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CANCER DIAGNOSIS AND TREATMENT USING MULTILAYER MICRO NANO CAPSULES

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Breast Cancer, Melanoma cancer, Early detection, Traditional cancer treatment, advanced diagnosis, Multilayer Micro Nano capsules

Abstract

In addition to being the most common disease in women, breast cancer accounts for almost half a million deaths globally every year. However, despite its relatively low mortality toll, melanoma cancer is still a major health concern. Cancer patients undergoing traditional cancer treatments confront a number of challenges, including a wide variety of adverse effects, and the treatment is not always effective. Using nanomaterials in in vivo breast cancer therapy applications has revolutionised many areas of the field, including the conjugation of natural compounds showing chemo preventive activity, such as curcumin, onto the nanoparticles, and the controlled drug release towards specific organs or at the tumour site, reducing the side effects, otherwise caused by conventional cancer therapy. Gold nanoparticles that have been "tagged" with folate have emerged as the most effective nanocarriers for cancer treatment and detection in recent years.

Early detection and diagnosis methods for breast and melanoma cancers have benefited greatly from the development of nano-based systems with enzyme mimetic properties, blood compatibility, and the ability to differentiate between normal cells and cancer cells. Targeting cancer cells that have an overabundance of folate receptors has led to an increased interest in delivering anticancer medications using polymer-coated gold nanoparticles (Au NPs) conjugated with folic acid (Fa) (C19H19N7O6). Due to its insolubility in water, folic acid must rely on nanoparticles to transport it to the tumour site. Here, we report a facile method for formation of folated silica NPs (Fa@Si NPs), folate conjugated to silica-gold core shell nanoparticles Fa@SiAu-PVP NPs, folate conjugated to amphipathic polymer coated gold nanoparticles (Fa@Au-PVP NPs) and Curcumin drug loaded folate conjugated to amphipathic polymer coated gold nanoparticles (Fa@CurAu-PVP NPs) with a size 185 nm, ~250nm, 40 ±12.5 nm and ~350nm with negatively charged

surface area. Functionalization using EDC/NHS as a cross-linking agent follows the coating of Au NPs with polyvinyl pyrrolidone (PVP) polymer and the development of a silica shell. Scanning electron microscopy (SEM), atomic force microscopy (AFM), and Fourier transform infrared spectroscopy (FTIR) all attest to the fact that NPs have been developed successfully. To replicate the peroxidase enzyme, a synergistic nanoconjugate that is highly catalytic in the presence of TMB and H2O2 has been developed. This target-functionalized nanoparticle has the potential to serve as a very sensitive nanoprobe for the rapid colorimetric detection of triple-negative and luminal-A breast cancer. Results from experiments show that the conjugate binds to albumin-like proteins, which increases the NP's cytoxicity and prolongs its circulation in the body during in vivo treatments, specifically for MDA MB 231, MCF 7 cells, 4T1 breast cancer cell lines, and B16F10 skin melanoma cells. Research on the percentage of haemolysis caused by cytotoxic concentrations of NPs on five distinct blood types was also conducted. The data showed that the N[s were devoid of any biological hazards. This is a major advance in cancer theranostics, since the folate molecule may now be used to guide the delivery of anticancer medications, and metal nanoparticles can be used to diagnose cancer via the invention of a colorimetric sensor.

1.Introduction

Developing and developed countries both are encountered by the most invasive, multifactorial and complex disease which is in second position in terms of death caused yearly athwart the globe. The most lethal types of cancer listed amongst both male and female are lung cancer, prostate cancer, breast cancer, liver cancer, colorectal cancer and skin cancer[1-3]

