The Pharma Innovation Journal 2023; 12(3): 4471-4474

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(3): 4471-4474

www.thepharmajournal.com
Received: 28-12-2022
Accepted: 31-01-2023

Prerna Sood
Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India

Manoj Kumar Singh
Scientist, ICAR-National Dairy Research Institute, Karnal, Haryana, India

Corresponding Author:
Prerna Sood
Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India

Effect of different concentrations of gold nanoparticles on the morphology of buffalo ovarian cells

Prerna Sood and Manoj Kumar Singh

Abstract
Nanoparticles (NPs) are now routinely used in a variety of fields, including consumer products pharmaceuticals and medicine. Due to the ease of synthesis and flexibility of surface functionalization, gold nanoparticles (AuNPs) have been extensively used in the practice and in advancement of chemistry, biology and medicine. AuNPs can be used to impact cellular physiological processes like proliferation and differentiation, according to recent studies. In this study, buffalo ovarian cells were co-cultured with two sizes of AuNPs (10 and 100 nm) at three different concentrations. In the experiment group, ovarian cells co-cultured with 100 nm gold nanoparticles had more impact on cytotoxicity of cells as compared with cells treated with 10 nm gold nanoparticles. Gold nanoparticles were found to be harmful to ovarian cells at concentrations 100 nm or more than 100 nm.

Keywords: Nanoparticles, concentrations, ovarian cells, proliferation, cellular toxicity

Introduction
Gold nanoparticles (AuNPs) are a particular type of nanomaterial that has demonstrated advanced biocompatibility, low cytotoxicity and strong oxidation resistance. They can be further engineered and altered to an ideal extent thanks to their controllable size and shape capabilities and the possibility of combining them with many different kinds of biological macromolecules (Hutter *et al*., 2011) [5]. Exhibiting stability and ease of decoration, gold nanoparticles (AuNPs) could be used in diagnosis, testing the cytotoxicity and disease treatment. They used it for therapeutic purposes (Mahdihassan, 1985) [9]. Additionally, surface alterations such as covering the core with a layer of silica can be used to optimize AuNPs (Jain, 2005) [6]. Nanoparticles need to cross the cell membrane before they can have toxicological effects. Due to potential toxicological and physiological effects, it is crucial to investigate how nanoparticles enter cells; nevertheless, there haven't been many studies on this subject to far. AuNPs have been used extensively in bioengineering and biomedicine, including drug delivery strategies, diagnostic imaging (Cheng *et al*, 2011) [3], antibody labelling, and targeting (Arvizo *et al*., 2010) [2]. By overcoming biological obstacles, AuNPs eventually influence physiological processes like cell division and proliferation by entering the cell nuclei (Posch *et al*., 2015) [10]. However, there were conflicting findings in relation to AuNPs' toxicity (Sperling *et al*., 2008) [12]. It was established that the cytotoxicity of AuNPs is size, concentration and time-dependent based on research findings showing specific sizes and concentrations of AuNPs have a stimulating influence on cell growth (Lu *et al*., 2010) [7].
Testing the toxicity of these materials is urgently needed due to the rising use of nanoparticles in research, medicine and the production of consumer goods. Although the impact of AuNPs on cell proliferation and differentiation has been investigated, there is no information available on the impact of AuNPs on buffalo ovarian cells. In the current work, we examined the impact of three different concentrations of two distinct sizes of AuNPs on the proliferation of buffalo ovarian cells

Materials and Methods
The ovaries served as the source of immature oocytes, follicular fluid. The buffalo ovaries were obtained from a nearby abattoir at Delhi. The culture media used in the present study, which included tissue culture medium-199 (TCM-199), Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (DPBs) and the additives which included bovine serum albumin (BSA) and antibiotics (gentamicin, penicillin and streptomycin) were purchased from Sigma-Aldrich Corp., St. Louis, MO, USA. All the cell culture media were in the form of ready-to-use liquid media.

