Appl Nanosci (2015) 5:443–448
DOI 10.1007/s13204-014-0336-z

ORIGINAL ARTICLE

Anticancer studies of the synthesized gold nanoparticles against MCF 7 breast cancer cell lines

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Received: 25 June 2014 / Accepted: 2 July 2014 / Published online: 12 September 2014

Abstract It has been previously stated that gold nanoparticles have been successfully synthesized using various green extracts of plants. The synthesized gold nanoparticles were characterized under scanning electron microscopy and EDX to identify the size of the nanoparticles. It was found that the nanoparticles were around 30 nm in size, which is a commendable nano dimension achieved through a plant mediated synthesis. The nanoparticles were further studied for their various applications. In the current study, we have made attempts to exploit the anticancer ability of the gold nano particles. The nanoparticles were studied against MCF 7 breast cancer cell lines. The results obtained from the studies of anticancer activity showed that gold nanoparticles gave an equivalent good results, in par with the standard drugs against cancer. The AuNP's proved to be efficient even from the minimum concentrations of 2 μg/ml, and as the concentration increased the anticancer efficacy as well increased.

Keywords Gold nanoparticles · i.MCF 7 cell lines · Breast cancer

Introduction

Gold nanoparticles have advantages over other metal nanoparticles due to their biocompatibility and non-cytotoxicity. Nanoparticles are nanometres in size. Gold is used internally in human from last 50 years due to their chemical inertness. The size of gold nanoparticles can be controlled during their synthesis and particle functionalization with different groups. Gold nanoparticles accumulate in the tumour cells and show optical scattering. Therfore, these can act as the probe for the microscopic study of cancer cells. These are also used in chemotherapy and diagnosis of cancer cell (Cai et al. 2008a, b). Gold nanoparticles are capable of delivering large biomolecules, without restricting themselves as carriers of only small molecular drugs (Ghosh et al. 2008).

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Synthesis of gold nanoparticles

The gold nanoparticles were synthesized using eight different green extracts of plants namely *Camellia sinensis* (green tea), *Coriandrum sativum*, *Mentha arvensis, Phyllanthus amarus*, *Artabotrys hexapetalus*, *Mimusops elengi*, *Syzygium aromaticum*, *C. sinensis* (black tea) extract (Annamalai et al. 2011; Ahmad et al. 2003; Begum et al. 2009; Lal and Nayak 2012). The synthesized nanoparticles were further studied under scanning electron microscopy (SEM) to get an insight of the morphology, size and distribution of the nanoparticles. Further, the nanoparticles were analyzed using energy dispersion X ray studies to get the description of the elemental composition of the synthesized nanoparticle.

Anticancer study of gold nanoparticles

Materials and methods

Materials

1. MCF 7(human breast cancer) cell lines
2. DMEM medium
3. Tamoxifen

4. Letroz
5. Trypsin
6. Short 96 well assay plate
7. Elisa reader

Cell lines

1. The MCF 7 cell lines were procured from Kings institute, virology department, Guindy.
2. The cancerous cells were seeded in the flask along with DMEM medium and incubated in a 5 % CO_2 incubator at 37 °C.
3. After 24 h of incubation period, the attached cells were viewed under the inverted microscope for the morphology.
4. The cell lines were further maintained in the CO_2 incubator with periodic change of the DMEM medium.

Cell lines against the gold nanoparticles (van de Loosdrecht et al. 1994)

Gold nanoparticles reduced the ATP content of the cell, caused damage to mitochondria and increased production of reactive oxygen species (ROS) in a dose-dependent manner.

Procedure

Short 96 well assay. Each condition should be done in triplicate or more.

Day one Trypsinize 1 T-25 flask and add 5 ml of complete media to trypsinized cells. Centrifuge in a sterile 15 ml centrifuge tube at 5,000 rpm.

- Remove the media and resuspend the cells to 1.0 ml with complete media.
- Count and record the cells per ml.
- Remember to remove the cells aseptically while counting.
- Dilute the cells to 75,000 cells per ml. Use complete media to dilute the cells.
- Add 100 µl of cells (7,500 total cells) into each well and incubate overnight.

