Second World Congress on Cancer 2010 (WCC-2010)
September 3, 4, 5, 2010 Kerala, India

Cancer Nanotechnology Treatment through Folate Conjugated Gold Nanoparticles (*)

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Abstract:

Here, we report our research on the optimum design, *in vitro* tests and *in vivo* applications of folate-conjugated gold nanoparticles (nanoconjugates) for selective targeting of folate receptor positive cancerous cells, which over express folate-receptor on their surface. Significantly high light absorption of these new nanoconjugate makes them promising materials for photo-activated cancer therapy and thermal ablation of tumors.
We have produced two nanoconjugates made of folate and gold nanoparticle (*AuNP*) with various linkers. We have used them for *in vitro* selective cancer cells targeting and destruction with excellent results. Experiments were conducted on human adenocarcinoma HeLa cell chosen as the model cancer cell line because of its folate-receptor over expression. In addition, MCF7 cell line was used as control because of its low level of folate-receptor expression.
Following photothermal treatment, more than 95% cell killing was achieved for HeLa cells. The following specific subjects will be reported here: 1) Synthesis and characterization and optimum design of folate-conjugated AuNPs using different linkers. 2) Our *In vitro* examination of the effects of light-particle interaction on cell lethality and optimization of the choice of linker and shape and size of gold nanoparticle. 3) Our ongoing *In vivo* research into ways to reduce the folate-conjugated gold nanoparticles antigenicity, increasing their hydrodynamic size, and studying their cytotoxicity.

Keywords: Gold nanoparticle, Folic acid, Folate, Hela cell, Mcf7 cell, Targeting, 4-aminothiophenol, 6-mercapto-1-hexanol

1- Introduction:

A new class of therapeutics for cancer treatment is being developed in recent years based on the applications of nanotechnology for selective targeting of cancer cells. Within this emergent class of targeted therapeutics, folic acid (or folate, the folic acid salt)—drug conjugates represent a highly promising group of nanomaterials. Cancer cells over express the folate-receptor because of their vast requirement for folate. Since folate is necessary for DNA nucleotide synthesis and cell division, a cancer cell will require much more folate than a healthy cell. It has been proposed that the folate-receptor makes for a suitable targeting agent because of its low expression level in healthy tissues and over expression in cancerous tissues [1-4].

On the other hands, gold nanoparticles (AuNPs) have found major applications for thermal ablation of cells. When AuNPs are excited by an electromagnetic field, they absorb an intense amount of energy in the form of heat attributed to the collective oscillation of electrons on their surface, which is termed as Plasmon resonance. In practice, when the light pulses are irradiated on an AuNP its temperature rapidly increases. This absorbed energy which is quickly transformed into a high temperature could cause destructive damage to cancer cells through local overheating effects. Accordingly, AuNP appears to be an effective agent for photo-activated cancer therapy and it can be used in vivo to destroy cancer cells and tissues in a non-invasive manner [3-7].

By combining the targeting characteristics of folate and the photothermal properties of AuNPs we have been developing a therapeutically targeted strategy based on folate conjugated AuNPs. Towards this goal, we have produced folate conjugated AuNPs using different linkers including in 4-Aminothiophenol (4Atp) and 6-mercapto-1 hexanol (MH). Here, we report our latest findings on these two nanoconjucates.

2- Materials and Methods:

The procedures for synthesis of both nanoconjugates are schematically shown in Figure 1 (a & b).