Amongst the lethal types of cancer, breast cancer is considered to be leading health problems especially in women. Whereas, it is also a major cause for annual 5 million mortality rate worldwide. Melanoma even being not the most lethal types, but still is the most common type of cancer occurring worldwide as the important risk factor for it is chronic exposure to UV light (even normal exposure to natural sunlight can be harmful for such patient's). Various drawbacks including a range of side effects are faced by the cancer patients going under conventional cancer therapy methods and thus lacks with positive and successful outcomes every time. The conventional cancer therapy includes surgery (preliminary approach), chemotherapy (systemic approach) and radiation therapy (pre-surgery/post-surgery/combinational therapy approach). Various anti-cancerous drugs inhibiting DNA replication and chain elongation have been approved by FDA for treating different cancer such as cis-diamine-dichloroplatinum (II) (cisplatin). But their still remains therapeutic challenge especially with breast cancer as they have the tendency to metastases as well as exhibit resistance to certain conventional anticancer drugs and show reoccurrence of lesions even after surgery3,4. Moreover, most of the conventional or chemotherapeutic drugs are not target specific which results numerous off targets harming the normal cells and tissues which thereby leads to more side effects and discomfort to the patient[4]. Photodynamic therapy using non-toxic photosensitizers which eradicates cancer cells by generating ROS is one of the unconventional therapies which has gained advantage over conventional therapy over the years[5] The only drawback faced by photodynamic therapy is the hydrophobic characteristics of most of the photosensitizers prevents them from delivering efficient result as they tend to aggregate in aqueous solutions exhibiting poor success in treatment5 . Conjugating such photosensitizers with NPs along with targeting ligands can not only increase their chances to delivery them at the correct site but also increase the efficacy of various treatments[6],[7]. Gold NPs have easy surface modifying chemistry due to which then can be functionalized with thiols, amines, proteins, nucleic

acids etc. Binding with various ligands such as folic acid can thus be possible to increase the chances to target breast cancer tumor combining the treatment with other therapies.[8]

2. Nanoparticles Based Therapies

Conventional therapies usually have an affect on the entire body treating affected as well as harming non-affected cells. Whereas nanoparticle-based target specific therapy targets only the affected area as depicted in figure 1., which is considered to be the greatest advantage till date.[9]

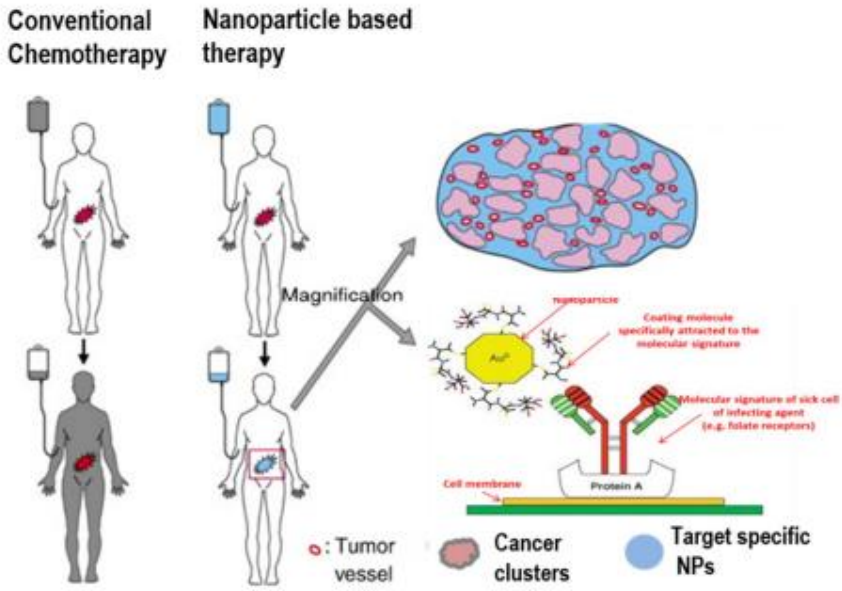

Figure 1: Diagram depicting advantage of NP based therapy over conventional therapy

