

# Inhibition of EML4-ALK Tyrosine Kinase of Lung Cancer by Development of Silica Nanoparticle

S.Dhanasekaran , P.Jayanthi, T.Pratheepa, B.Sarani, B.Jansirani

**Abstract**— Cancer is a potentially critical disease caused mostly by environmental factors that transmute genes programming dangerous cell-regulatory proteins. Lung cancer is one of the most common types cancer and cases in increasing yearly. Lung cancer is usually a “systemic disease”. Nanoparticle medicine delivery is one area of current research significance since incorporation of drug to nano particles enhances beneficial effectiveness and reduces side effects of the drug payloads improving their pharmacokinetics. In the present study Mesoporous silica nanoparticles were synthesized, loaded with anticancer drug 5FU and characterized by Zeta analysis to have a average size of 288 nm

**Index Terms**— Transmute genes, cell regulatory proteins, reduces side effects, anti cancer drugs ,pharmacokinetics, silica nanoparticles.

## I. INTRODUCTION

### NANOPARTICLES

Nanoparticle have emerged as valuable vehicles for inadequately soluble agents such as 5fu. Nanoparticles made of magnetic material can also be used to deliberate agents at growth sites using an outwardly applied magnetic field. The nanostructure working as drug delivery system have multiple advantages which makes them superior to conventional liposomes, Nano capsules, Nano emulsions, solid lipid nanoparticles etc. are for delivery of drugs.

## II. MATERIALS AND METHODS:

### A. Production of silica nanoparticles

In a round bottom flask 1.5 ml of tetraethyl orthosilicate (99%) and 50ml of ethanol was added. After the assortment attained the temperature of 64<sup>0</sup>c, 2.5ml of ammonium was added and centrifuged at 8000 rpm 10 minutes, washed with ethanol and sediment was dried to obtain silica nanoparticles

**Manuscript received January 01, 2015.**

**Mr. Dhanasekaran S.**, Department of Biotechnology, Vivekanandha college of Engineering For Women , Namakkal , India, Mobile No:9655849404.

**Jayanthi P.**, Department of Biotechnology, Vivekanandha College of Engineering For Women, Namakkal, India, Mobile No:8807460450.

**Pratheepa T.**, Department of Biotechnology, Vivekanandha College Of Engineering For Women, Namakkal, India, Mobile No:9042743543.

**Jansirani B.**, Department of Biotechnology, Vivekanandha College Of Engineering For Women, Namakkal, India, Mobile No: 9976414942.

**Sarani B.**, Department of Biotechnology, Vivekanandha College Of Engineering For Women, Namakkal, India, Mobile No:9894855467.

### B. Dulbeccos customized eagles media (DCEM) preparation

Perched 0.67 g in 25ml tissue culture grade water with constant rousing until the powder is totally dissolved. The water should not be heated. 0.187 g of NaHCO<sub>3</sub> powder was supplemented and stimulated until dissolved. The pH was attained to 0.2-0.3 pH units below the preferred pH (pH 7.4) using IN HCL or IN NaOH since the pH tends to rise during filtration.

### C. Determination of cytotoxicity

To determine cytotoxic effects of cur cumin nanoparticles in A549 cell line, different concentrations of samples 1,5 and 10µl from stock (10mg/ml) were added to the A549 cells and incubated for 24 hours. The A549 medium was prepared as per standard procedure described above and the cells after 72 hours growth were harvested and used for further studies.

### D. MTT cell capability assay

The viable cells were washed with IX PBS and 200 µl of MTT solution was added to the culture and incubated at 37<sup>0</sup>c for 3hours. MTT solution was eliminated, then washed with IX PBS and added 300µl DMSO to each culture flask, awaiting all cells get lysed. The culture was centrifuged at top speed for 2 mins to precipitate cell debris. Optical density 540nm.

### E. Drug loading and release

The hollow silica samples were covered with water in 5FU drug with a certain concentration, 100mg of silica powders were soaked in 50ml solution of 120ppm of 5FU. Drug loaded nanoparticles were centrifuged and washed with acetone 3 times and then dried under vacuum at 40<sup>0</sup>c for 3hr

### F. Evaluation of drug release by dialysis

5FU /PEG-PB LG nanoparticles were positioned into DMF solution. After agitation at 37<sup>0</sup>c for 3 hr, dialyzed sample was determined for drug concentration by measuring the absorbance at 269 nm. For in vitro release studies 5FU /PEG-PB LG were placed into dialysis bags and the bags were introduced into PBS at P<sup>H</sup>=6,7,9. The medium was stirred at 37<sup>0</sup>c.