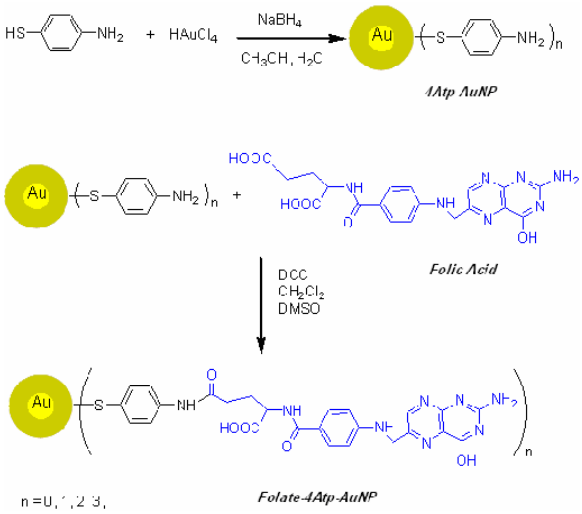


Figure 1- (a) Scheme for the synthesis procedure of Folate-4Atp-AuNP nanocongugate.

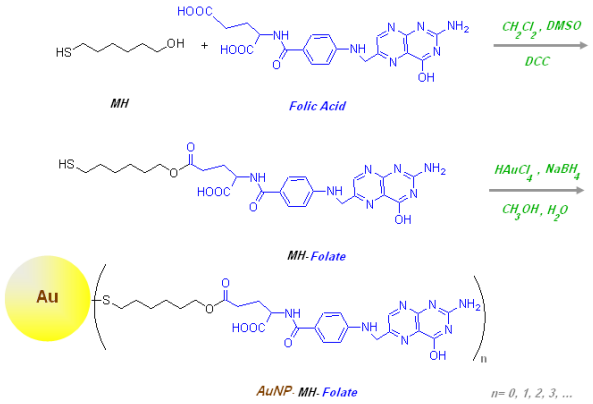


Figure 1- (b) Synthesis route of Folate-MH-AuNP.

After characterizing the synthesized nanoconjugates, we performed in vitro tests on Hela and MCF7 cell lines. The former was selected because of its high level of folate receptor and the later for its low level of folate receptor expression.

An intense pulse light source was used as the provider of electromagnetic field. Cell viability was assessed through MTT assay.

3- Results:

The results of our researches on investigation of nanoconjugate characteristics are shown in Figures 2 and 3.

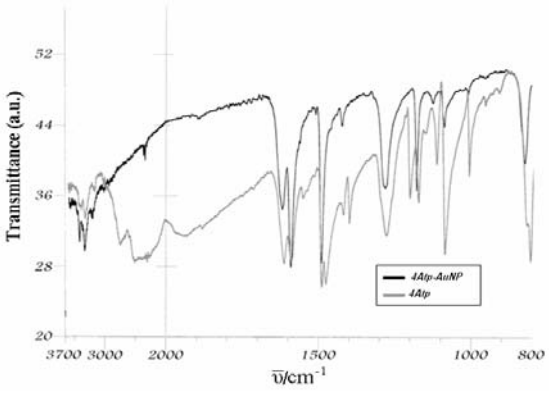


Figure 2- (a) FTIR spectra of 4Atp-AuNP in comparison to 4Atp.

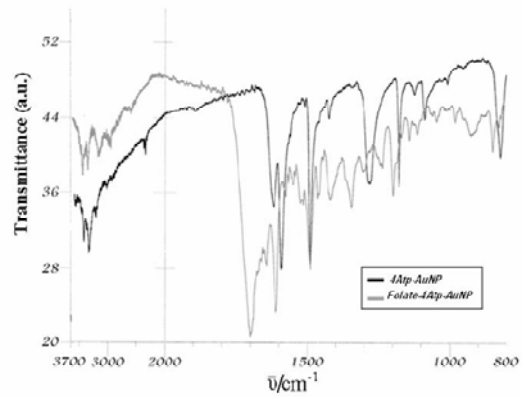


Figure 2 (b) FTIR spectra of Folate-4ATP-AuNP in comparison to 4ATP-AuNP.