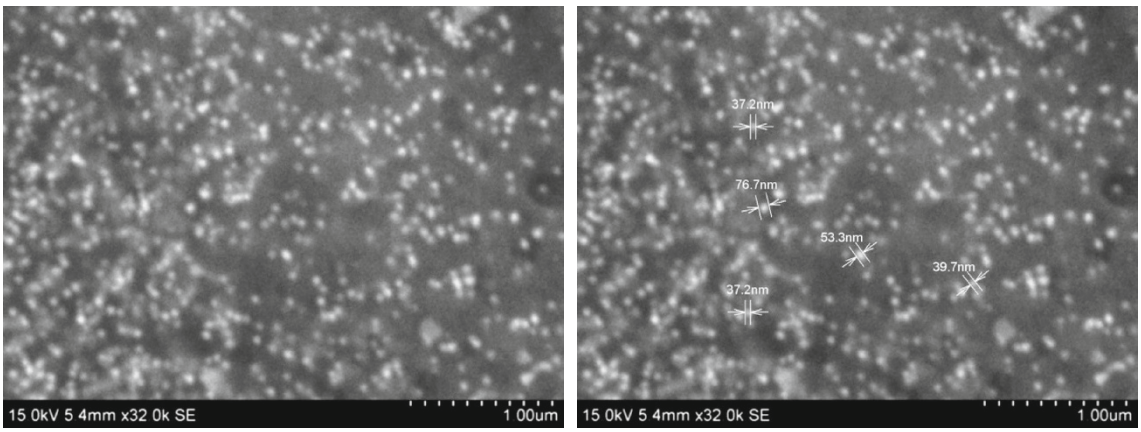
Day two Treat the cells on day two with agonist, inhibitor or drug.

- If removing media, do it carefully.
- Final volume should be 100 µl per well.

Day three Add 10 µl of 5 mg/ml of MTT to each well. Include one set of wells with MTT but no cells.

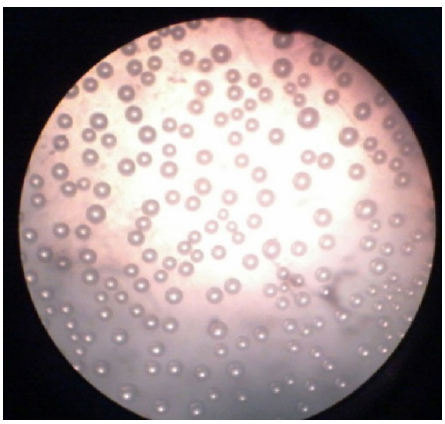
- To this different concentration of (2, 4, 6, 8, 10, 5, 10, 15, 20, 25, 30) gold nanoparticles were added to each well, in the same ways standard tamoxifen drug and Letroz drug were added in different concentrations to other set of each well. All should be done aseptically.
- Incubate for 24 h at 37 °C in the CO_2 incubator.
- Carefully remove the media. Do not disturb the cells and do not rinse with PBS.
- Add 150 µl of MTT solvent.
- Cover with tinfoil and agitate the cells on orbital shaker for 15 min.
- Read absorbance at 590 nm with a reference filter of 620 nm.
- Percentage of cell viability and IC_{50}were calculated.
- Formula for cell inhibition = (1 − O.D of treated cells/O.D of control) × 100
- Formula to check cell viability = (100 − cell inhibition)

Results and discussion

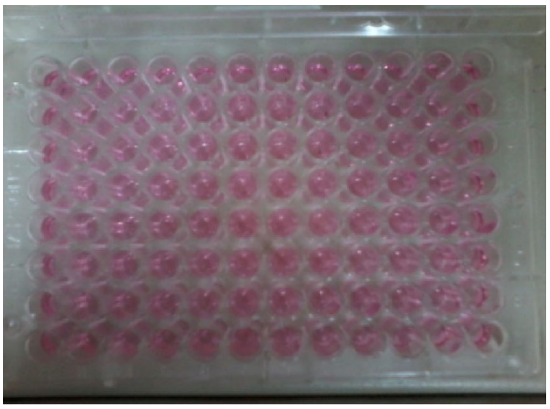


SEM image of the synthesized gold nanoparticles

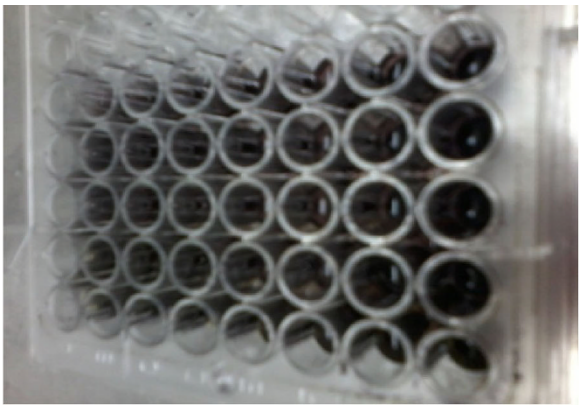
The results obtained from the studies of anticancer activity showed that gold nanoparticles gave an equivalent good results, on par with the standard drugs against cancer. The AuNPs proved to be efficient even from the minimum concentrations of 2 μg/ml, and as the concentration increased the anticancer efficacy as well increased. The results obtained from the studies of anticancer activity showed that gold nanoparticles gave an equivalent good results, in par with the standard drugs against cancer. The AuNPs proved to be efficient even from the minimum concentrations of 2 μg/ml, and as the concentration increased the anticancer efficacy as well increased.