Cell passaging was done so that we got 80-90 % confluency of cells. Cell were examined using an inverted microscope (NIKON, Japan, Model TMD) to track the development, morphology and health of the cultivated cells. Of the confluent flask 25000 cells/ well in 6 wells were taken after counting the cells by cell counter. Then these 6 wells were treated with two diameters of AuNPs i.e 10 and 100 nm after 2 days of seeding. Three concentrations of 10 and 100 nM were taken (0.5, 2.5 and 10 ug/ml) and one set of control was taken in which no treatment was given. Then RNA and DNA of treated and control cells were removed. A thermal cycler (My Cycler, BIO-RAD, Hercules, CA, USA) was used for synthesizing cDNA from mRNA of buffalo embryos of different development stage through reverse transcription in the amplification presence of reverse transcriptase enzyme for genes of interest with gene specific primers and heat stable taq polymerase. It gave 2n number of DNA strands, where n= number of cycles. Ovarian cultured cells were grown for four days after being treated with and without AuNPs. RNA was extracted using the Trizol Reagent, and RNA concentration was determined using spectrophotometry (Eppendorf Bio Photometer, Germany). The RNA Reverse Transcription (RT) kit was used in accordance with the manufacturer's instructions to create first strand cDNA. During the 40 cycles of the amplification process, the following temperatures were used: denaturation at 94°C for two minutes, annealing at 58°C for 30 seconds and extension at 72°C for 45 seconds.

Results and Discussion

Due to the similarities between AuNPs and the nanostructured nature of the microenvironment, they have drawn significant attention in tissue engineering investigations. Similar to other nanomaterials, AuNPs can enter cell nuclei, cross biological barriers, and influence cellular processes like differentiation and proliferation (Alkilany *et al*., 2013) [1]. Gold nanoparticles are distinguished by their special volume, surface, macroquantum tunnelling effect, optical property and other nanoparticle characteristics. It has been employed extensively in the biomedical field in addition to chemistry and physics (Lu *et al*., 2010) [7].

Table 1 showed the effect of 10 nM size of gold nanoparticles at three different concentrations (0.5, 2.5 and 10 ug/ml) on the live cells and % age of cultured cells of buffalo ovarian cells. Table 2 showed the effect of 100 nM size of gold nanoparticles at three different concentrations (0.5, 2.5 and 10 ug/ml) on the live cells and % age of cultured cells of buffalo ovarian cells. The 100 nm size of AuNPs have more impact on live and % age of cultured buffalo ovarian cells. According to studies, the concentration and size of the particles have a significant impact on how AuNPs behave in cells. According to Lu *et al*. 2010 [7], a low concentration of 34 nm gold nanoparticles can speed up keratinocyte growth. Gold nanoparticle concentrations greater than 10 ppm considerably boosted the cytotoxic effect. Another study demonstrated that the immobilisation of 24 nm gold colloid onto pig hepatocytes produced a hepatocytes/gold colloid interface that could effectively and quickly promote hepatocyte proliferation (Gu *et al*., 2004) [4]. Regardless of the type of cell studied, Lu *et al*. discovered that gold nanoparticles as small as 1-2 nm were extremely toxic, but smaller gold compounds and bigger 15 nm gold colloids were both relatively non-toxic (Lu *et al*., 2010) [7].

After entering in the circulatory system via various items (food/oral ingestion, inhalation, skin penetration), they can exert their negative toxic effects on cells by increasing the production of reactive oxygen species (ROS), damaging DNA and mitochondria and even inducing cell death (Verma *et al*., 2010) [13]. However, AuNPs were internalized by cultured buffalo granulosa cells and operated as ovarian endocrine disruptors by perturbing steroidogenesis. In particular, progesterone production was significantly increased, probably as a consequence of increased mitochondria permeability due to particle movement across the cells and other membranes. Such a disturbance of the mitochondrial membranes and homeostasis generates ROS that, in turn, causes oxidative stress and even cell death by stimulating the expression of pro-apoptotic genes (Stelzer *et al*., 2009 and Lyngdoh *et al*., 2020) [11, 8]. Fig 1 showed the control cells without treatment of AuNPs. Cells are spindle or triangular in shape and typically well-spread. Fig 2 showed the cells treated with 10 nM AuNPs at 0.5, 2.5 and 10 ug/ml concentrations for 24 hours. Fig 3 showed the cells treated with 100 nM AuNPs at 0.5, 2.5 and 10 ug/ml concentrations for 24 hours. Zhang *et al*. published comparable findings from their own tests. They discovered that in vitro, AuNPs could considerably accelerate osteoblast proliferation, improve ALP activities, and increase the quantity of bone nodules and calcium content.

From the present study it can be concluded that 100nm-diameter AuNPs at a concentration of 10ug/ml could stimulate the proliferation and cytotoxicity of ovarian cells while having less discernible impact at 10 nm diameter size of AuNPs.