Different tumor immune-suppressive cells such as regulatory T cells, myeloid derived suppressor cells and various growth factors VEGF (vascular endothelial growth factor) and TGFβ (transforming growth factor-beta) accumulated in the tumor microenvironment due to rapid tumor growth can inhibit the functioning of dendritic cells, trigger the transfer of macrophages to M2 phenotype (pro-tumorigenic) and also lead to aberrant fibrosis. The entire phenomena can be reversed so that the immunosuppressive environment can turn into immune-supportive by introduction of nanoparticles with specific design to effectively work as drug delivery system.[10] Nanoparticles can easily passivate through the tumor tissue with prolonged retention time and can accumulate in tumor much more apparently than in normal tissue due to abnormal vasculature and leaky lymphatic nature of tumor cells. Surface modification of the nanoparticles with specific biomarkers, ligands, drugs, peptides and controlling the size, shape and charge on the nanoparticles can make them an efficient tool to deliver drugs at the target site. FDA has approved several nanodrugs such as Doxil and Abraxane38 for their use in clinical trials[11]. Real time in vivo tracking, photoacoustic,

luminescence imaging and immunotherapy by using gold nanocages along with CD11c encapsulated on dentritic cells within the liposomal cells was achieved by Liang et al.39 Au nanocages were accumulated at the lymph nodes within 1 hr to 12 hr of injection which enhanced dendritic cell maturation and also activated cytotoxic CD8+ T cells.

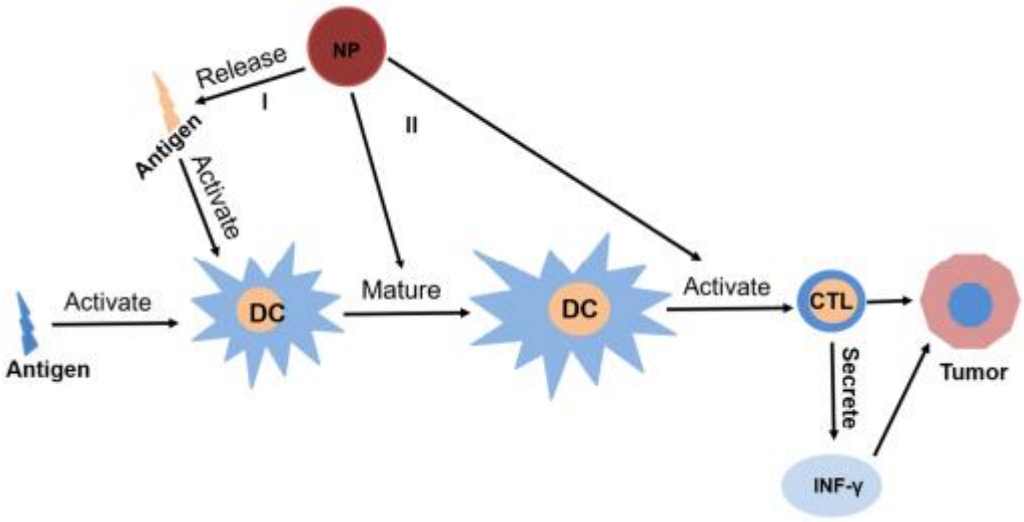

Figure 2. Role of NPs in DC's mediated tumor immunity.

I) Nanoparticles conjugated with antigens are delivered specifically to DC. II) DC's recognize this antigen which is presented by them to cytotoxic T cells (CTL) in the process of DC's getting matured and leading to CTL activation. Activated CD4+ and CD8+ acquires cytotoxic properties and secrets INF-γ that results in tumor death.[12].

3.Targeting Nanoparticles to Tumor Site

Passive tumor targeting is one of the extensively studied areas which advents via EPR (enhanced permeability and retention) effect for delivering nanoparticles at the tumor site. The heterogeneity of tumor microenvironment altering the EPR effect, various physiological barriers (example: blood brain barrier) and varied physiochemical characteristics of NPs altogether, severely affect the potential application of active targeting mechanism. Fabricating and optimizing the surface modification of the NPs by controlling their size, shape, surface charge will help in conjugating their surface with specific targeting ligands.[13] This ligand can recognize the receptors on cancer cells and attaching anti-cancer drugs to NPs can overcome the limitations faced until now and thus the efficacy of active target mechanism can be enhanced by accumulating well designed NPs at the tumor site . The choice of targeting moiety to be conjugated onto the surface of nanoparticles or to be encapsulated in the nanoparticle is very important in terms of optimizing the efficacy of active targeting. A wide range of targeting moieties, that can be attached to the NP structures by using either physical adsorption or chemical conjugation strategies. Different targeting moieties used to