### G. Neutral red assay

A549 cells were incubated for 2 h with neutral red dye (100 g/ml) dissolved in serum free medium (DMEM for A549cells). Cells were then washed with Phosphate Buffered Saline and the addition of 1 ml of elution medium (EtOH / AcCOOH, 50%/1%) followed by gentle shaking for 10 min to achieve complete dissolution. Aliquots of the resulting solutions were transferred to 96-well plates and absorbance at 540 nm

H. LDH leakage assay

Cytotoxicity was assessed by lactate dehydrogenase, leakage into the culture medium. Following exposure to the metal the culture medium was aspirated and centrifuged at 3000 rpm for 5 mins. The development of NADH from the results in a change in absorbance at 340 nm. Aliquots of media and warm reagent were mixed in a 96-well plate(Fisher) and absorbance was recorded using a spectrophotometry (Spectra max190-Molecular Devices).OD was read at 540nm (ErbaLisa reader) using DMSO as blank.

III. DETERMINATION OF APOPTOSIS BY ACRIDINE ORANGE (AO) & ETHIDIUM BROMIDE (EB) DOUBLE STAINING METHOD.

DNA-binding dyes AO and EB (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells (Zhang et al, 1998). AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into double stranded nucleic acid (DNA). EB is taken up only by non-active cells and emits red fluorescence by intercalation into DNA. After treatment with different concentrations of curcumin nanoparticles, the cells were washed by cold PBS and then stained with a mixture of AO (100 µg/ml) and EB (100 µg/ml) at room temperature for 10min

IV. RESULTS

A. MTT Cell Viability Assay

The MTT cell viability assay is normally considered as a specific technique in determining in vitro cell cytotoxicity. In the current study the cell viability was reduced to 68 % by treatment of 10mcg of nanoparticles which confirms the decreased toxicity of silica nanoparticles.

Table I : . MTT Cell Viability Assay on A549

sample	Concentration	% viability
Strandard 5fu	1 µg	100%
	5 µg	82.23%
	10 µg	72.85%
Silica nanoparticles Loaded with 5fu	1 µg	65.92%
	5 µg	81.91%
	10 µg	67.76%

Graph :1 MTT Cell Viability Assay on A549

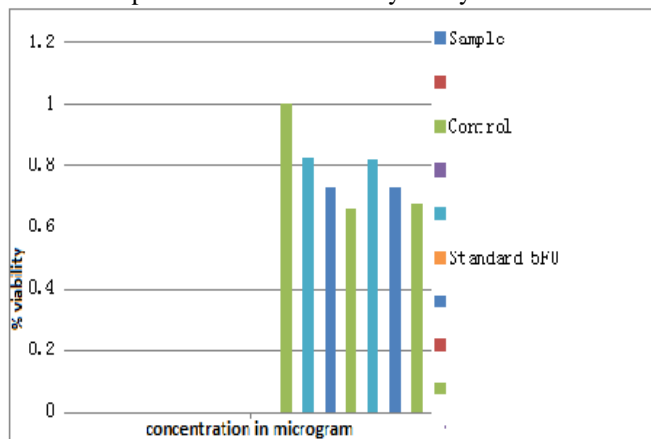
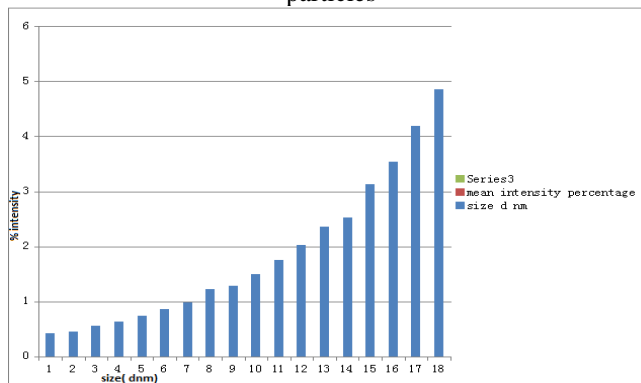