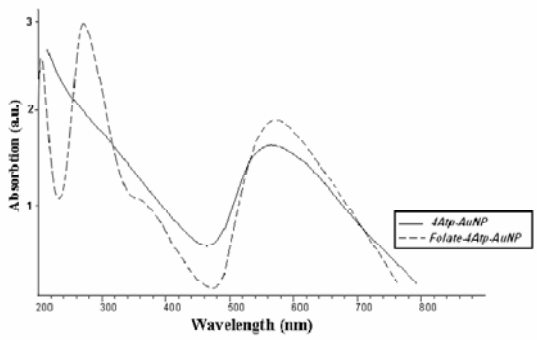


Figure 2 (c) UV-Visible absorption spectra of 4Atp-AuNP and Folate-4Atp-AuNP.

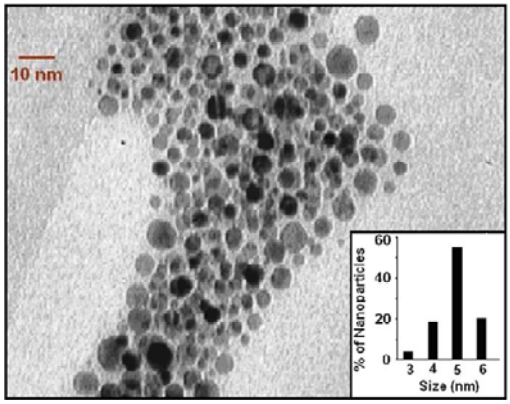


Figure 2 (d) TEM photograph of Au nanoparticles in Folate-4Atp-AuNP nanocongugate and histogram for the size distribution of Au nanoparticles.

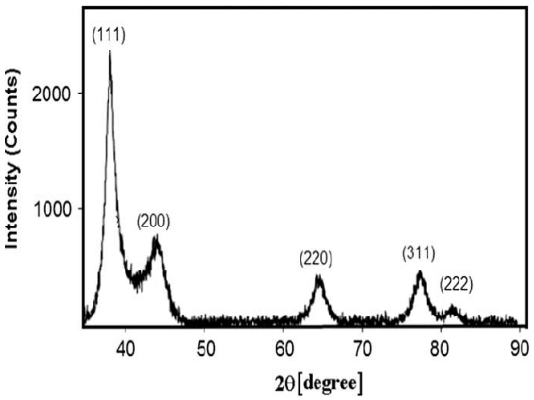


Figure 2 (e) XRD pattern of Au nanoparticles in Folate-4ATP-AuNP.

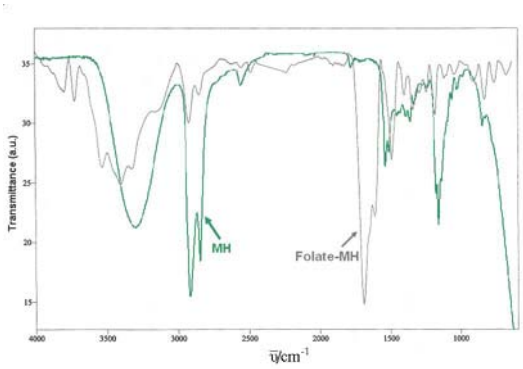


Figure 3 (a) Infra Red spectra of Folate-MH in comparison with MH.

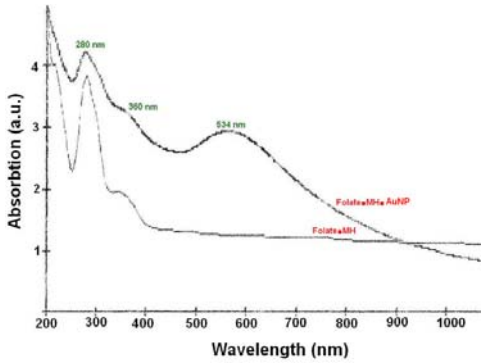


Figure 3 (b) UV-Visible absorption spectra of Folate-MH and Folate-MH-AuNP.