deliver anti cancerous drugs in the in vitro and/or in vivo models includes proteins, protein domains, synthetic peptides, nucleic acid and nucleic acid aptamers, small molecules, monoclonal antibodies etc., Antibodies, especially monoclonal antibodies are considered to be the smart ligands with higher specificity and greater success in diverse range of tumors[7][9] . Targeting tumor activated macrophages with nucleic acids and peptides although account for the traditional therapies with various limitations such as reduced circulation time, reduced specificity towards target site as well as reduced drug solubility. Contrarily, there are small chemical molecules such as folate , phenylboronic acid, anisamide, plant polyphenols, quinic acid etc., which are smaller in size although has reduced specificity as their targeting receptors are also expressed in normal cells with reduced expression. Due to different vasculature structure of tumor membrane the nanocarriers with these types of targeting moiety has shown efficacy in active targeting mechanism in the in vitro as well as in vivo model system.[10]

4. Development of Folate-Amphipathic Polymer Functionalized Gold Nanoparticles: Acting as A Nanoprobe Mimicking Peroxidase Activity

Au NPs have unique optical properties and therefore by tuning their shape and size can shift there plasmon band to longer wavelengths. Such modified Au NPs having surface plasmon resonance which can convert light energy into heat energy of greater efficiency thus, the treatment can be targeted to specific tumor site (even in deep tissues) to prevent off target effects which allows use of AuNPs photo thermal therapy along with targeted drug delivery. The surface chemistry, stability and morphology of AuNPs can be further controlled by using additives such as certain polymers including PVA, PEG etc. Such polymer coating is commonly used to develop hydrophilic folate conjugated nanoparticles for targeting cancer cells, but the drawback lies in the large size and colloidal stability of such conjugates which restrict their intravenous delivery. For easy passage of nanoconjugates through blood stream towards the target site monitoring their smaller size and colloidal stability is an important factor. To overcome this problem, a simple one pot method has been PVP is one such ligand with unique properties like an eco-friendly stabilizer; non-ionic in nature containing strong hydrophobic amide groups and hydrophilic pyrrolidone moiety which are present in their monomers. It prevents aggregation of nanoparticles due to the repulsive nature of hydrophobic carbon groups elongating into the solvent used for dissolving the polymer5 .PVP also prevents the opsonization of Au NPs by macromolecular phagocytic system[11].

For Au NPs to function as a targeting molecule they can be conjugated with a biomarker for instance, folic acid (FA), which is a navigational molecule that will help the nanoparticles to actively target cancer cell surfaces. FA is a well-known ligand used by several researches for conjugating with positively charged liposomes, chitosan, poly(lysine) and polyethyleneimine[12]

Formulation of Curcumin Loaded Drug-Folate-Targeted PolymerCoated Gold Nanoconjugates (Fa@CurAu-PVP NPs): Studies on Drug Delivery And In vivo Cancer Therapy

Despite chemotherapy's widespread usage, concerns about its safety and effectiveness have prompted intensive research into alternative cancer therapies based on plant-based ingredients.Therefore, the National Cancer Institute has made an effort, leading to the discovery of natural substances with chemopreventive action. Figure 2 shows the progress made using folated curcumin loaded nanoconjugates in the treatment of breast cancer. In breast cancer treatment, folated curcumin nanoparticles with targeted drug delivery as their primary use are employed[8][9]. These nanoparticles include micelles, magnetic NPs, solid lipid NPs, mesoporous silica NPs, TiO2 NPs, chitosan NPs, microspheres, silica-coated graphene sheets, SPIONS, PLGA NPs, Au-PVP NPs, etc. Because of the unique receptor overexpression on certain cancer cell types, ligands like folic acid may be used to build nanocarrier coupled with medications for active targets. It is well knowledge that the B vitamin folic acid (FA) promotes cell proliferation and may be used as a biomarker for a particular target. The anticancer medication curcumin may be more effectively delivered to its cellular targets when the formulation is functionalized with folic acid. Interest in folic acid conjugated nanomaterials for use in cancer biology has increased, with a particular emphasis on their potential in the areas of diagnostics and targeted medicine administration.

