DOI: https://doi.org/10.53350/pjmhs22169408

ORIGINAL ARTICLE

Therapeutic and Diagnostic Analysis of Cadmium Sulfide Quantum Dots Conjugated with Cysteine, Anticancer Drug and Folate

MEHREEN FATIMA[1], ANADIL NOEL[2], ZEESHAN ABDULLAH[3], KAINAT BIBI[2], SHUMAILA YOUSAF[5], MUHAMMAD NOUMAN AZIZ[1], MUHAMMAD FARHAN ZAFAR CHAUDHRY[6], MADIHA SHAHZAD LODHI[2], FATIMA KHALID[7], AYESHA AMEEN[8], SANA FAROOQ[9], Qudsia Mushtaq[10]

[1]*Department of Life Sciences, University of Management and Technology, Lahore, Pakistan.*
[2]*University College of Medicine and Dentistry, University of Lahore, Pakistan*
[3]*Surgery Department, Shaikh Zayed Hospital, Lahore*
[5]*Nuclear Institute for agriculture and Biology, (NIAB), Faisalabad*
[6]*Department of Microbiology, Government College University, Faisalabad*
[7]*School of Biochemistry and Biotechnology, University of the Punjab*
[8]*Office of Research Innovation and Commercialization, University of Management and Technology, Lahore, Pakistan*
[9]*School of Science, Engineering and Environment, University of Salford*
[10]*Department of Zoology, University of Punjab, Lahore*
Corresponding author: Ayesha Ameen, Email: ayesha.ameen@umt.edu.pk, Cell: 03054095036

ABSTRACT

Background: Nanotechnology is evolving in the field of cancer drug design and cancer therapy. The ultrafine nanoparticles that have a size range of 1-10nm are called Quantum Dots. The ability to fluorescence has deemed quantum dots as suitable candidate for diagnostic imaging. Upon proper structural arrangement these quantum dots can be engineered to detect specifically cancer cells and deliver anticancer agents to the targeted site for cancer cell apoptosis.

Aim: Cadmium sulfide (CdS) nanoparticles conjugated with cysteine were prepared by wet method under high temperature and pressure

Methods: Folic acid receptor has shown overexpression in multiple types of cancers therefore the binding of folate to the composite ensures that only the cancer cells are targeted and normal cells remain unaffected. HeLa cells were opted to check the composite activity.

Results: Composite showed 23% cell death in the first 2 hours of delivery. FTIR analysis confirmed conjugation of CdS with cysteine and further conjugation of dacarbazine and folate to cysteine. The molecules of folic acid attached to cysteine could be measured to be 2.545×10^{18} molecules which were attached to 0.996×10^{22} molecules of cysteine that were attached 1.66×10^{20} molecules of CdS. Similarly, 2.3103×10^{19} of doxorubicin were attached to 0.996×10^{22} molecules of of cysteine attached to 1.66×10^{20} molecules of CdS. Furthermore, the cytotoxic nature of Cadmium Sulfide also gives an advantage two way cancer cell death.

Conclusion: Quantum dots attached to specific ligands that bind to cancer antigens, antibodies, proteins, Receptors etc. can be used for effective diagnosis of cancer. They can act as probe and detect thousands of molecules simultaneously by using only a small quantity of nanoComposite.

Keywords: Cadmium sulfide, Cancer, Quantum dots, Dacarbazine

INTRODUCTION

Drug delivery through targeted methods to cancer cells in a new and promising field of cancer therapeutics that involves use of nanocomposite in combination with the drug to be delivered. The nanoparticles have shown promise in the field of targeted drug delivery and also have reduced the side effects caused by unspecific delivery of the drug to healthy cells.

Fluorescent nanoparticles are of keen interest in cancer imaging and diagnosis (in vitro and in vivo).the florescent probes that are greater than 1nm size have the characteristics to be easily visualized in proper microscopic setup. One benefit of using these probes is that nanosized probes do not exceed the size of protein and therefore provide platform for high quality imaging at a molecular level. (Davis & Shin, 2008)

Quantum that are light activated give a choice to track the outcome of the treatment and ensure that the composite is bound to the target cells.(Iannazzo, et.al,2021) Thus the immunohistochemical analysis will provide proof that the composite indeed has the ability og bind specifically to cancerous cells. (Sudimack & Lee, 2000)

The multifunctional nanomedicine is normally opted for the enhanced cancer treatment and it provides an integrated platform for various pathways of treatment. The multifunctional nanocomposite has the advantage of being size specific and targeting the cancer from various pathways. (Davis & Shin, 2008)