The structure of MCF 7 breast cancer cell lines



At day 1 of cell lines study



At day 3 of cell lines study

Anticancer activity of gold nanoparticles

Concentration of AuNP's (μg/ml)	O.D value	Cell inhibition %ml	Cell viability %ml
2	0.036	81.9	18.1
4	0.049	69.1	30.9
6	0.029	68.6	36.4
8	0.031	67.3	32.7
10	0.134	67.3	52.7
5	0.198	80.0	20
10	0.058	71.8	78.2
15	0.036	71.9	28.1
20	0.180	73.7	26.3
25	0.034	55.5	44.5
30	0.020	67.3	32.7

MIC = 2 (μg/ml)

Anticancer activity of Tamoxifen

Concentration of AuNP's (μg/ml)	O.D value	Cell inhibition %ml	Cell viability %ml
50	0.702	46.9	53.1
40	0.855	35.3	64.7
30	0.866	34.5	65.5
20	0.977	26.1	73.9
10	1.086	17.8	82.2

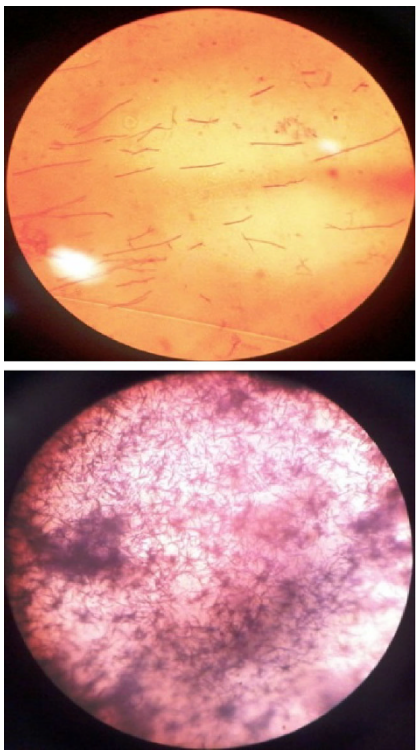
MIC = 50 (μg/ml)

Anticancer activity of Letroz

Concentration of AuNP's (μg/ml)	O.D value	Cell inhibition %ml	Cell viability %ml
50	1.687	85.1	14.9
40	1.174	28.8	71.2
30	1.315	44.1	55.9
20	0.727	17.5	82.5
10	0.577	36.7	63.3

MIC = 50 (μg/ml)

After the activity of AuNP's on MCF 7 cell lines



MCF 7 cell lines after the addition of gold nanoparticles. It can be noted that the morphology of the cell lines have been severely affected by the action of the gold nanoparticles. From the study, we can state that the gold nanoparticles can be further studied and can be employed as an effective anticancer agent

Conclusion

The increasing demand for the nanoparticles applications opens up new arenas in the process of its synthesis. We have dealt with a green synthesis in this study using various feasible, amply available and cost effective resources, the plant extracts. We attained good results with the nanoparticles synthesis and the size of the nanoparticles were holding commendable nanodimensions, that makes the particles more attractive towards incredible applications. Here in the study, some of the applications of the synthesized nanoparticles were investigated, still more of the properties need to be exploited for beneficiary outcomes.

Indeed, the gold nanoparticles have enormous potentialities, that makes them to be inevitable in almost all sectors, in the years to come.

Summary

This integration of nanoparticles with biological molecules has lead to the development of diagnostic devices, contrast agents, and important tools in cancel therapy. Currently, there are many gold nanoparticles industrial uses that resulted in a boost in its demand and production. Recent advancement in technology has introduced gold nanoparticles into the medical field. As studies of gold nanoparticles improve, several gold nanoparticles medical applications have been developed to help prevent the onset of infection and promote faster wound healing. The targeted drug delivery is one recent gold nanoparticles medical application in study. The anticancer abilities of gold nanoparticles were studied with MCF 7 breast cancer cell lines with reference of standard anticancer drugs like Tamoxifen and Letroz. The results obtained from the studies of anticancer activity showed that gold nanoparticles gave an equivalent good results, in par with the standard drugs against cancer. The AuNPs proved to be efficient even from the minimum concentrations of 2 μg/ml, and as the concentration increased the anticancer efficacy as well increased. All the above mentioned studies yielded reliable results in favor of the gold nanoparticles. This implies that the research on gold nanoparticles will be definitely fruitful to the people and betterment of the society.

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