Salmaso and colleagues developed a targeted delivery method by attaching folic acid to one end of a polymeric carrier that also included a cyclodextrin-curcumin combination. Due to targeting overexpressed folate receptors, the nanoconjugate complex entered the clathrin-independent route leading to cell endocytosis, although it shows only modest cellular uptake, therefore its drug release effectiveness needs further study.

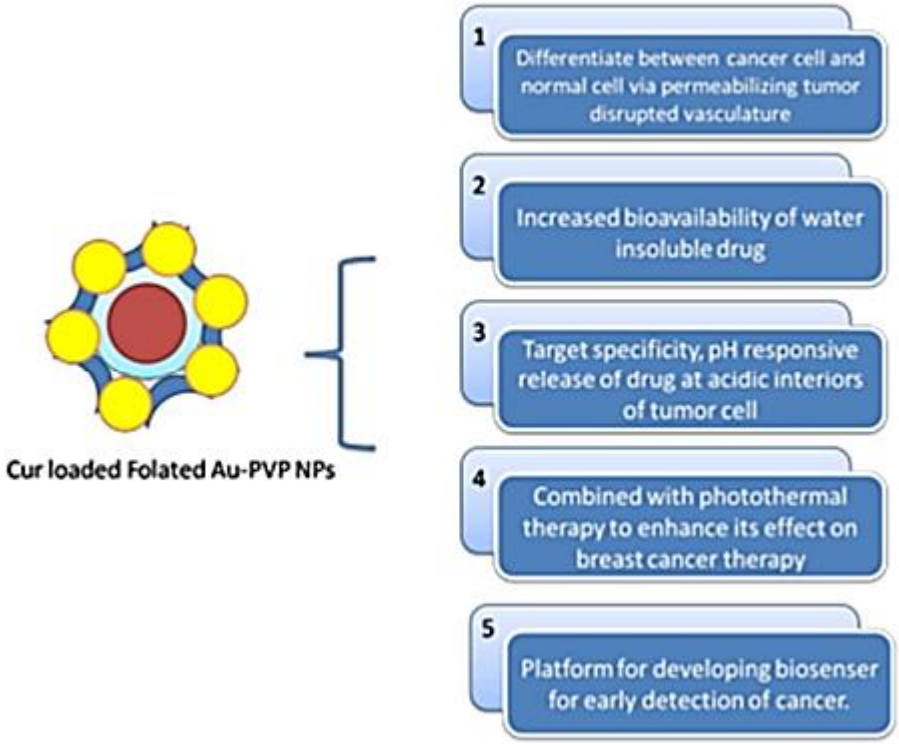

Figure 3. The properties of curcumin loaded folated gold-PVP nanoconjugates

Nanocarriers developed and optimised by chemical engineering may be used as effective medication delivery vehicles. Gold nanoparticles were widely employed as nanocarriers because of their superior targeting and drug release capabilities. Curcumin's poor solubility and non-specificity are addressed by attaching it to one of the side chains of a soluble polymer like PEG, polyvinyl pyrrolidone (PVP), or chitosan, and then attaching the other chain of the polymer to a gold nanoparticle. We have investigated targeting breast cancer cells by conjugating folic acid and loading curcumin onto PVP-functionalized AuNPs, which results in a high cell kill capacity. To this end, there is a lot of interest in the cutting-edge technique of adsorbing anti-cancer drugs onto the solid surface of gold nanoparticles using a sterically-stabilizing polymer with an opposite charge, such as polyvinylpyrrolidone (PVP). So, utilising carbodiimide coupling chemistry to load activated folate onto the above produced conjugate may further improve the LBL assembly. In order to create an amide bond by coupling processes, the crosslinking agents EDC/NHS (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide)/(n-hydroxysuccinimide) are used. Manju et al. showed that DCC/DMAP may be used as a catalyst and coupling agent for the conjugation of hyaluronic acid and curcumin.

5. UV Visible analysis

The presence of a surface plasmon band at 223 nm and 532 nm on Cur-conjugated AuNPs indicates that Cur has been loaded onto Au-PVP NPs.Cur interacted with Au-PVP NPs, as seen by the appearance of a new peak. The UV-visible spectra of folate-conjugated nanomaterials showed two absorption maximum peaks, at 285 nm and 364 nm.