Table :2 – Zeta potential analysis

Size dnm	Mean intens ity%	Size dnm	Mean intensity %	Size dnm	Mean intensity%
0.4225	0	5.589	0	74.54	2.5
0.4532	0	6.529	0	92.28	2.4
0.5475	0	7.426	0	105.36	2.2
0.6314	0	80854	0	126.8	2.3
0.7285	0	10.15	0	141.25	2.5
0.8542	0	11.98	0	164.28	3.2
0.9745	0	13.56	0	190.2	3.9
1.2274	0	18.45	0	220.36	4.8
1.2859	0	21.75	0	225.87	5.8
1.488	0	24.83	0	295.58	6.7
1.756	0	28.35	0	358.69	7.2
2.018	0	32.86	0.3	458.36	7.4
2.359	0	43.78	0	589.25	7.2
2.524	0	50.96	0	624.58	7.3
3.128	0	58.47	1.2	712.36	6.7
3.536	0	59.36	1.5	842.56	5.7
4.189	0	62.58	1.7	945.32	4.3
4.859		63.57	2.3		

Graph 2:zeta potential analysis of 5fu loaded silica nano particles



B. MTT assay in A549

The MTT cell viability assay is generally considered as a precise technique in determining in vitro cell cytotoxicity.MTT assay showed decreased cell viability for silica nanoparticles when compared to standard 5F.

C. LDH assay in A549 cells

LDH has proved to be an particularly useful invitro marker for cellular toxicity. Therefore it is used as a quantitative marker enzyme for intact cells,its activity providing information on cellular glycolytic capacity. The result shows that the cell leakage of Silica nanoparticles shows increased membrane damage when compared with standard 5FU.

D. Neutral red uptake assay in A549

Neutral red is extensively used in vitro test for the determination of cytotoxicity. neutral red measures the intracellular integrity after the treatment .In current study, the cell viability was reduced more by nano curcum.

## V. DISCUSSION

Of the several new chemotherapeutic agents introduced recently, the taxanes have had a deep impact in a wide variety of malignancies. 5FU is accepted for clinical use by the food and Drug Administration(FDA) board for the treatment of breast cancer, ovarian cancer, non-small cell lung cancer (NSCLC) and prostate cancer. The toxicity profiles for these agents are somewhat different 5FU has been most widely associated with peripheral neuropathies and myalgias /athralgias, whereas docetaxel most commonly results in cumulative fluid retention that may be dose limiting in some cases. The problems associated with carriers such as polyoxyethylated castor oil vehicle, and dehydrated ethanol which is the common method used nowadays prompt many adverse conditions such as high incidence of acute hypersensitivity reactions characterized by respiratory distress, hypotension. Angiodema, generalized urticaria and rash. Initially investigated alternative excipients such as polyethylene glycol for 5FU solubilization; however, this compound appeared to decrease the antitumour activity of paclitaxel in murine models. Hence the development of a novel carrier material which offers lesser toxicity like silica nanoparticles for delivery of 5FU will be more useful. Mesoporous silica nanoparticle have been highlighted as an interesting drug delivery platform, due to their flexibility and high drug load potential. One in every three cancers diagnosed is a skin cancer.