The increase in cancer fatalities is due the the hetrogenous behavior of the cancer and the difference of phenotypic expression of cancer in different organs.this causes problems in cancer therapeutics. Furthermore, if the cancer has metastasized and formed metastatic colonies, this leads to further complications in the cancer treatment.(Sudimack & Lee, 2000) Paramagnetic and superparamagnetic agents e.g. iron oxide nanoparticles are currently used in cancer diagnosis by medical imaging. (Jun, Lee, & Cheon, 2008)

Nanocomposite for cancer therapeutics provides a new hope in the field of cancer research and treatment. But the health issues should also be considered.(Lu & Low, 2012)

Theranostic nanomedicine i.e. diagnostic and therapeutic functions are integrated into the same platform, have an advantage over conventional method as they provide simultaneous cancer treatment and diagnosis of the cancerous tissue or organ. These Nanocomposites have molecules for cancer diagnosis and treatment integrated into the same composite system .(Duncan & Spreafico, 1994)

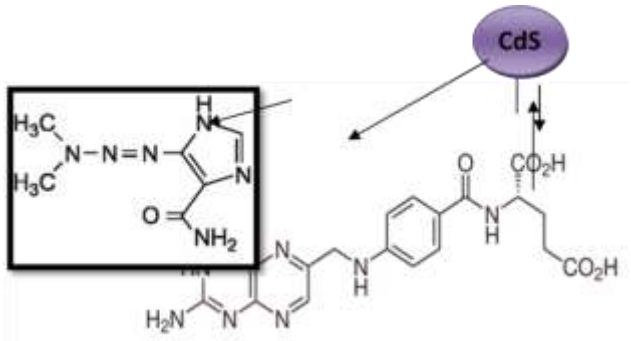

Figure 1: Proposed model of nano Composite.

CYS COOH NH **Dacarbazine**

In this study, the nanoComposite of anticancer drug i.e. Dacarbazine was prepared conjugation to cadmium sulfide quantum dots coated with cysteine. The Cysteine gave the extra C and N terminal to which the anticancer drug and folic acid was conjugated. Attachment of folic acid was done to target folic acid receptors on cancer cells. HeLa cells were used to carry out a comparative study of composite activity with B-cells. The nanocomposite has shown enhanced activity against HeLa cells without harming normal cells.(Murali, et.al, 2022)

METHODOLOGY

Immobilization of cysteine on CdS quantum dots: Modified wet method was used for preparation of cadmium sulfide quantum dots coated with cysteine at high heat and pressure with constant stirring spectophotometric analysis of the particles was carried out to check the absorption pattern of the quantum dots. The final Composite was termed Cd-Cys quantum dot.

Conjugation Dacarbazine to Quantum dots: Conjugation of Dacarbazine was carried out by using carbodiimide method. (M.S.Lodhi &Z.Q Samra, 2019). Particles were stored in PBS at 4 C°.

Conjugation of folic acid with quantum dots: Conjugation of folic acid with the quantum dots was carried out by Gluteraldehyde method (M.S.Lodhi &Z.Q Samra, 2019) and the sample was stored in PBS for further processing.

Development of anti Dacarbazine antibodies: Ovalbumin and BSA conjugated Dacarbazine was prepared by two step gluteraldehyde method. Anti Dacarbazine antibodies were prepared by subcutaneously immunizing the mice witnh BSA activated formulation of the anticancer drug on a weekly basis and then applying an biooster injection of the drug, Cardiac puncture technique was used to extract the blood from the mice from which srum was prepared and further analysed. The antibody presence was detected using immuno dot blot technique and ELISA. (Samra et al., 2012).

Mice were euthanized by chloroform and cardiac puncture was performed.

Characterization of Composite attachment on HeLa cells: Hela cell line was obtained from School of biological sciences, university of the Punjab, Lahore, Pakistan.

The activity of the nanocomposite was frther analysed by MTT assay and Trypan blue test. Cell apoptosis assay was also performed using a control sample, a drug conjugated CdS sample and an only CdS without drug sample as comparative parameters to compare the activity of the Composite in controlled environment.

Cell immunohistochemistry analysis was also performed to confirm presence of drug onto the Composite using Taxus red labeled secondary antibody.

MTT assay: MTT assay was carried out using standard protocol. Absorbance of samples was recorded at 570nm.

Trypan Blue Test: Cell density was determined using hemocytometer. Trypan blue assay standard protocol was used. Cell viability was found using formula.

Total no. of live cells in all squares / total no. of live cells X total no of dead cells X 100= cell viability.