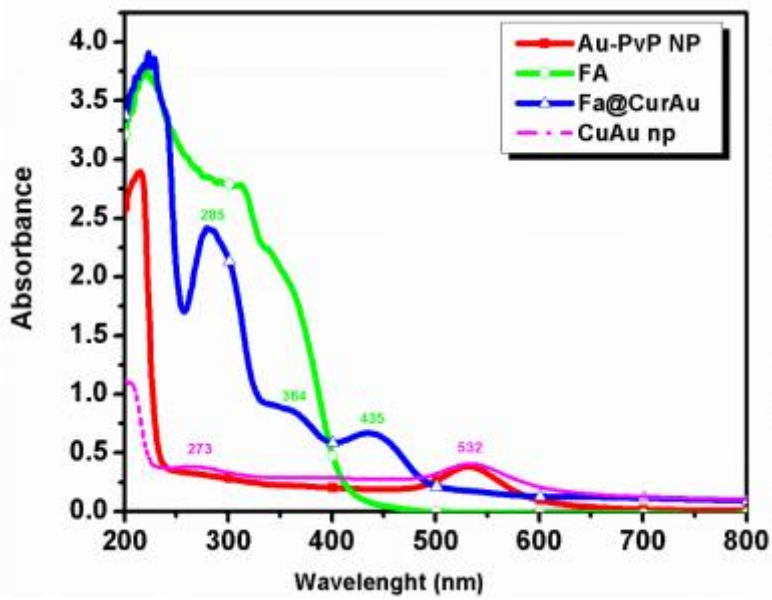

Figure 4: UV-visible spectra of AuNPs and AuNPs coated with PVP

The spectra of FA@CurAu-PVP NPs exhibits three prominent peaks. This suggests that the FA ligand was successfully bonded to the CurAu-PVP NPs, since two of the three absorption peaks were at 285 nm and 364 nm, indicating folate covalent connections. The spectra of FA@CurAu-PVP NPs exhibits peaks at 273, 285, 364, and 435 nm. The findings also show that the sample size grew following adaptation and conjugation.

It can be shown that FA was effectively conjugated with CurAuPVP NPs since their spectra have the same distinctive peaks at 285 nm and 364 nm, which are attributed to the * transition of the pterin ring of FA molecule.

The unaltered AuNPs exhibit typical surface plasmon absorption at 533 nm, but after being coated with a polymer PVP, the peak location shifted to 532 nm, indicating effective coating. One wide absorption band at 285 nm was seen in the UV-visible spectra of pure FA, which corresponded to the

n-n *transition of the C-C bond, and another large shoulder about 364 nm was observed in the spectra, which was associated with the n-p *transition of the C-O bond in the enone moiety of FA.

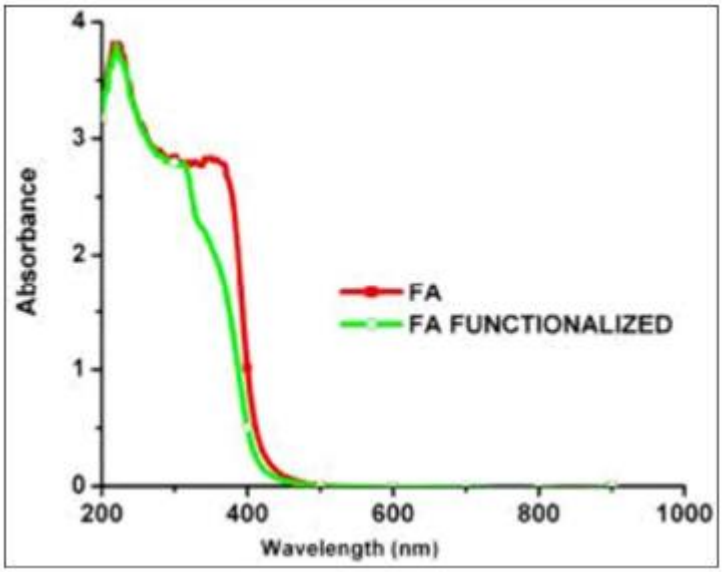

Figure 5: UV-Visible spectra representing activation of folic acid into folate

Two wide peaks appear in the spectrum of functionalized FA, however these peaks have moved from 285 to 312 nm and 364 to 376 nm, respectively, due to the bathochromic effect. Thus, the difference between FA's pre- and post-functionalization spectra indicates that functionalization was effective.