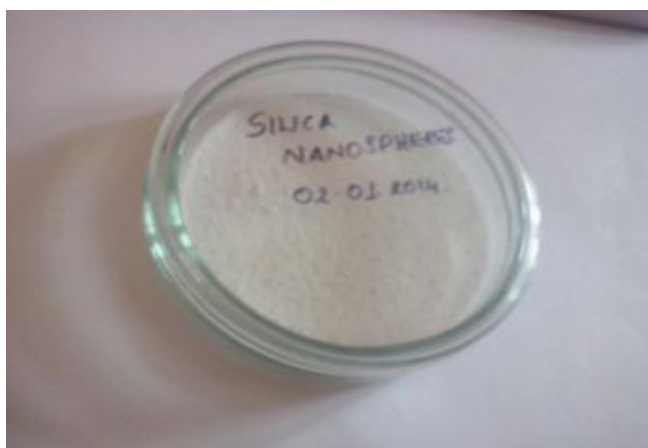


Figure I :Silica Nanospheres

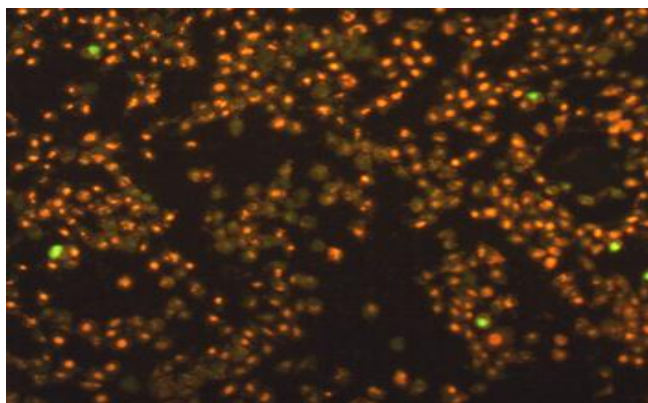


Figure II :Apoptosis A549cells treated with MSFU

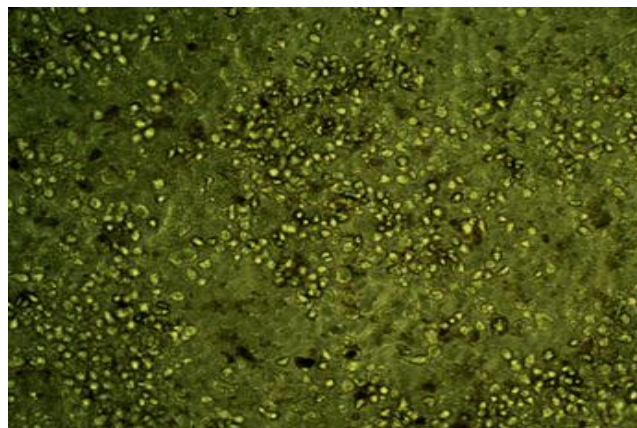


Figure III:phase contrast analysis of A549 cells treated with 10mcg/ml 5fu

## REFERENCE

- [1] Eliza Panzarini, Valentina Inguccio Mysore S Veena, Marilene B Wang, Eri S Srivatsan (2013): A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma using nanomaterials.
- [2] M. Rajan, Pron G, Poddevin B, and Mir L-M (2013) Electro permeabilization of cells and tissues assessed by the qualitative and quantitative, by chitosan nanocomposites.
- [3] Macmillan cancer support (2013) Electrochemotherapy, a new antitumour treatment. First clinical phase I-II trial. *Cancer* 72, 3694-3700.
- [4] Chen, Formanek M, and Regele H (2010) Electroporation therapy in head and neck cancer (curcumin) *Acta Otolaryngol* 123, 264-268.
- [5] Basu, S, Miklavcic D, Vodovnik L, Jarm T, Rudolf Z, Stabuc B, Cufer T, and Sersa, G (2011) Improved therapeutic effect of electrochemotherapy with cisplatin by intratumoral drug, stress modulated signaling pathway.
- [6] Ono, and Miklavcic D (2013) Electrochemotherapy with cisplatin in the treatment of tumor cells resistant to cisplatin, (traditional medicine) *Anticancer Res* 18, 4463-4466
- [7] Sumathi, S, and Sersa G (2013) Intrinsic sensitivity of tumor cells to bleomycin as an indicator of tumor response to electrochemotherapy. *Jpn J Cancer Res* 89, 328-333.
- [8] Agnieszka Z. Wilczeska, Milacic R, Miklavcic D, Dolzan V, and Sersa G (2012) Intratumoral cisplatin administration in electrochemotherapy: antitumor effectiveness, sequence dependence and platinum content, controlled drug delivery system. *Anti-Cancer Drugs* 9, 525-530.
- [9] R. Seda, Golouh R, and Sersa G (2012) Increased platinum accumulation in SA-1 tumour cells after in vivo electrochemotherapy with cisplatin and chitosan nanoparticles *Br J Cancer* 79, 1386-1391.
- [10] Dimirtis G Fatourous, Holder A-L, Chaplin D-J, Tozer G-M, and Sersa G (2011) Electroporation of human microvascular endothelial cells: evidence for an anti-vascular mechanism of electrochemotherapy, microporous zeolites *Br J Cancer* 84, 565-570.
- [11] Dai SJ, Pang WB, Sheen L, "New norditerpenoid alkaloids from *Scutellaria barbata* with cytotoxic activities". *Nat. Prod. Res*, 15(2011) 1-6.