Cell apoptosis assay: Cells were seeded in 24 well plates and incubated overnight. Composite, drug, CdS were added into different wells (100ul) and incubated for 30 min, 1 hour, 1h 30min and 2 hours and control was processed the same way. All the cells were stained by 0.4%trypan blue and cell viability of each sample was checked by the following formula.

(Total no. of live cells in all squares / total no. of live cells X total no of dead cells) X 100= cell viability.

Cell immunohistochemistry: Overnight seeding of the cells was carried out in a microwell plate. The seeded cells were then suspended in the cell culture media with the help of micropipette. 10ul of cells were added onto albumin coated slide and 3 slides were made. 100ul of 1% gluteraldehyde was added onto the slides and slides were incubated in humidified chamber for 5 minutes (cell fixation). Fixed cells were washed with PBS. CdS final Composite (100ul) was added in all the slides and incubated for 1 hour at room temperature. Samples were washed with PBS and visualized under fluorescent microscope. Skim milk TBS for blocking was added in the 2nd and 3rd slide and incubation was carried out for 1 hour. Then primary antibody (mouse anti drug antibody) 100ul was added and incubated for one hour. Slides were washed with PBS. Then Taxus red conjugated secondary antibody onto both slides and incubated for 1 hour. Slides were washed and observed in fluorescent microscope.

Immunohistochemistry: Immunohistochemistry was performed on formalin fixed mouse epithelial tumor sample. Fixed tissue sample was visualized under microscope without any dye, with taxus red and with simple DAP staining to compare binding of Composite with normal tissue sample.

Fixation and Embedding: Formaldehyde fixed tumor tissue samples (tumor of treated mice, tumor of untreated mice, liver and kidney of both normal and tumor mice) were cut into cubical pieces of 4mm width. These tissue samples were then separately added in 5% sucrose in Tris buffer saline (TBS) and kept in shaking incubator 6 hours at room temperature. After 6 hours sucrose solution was discarded and same procedure was carried out with increasing quantity of sucrose ie. with 10%, 20% and 30% sucrose solution in TBS. After dehydration of tumor samples the tissue sections were embedded in optimum cutting temperature compound (OCT) in the molds prepared from aluminum foil and molds were kept at -20.

Section cutting: Sections of frozen tissues were cut by using cryostat at -10℃ using OCT compound. Sections were transferred on albumin coated slides and stored at -20℃ in slide in rack for further analysis.

Immunohistochemistry: Slides having tissue samples were processed for immunohistochemistry. Firstly blocking was carried out by adding a drop of 3% BSA on each tissue section and incubates for 1 hour. Then washing was carried out by TBS and slides were incubated with CdS nanoComposite and incubated for 1 hour at room temperature, the slides were visualized under fluorescent microscope.

RESULTS

Spectrophotometric analysis of CdS particles conjugated with cysteine: Spectrophotomometric analysis of cadmium sulfide quantum dots coated with cysteine was carried out at different wavelengths to analyse the wavelengthy at which the sample will exhibit maximum absorption.

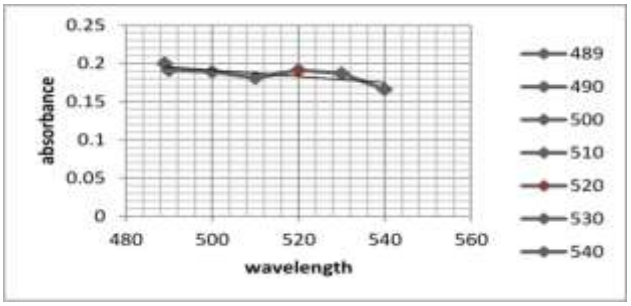

Figure 1.1 Graphical analysis of quantum dot spectrophotometric test

Microscopic analysis of CdS:

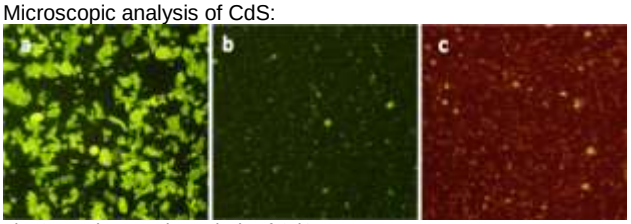

Fig: 2.1 : Microscopic analysis of CdS:

a. CdS crystals in white light
b. CdS nanoparticles in white light
c. CdS nanoparticles in infrared light

Cadmium sulfide nanoparticles were alalysed at 520nm and green fluorescence was observed to be emitted by the nanoparticles. Cadmium sulfide particles were further observed in infrared light. Orange red fluorescence was emitted by the particles in infrared light.