Table 1: Effect of 10 nM size having three different concentrations of AuNPs on the live cells and % age of cultured cells of buffalo ovarian cells

10 nM	Total count	Live cells	Live% age
0.5 ug/ml	7.77×10^5	5.70×10^5	73
	4.94×10^5	4.69×10^5	95
	4.69×10^5	4.44×10^5	95
2.5 ug/ml	4.19×10^5	3.83×10^5	92
	4.34×10^5	3.68×10^5	85
	6.00×10^5	5.35×10^5	89
10 ug/ml	7.36×10^5	7.16×10^5	97
	6.37×10^5	8.02×10^5	96
	9.38×10^5	8.52×10^5	91

Table 2: Effect of 100 nM size having three different concentration of AuNPs on the live cells and % age of cultured cells of buffalo ovarian cells

100 nM	Total count	Live cells	Live% age
0.5 ug/ml	5.09×10^5	4.54×10^5	89
	5.04×10^5	4.44×10^5	88
	5.09×10^5	4.10×10^5	83
2.5 ug/ml	8.27×10^5	7.11×10^5	86
	8.47×10^5	6.56×10^5	77
	8.32×10^5	7.16×10^5	76
10 ug/ml	1.07×10^5	8.78×10^5	82
	1.15×10^5	8.62×10^5	75
	1.04×10^5	8.37×10^5	70

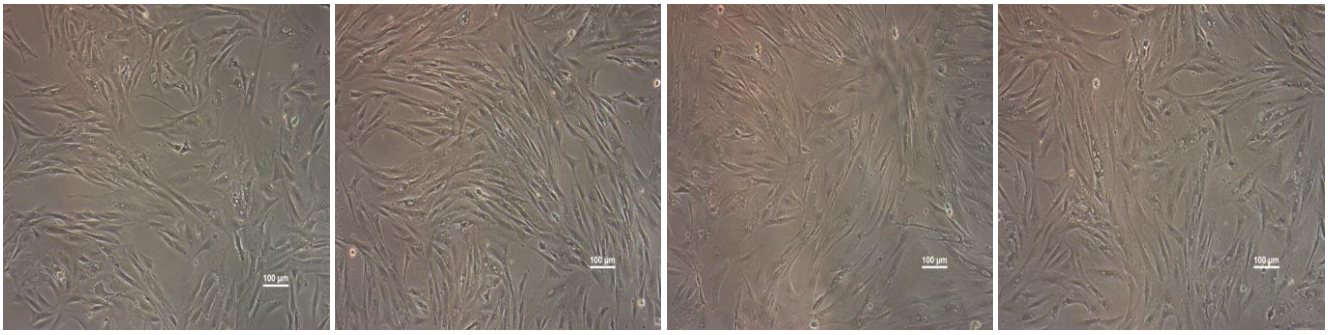

Fig 1: Control cells without treatment of AuNPs. Cells are spindle or triangular in shape and typically well-spread

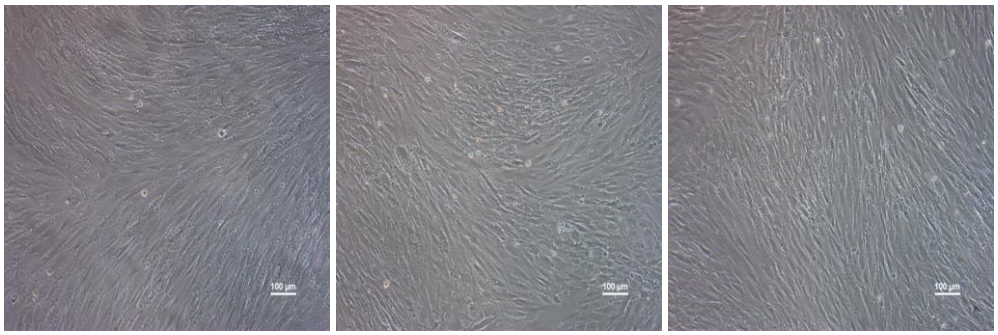

Fig 2: Cell morphology: Cells treated with 10 nM AuNPs at 0.5, 2.5 and 10 ug/ml concentrations for 24 hours

