90: 217-26.
43. Watari F, Takashi N, Yokoyama A, et al. Material nanosizing effect on living organisms: Non-specific, biointeractive,

physical size effects. J R Soc Interface. 2009;6(Suppl 3):S371-88.
44. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int. 2013;2013:942916.
45. Papageorgiou A, Rapley J, Mesirov JP, Tamayo P, Avruch J. A genome-wide siRNA screen in mammalian cells for regulators of S6 phosphorylation. PLoS One. 2015;10(3):e0116096.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21354