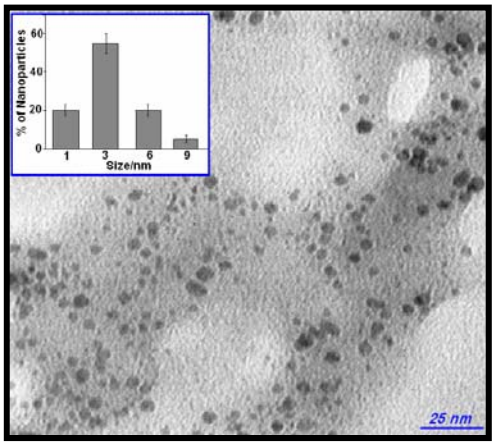


Figure 3 (c) TEM photograph of Au nanoparticles and their size histogram (inside) in Folate-MH-AuNP.

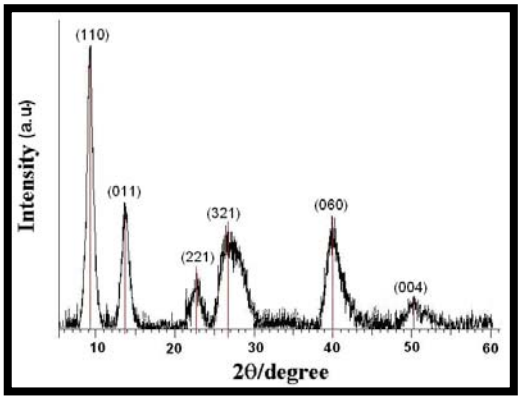


Figure 3 (d) XRD pattern of Au nanoparticles in Folate-MH-AuNP.

In the next step of our studies, we did in vitro tests on Hela and MCF7 cell lines. The condition for exposing Folate-4Atp-AuNP were: {20 IPL pulses; Operating modes: single; Spot size: 8 mm × 15 mm; Fluence: 15 J/cm^2; Filter (wavelength): 560 nm; Pulse duration: 3 ms}. In addition, the

conditions for exposing Folate-MH-AuNP were similar to Folate-4Atp-AuNP except that the filter was selected at 560 nm. Figures 4 and 5 present our results in this area.

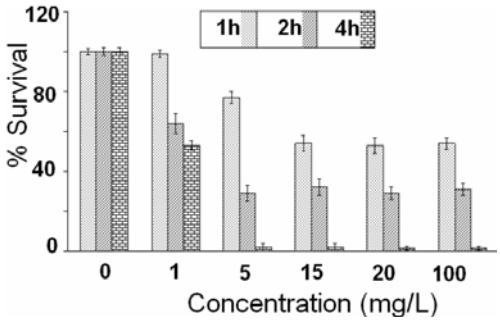


Figure 4 (a) The percentage of survival for HeLa cells obtained following photothermal treatment using Folate-4Atp-AuNP.

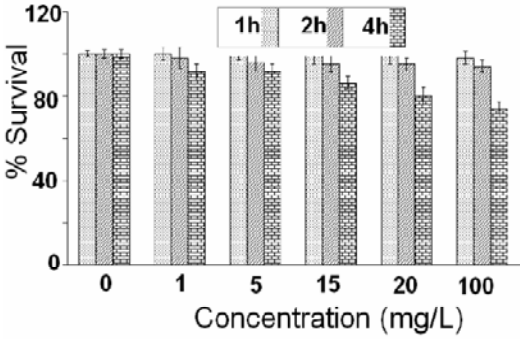


Figure 4 (a) The percentage of survival for MCF7 cells obtained following photothermal treatment using Folate-4Atp-AuNP.

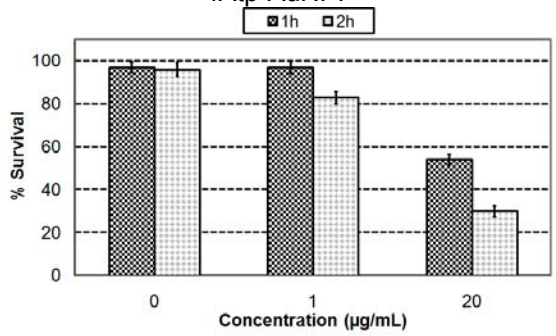


Figure 5 (a) The percentage of survival for HeLa cells obtained following photothermal treatment using Folate-MH-AuNP.