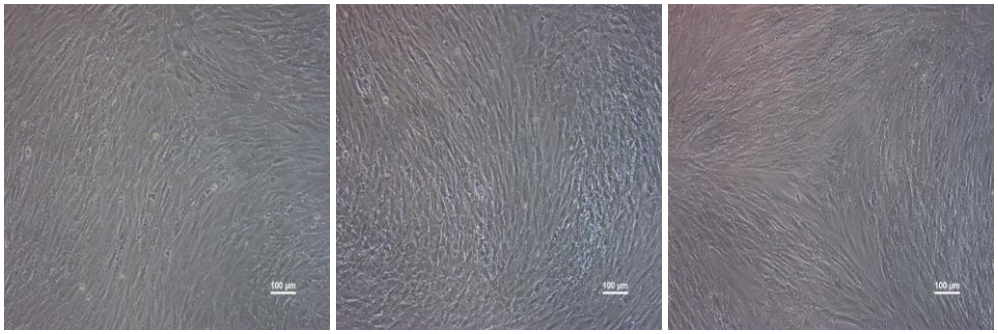

Fig 3: Cell morphology: Cells treated with 100 nM AuNPs at 0.5, 2.5 and 10 ug/ml concentrations for 24 hours, no spindle shape and and tightly spread

Acknowledgements
Authors are thankful to the Head Department of Animal Biotechnology, NDRI Karnal and Head Department of Zoology for providing facilities for this study.

Financial Support
Authors are thankful to the Head Department of Animal Biotechnology, NDRI Karnal for providing financial assistance for the above work.

Author Contribution Statement.
PS designed research. PS conducted experiments. PS wrote and approved the manuscript.

Conflicts of Interest
There is no conflict of interest.

References
1. Alkilany AM, Lohse SE, Murphy CJ. The gold standard: Gold nanoparticle libraries to understand the nano-bio interface, Accounts of Chemical Research. 2013;46(3):650-661.
2. Arvizo R, Bhattacharya R, Mukherjee P. Gold nanoparticles: opportunities and challenges in nanomedicine, Expert Opinion on Drug Delivery. 2010;7(6):753-763.
3. Cheng Y, Meyers JD, Broome AM, Kenney ME, Basil JP, Burda C. Deep penetration of a PDT drug into tumors by noncovalent drug-gold nanoparticle conjugates. Journal of the American Chemical Society. 2011;133(8):2583-2591.
4. Gu HY, Chen Z, Sa RX. The immobilization of hepatocytes on 24 nm-sized gold colloid for enhanced hepatocytes proliferation Biomaterials. 2004;25(17):3445-3451.
5. Hutter E, Maysinger D. Gold nanoparticles and quantum dots for bioimaging Microscopy Research and Technique 2011;74(7):592-604.
6. Jain KK. Nanotechnology-based drug delivery for cancer. Technology Cancer Research Treaty. 2005;4:407-416.
7. Lu S, Xia D, Huang G, Jing H, Wang Y, Gu H. Concentration effect of gold nanoparticles on proliferation of keratinocytes, Colloids and Surfaces B: Biointerfaces. 2010;81(2):406-411.
8. Lyngdoh EL, Nayan V, Vashisht M, Kumari S, Bhardwaj A, Bhatia T, *et al*. Gold nanoparticles modulate the

steroidogenic and apoptotic pathway in a buffalo granulosa cell model. Biotechnology Letters. 2020;42:1383-1395.
9. Mahdihassan S. Cinnabar-gold as the best alchemical drug of longevity, called Makaradhwaja in India. American Journal of Chinese Medicine. 1985;13:93-108.
10. Posch C, Latorre A, Crosby MB. Detection of GNAQ mutations and reduction of cell viability in uveal melanoma cells with functionalized gold nanoparticles, Biomedical Microdevices. 2015;17(1):144-48.
11. Stelzer R, Hutz RJ. Gold Nanoparticles Enter Rat Ovarian Granulosa Cells and Subcellular Organelles, and Alter In-Vitro Estrogen Accumulation. Journal of Reproduction Development. 2009;55:685-690.
12. Sperling RA, Gil PR, Zhang F, Zanella M, Parak WJ. Biological applications of gold nanoparticles, Chemical Society Reviews. 2008;37(9):1896-1908.
13. Verma A, Stellacci F. Effect of surface properties on nanoparticle-cell interactions. Small. 2010;6:12-21.
14. Zhang D, Liu D, Zhang J, Fong C, Yang M. Gold nanoparticles stimulate differentiation and mineralization of primary osteoblasts through the ERK/MAPK signaling pathway, Materials Science and Engineering. 2010;42:70-77.