6. FTIR analysis

FTIR spectroscopy was used to characterise the nanoconjugates and provide additional confirmation and qualitative analysis of the conjugation of FA on CurAu-PVP NPs. The 1651 cm1 peak is attributed to the stretching vibration of -C=O groups in both unmodified and modified FA. C=C stretching vibration peaks for the aromatic ring backbone were found to be between 1500 and 1600 cm-1 on a spectral plot.

Intense absorption is seen at 1429 cm1, 2911 cm1, and 2999 cm1 in both the IR spectra of FA and FA@CurAu-PVP NPs. The peak at 1508 cm-1 is attributed to the free amine group of folate on FA@CurAu-PVP NCs, which causes a bending vibration of the NAH. The hydroxyl (OH) stretching bands, seen between 3000 and 3600 cm1, were thought to be displaced when FA was conjugated with CurAu-PVP NP. This was observed for FA, CurAu-PVP NP, and FA@CurAuPVP NPs.

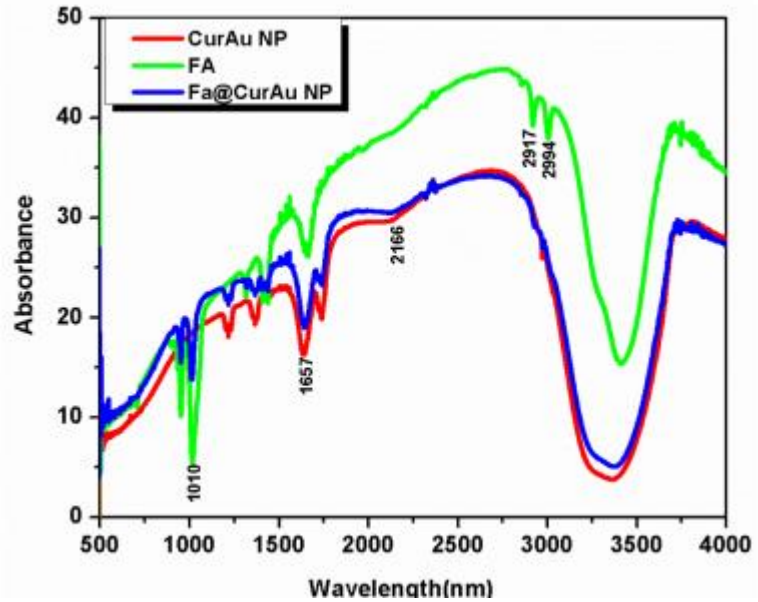

Figure 6: FTIR Spectra of variation on forming different layers of NP.

7. Conclusion

Firstly, the primary advantage of using NPs in biomedical application is their tunability due to which they can be formulated in desired shape, size and functions. Surface modification of NPs help to load them with various target specific moieties and also conjugate with various anti cancerous drugs to achieve target specific delivery of drugs and other therapeutic agents. Secondly, due to enhanced permeability retention effect caused in cancer cells i.e leaky vasculature and lymphatic drainage, NPs tend to accumulate in larger amount at the affected site rather than the normal tissue. This helps to achieve maximum accumulation of drugs and therapeutic agent at specific tumor site thus reducing the side effects otherwise caused. Finally, drug release efficiency defines release of drug at specific pH (mostly acidic, due to acidic interiors of the cancer cell), specific place and at specific time is prerequisite considering the complexity of tumor development. NPs modified to have unique properties can help to prevent drug aggregation and degradation before reaching the target site as well as tend to release drugs with specific stimuli such as pH, hypoxia or H2O2

We have well described and proved how the nanoparticles act as efficient curcumin drug delivery system after modification with folate as a target specific ligand. Such NPs and NPs formulates can influence aberrant structures and functioning of tumor microenvironment thereby reducing resistance caused by drugs and thus enhancing the therapeutic efficiency of other conventional cancer therapies.