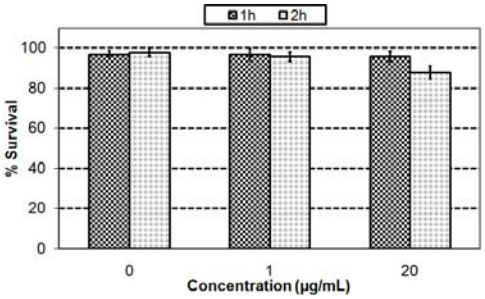


Figure 5 (b) The percentage of survival for MCF7 cells obtained following photothermal treatment using Folate-MH-AuNP.

4- Conclusion:

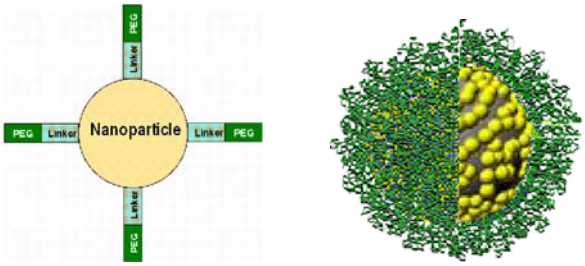
In order for the folate-conjugated gold nanoparticle to be selective in cancer cells targeting and destruction it must have long enough circulation residence time and no cytotoxicity on healthy cells. Our ongoing research is to develop ways to reduce the nanoconjugate antigenicity, increasing its hydrodynamic size in order to prolong its circulatory time and reducing its renal clearance and research into its cytotoxicity. Our research findings using solid gold nanoparticles (AuNPs) indicate that this procedure is quite effective for cancerous cells targeting and destruction. We are quite aware of the research works which described the use of Au nanorods [8] or Au nanoshells [9] for targeted cancer photothermotherapy. For the *in vivo* stage of our proposed work we are using different shape and size AuNPs including nanoshells and nanorods.

Au nanorod [8]
(black dots are Au atoms)

Nanoshell [9]

We are also considering the well-established Polyethylene glycol (PEG), chemical formula $(–CH2CH2O–)_n$, (MW ≤ 10 kD), for coating of Au nanoparticles to make them compatible for *in vivo* applications. PEG

coating technology for nanoparticles, drug, etc. is now well developed. For coupling nanoparticles to PEG, usually monomethoxy PEG [CH3 (–O–CH2–CH2)n–OH] is first activated by means of an activating agent (linker) before attachment to nanoparticles [2].



(a) (b)

Illustration of a PEG coated nanoparticles: (a). (b).Illustration of a PEG*lated* nanoparticles.[2]

The proposed cancer nanotechnology approach for targeting the cancerous cells' folate receptors constitutes a highly promising method for cancer treatment. This aspect of the method is unique among all the existing technologies and those in various development stages. It will become a front-runner in targeted nanotechnology cancer treatments with the completion of the proposed stages of research. It will help prevent human suffering and death from cancer. Cancer is one of the deadliest illnesses suffered by mankind. We are confident with implementation of the proposed research we will be able to optimally design folate-conjugated AuNP for effective elimination of cancerous cells which overexpress folate-receptor on their surface.

In total, the procedures developed in our studies seem to be effective for cancer therapy. We are doing researches for the application of nanoconjugates in vivo.

5- References:

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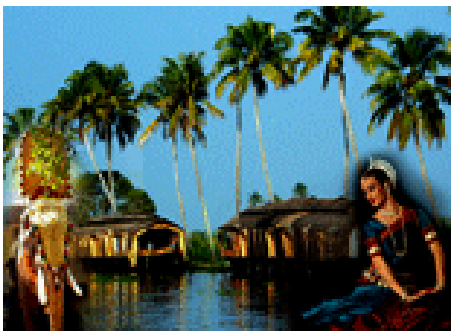

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