As an end note, through our work we definitely conclude that the nano-formulation formulated in this study will surely revolutionized in vivo cancer therapy in near future.

References

[1] Dreaden, E.C. et al. (2012) Size matters: gold nanoparticles in targeted cancer drug delivery. Ther. Deliv. 3, pp 457–478.

[2] Ali, M.R.K. et al. (2019) Gold nanorod-assisted photothermal therapy decreases bleeding during breast cancer surgery in dogs and cats. Cancers 11, pp 851.

[3] Zhao, C.Y. et al. (2018) Nanotechnology for cancer therapy based on chemotherapy. Molecules 23, pp 8266.

[4] Stacey M. Louie, Justin M. Gorham, Jiaojie Tan and Vincent A. Hackley, "Ultraviolet photo-oxidation of polyvinylpyrrolidone (PVP) coatings on gold nanoparticles" ,Environ. Sci.: Nano, 2017, 4 , pp 1866-1875 .

[5] Kallum M. Koczkur, Stefanos Mourdikoudis, Lakshminarayana Polavarapu and Sara E. Skrabalak, "Polyvinylpyrrolidone (PVP) in nanoparticle synthesis", Dalton Trans., 2015,44, pp 17883-17905.

[6] Kandoth Kandy Jesna, Malaichamy Ilanchelian, "Photophysical changes of thionine dye with folic acid capped gold nanoparticles by spectroscopic approach and its in vitro cytotoxicity towards A-549 lung cancer cells", Journal of Molecular Liquids 242 (2017), pp 1042–1051.

[7] Sathish Sundar Dhilip Kumar, Ayyavu Mahesh, M. Gover Antoniraj, Hanumant Singh Rathore, N.N. Houreld, Ruckmani Kandasamy, "Cellular imaging and folate receptor targeting delivery of gumkondagogu capped gold nanoparticles in cancer cells", International Journal of Biological Macromolecules 109 (2018), pp 220–230.

[8] Sunil Pandey, Goldie Oza, Ashmi Mewada, Ritu Shah, Mukeshchand Thakur and Madhuri Sharon, "Folic acid mediated synaphic delivery of doxorubicin using biogenic gold nanoparticles anchored to biological linkers", J. Mater. Chem. B, 2013, 1, pp 1361.

[9] Hanjie Wang, Peiqi Zhao, Xiaofei Liang , Xiaqun Gong, Tao Song, Ruifang Niu, Jin Chang, "Folate-PEG coated cationic modified chitosan – Cholesterol liposomes for tumor-targeted drug delivery", Biomaterials 31 (2010), pp 4129– 4138.

[10] Puwang Li,Yichao Wang, Fanbo Zeng, Lijue Chen, Zheng Peng, Ling Xue Kong, "Synthesis and characterization of folate conjugated chitosan and cellular uptake of its nanoparticles in HT-29 cells", Volume 346, Issue 6, 1 May 2011, pp 801-806.

[11] Kyung Chul Cho, Sun Hwa Kim , Ji Hoon Jeong ,Tae Gwan Park, "Folate Receptor-Mediated Gene Delivery Using Folate-Poly(ethylene glycol)-Poly(L- lysine) Conjugate",Volume5, Issue6, June 24, 2005, Pages 512-519.

[12] Xiaosheng Ye, Hui Shi, Xiaoxiao He, Kemin Wang, Dinggeng He, Lv'an Yan, Fengzhou Xu, Yanli Lei, Jinlu Tang, and Yanru Yu, "Iodide-Responsive Cu−Au Nanoparticle-Based Colorimetric Platform for Ultrasensitive Detection of Target Cancer Cells", Anal. Chem. 2015, 87,pp 7141−7147

[13] Shenguang Ge,a Fang Liu,a Weiyan Liu,b Mei Yan,a Xianrang Songc and Jinghua Yu, "Colorimetric assay of K-562 cells based on folic acid-conjugated porous bimetallic Pd@Au nanoparticles for point-of-care testing", : Chem. Commun., 2014, 50,pp 475.