3. Stoichiometry analysis:

0.04g of CdS contain 1.66×10^{20} molecules.

Attachment of cysteine:

Cysteine attaches to Cd ions at a ratio of 1:60

Therefore no. of molecules of cysteine attached to CdS=1.66×10^{20} x 60=$0.996x10^{22}$

Binding of Folic Acid (100mg) with CdS

2.545×10^{18} molecules of folic acid were attached to 0.996×10^{22} molecules of of cysteine attached to 1.66×10^{20} molecules of CdS.

Binding of Dacarbazine (200mg) with CdS

2.3103×10^{19} of dacarbazine were attached to 0.996×10^{22} molecules of of cysteine attached to 1.66×10^{20} molecules of CdS.

FTIR Analysis:

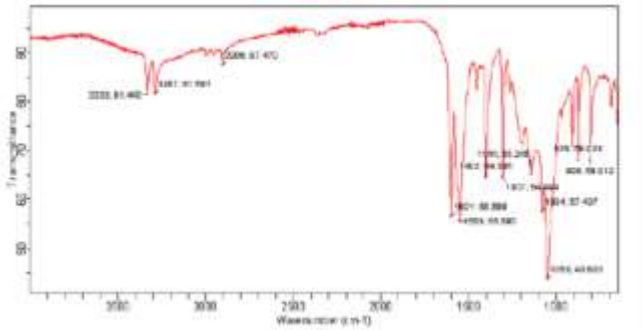

Figure 3.4.1: FTIR analysis of CdS + Cysteine+Dacarbazine+Folic acid

Figure 3.4.4 Binding of folic acid and dacarbazine to the Composite is shown in the form of peaks in both the specific regions of the FTIR analysis graph thus showing positive formation of the Composite for further analysis.

Synthesis of anti dacarbazine antibody in mice: Immunodot blot of the mose seum indicated that the antibody present in mouse serum is mouse anti dacarbazine antibody.

ELISA to detect anti dacarbazine antibodies: Positive result of ELISA using enzyme linked antibodies indicated presence of mouse anti Dacrbazine antibody in mouse serum.

MTT Assay: Color change was observed after addition of MTT reagent and detergent reagent in test and control sample. Intensity of color depicts amount of live cells present in culture.

Absorbance of control at 570nm = A570= 1.694

Absorbance of test at 570nm= A570=1.649

Decrease in absorbance indicates decrease in NADP synthesis which means that cell proliferation is decreasing on addition of nanoComposite and cell death is being carried out by the composit whereas cell proliferation is normal in control sample.

Trypan blue staining:

Control sample

Number of cells per square in control=9+9+7+13/ 4=9 cells

Cells per ml in control= 90000

Number of dead cells in control= 1+0+2+1=1 cell per square

Number of live cells in control= 8+9+5+12= 8cells

Percentage viability= (8/9) x100=88.8%

Test sample

Number of cells per square= 10+8+7+12/4= 9.25 apx 9cells per square

Number of live cells =3+4+5+3/4=3.75 apx 4 cells

Number of dead cells = 9-4=5 cells

Percentage viability in test sample =(4/9)x100= 44.4%

Cell apoptosis assay:

Table 2.1

Sample	% viability 30 min	% Viability 1 hour	% viability 1h 30min	% viability 2 hour
Drug	86.5%	84.8%	82.9%	82.4%
CdS+Folic acid	87.3%	86.3%	85%	82.6%
CdS+ Folic acid +drug	85.6%	83.7%	81.8%	77.9%

According to cell apoptosis assay the Composite is more potent in causing cancer cell death than CdS or Drug acting alone on the cancer cells. Composite shows 23% cell death within 2 hours of incubation.

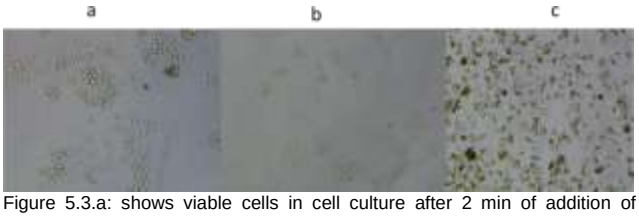

Figure 5.3.a: shows viable cells in cell culture after 2 min of addition of Composite into the cell culture

Figure 5.3.b: shows increase in cell death after 2 hours of incubation of cells with Composite.

Figure 5.3.c: shows complete cell death after 24 hours of incubation of HeLa cells with Composite.

Figure 5.3: cell apoptosis assay

a. HeLa cells with Composite after 1 min
b. HeLa cells with CdS after 2 hours
c. HeLa cells with CdS after 24 hours

Cell immunohistochemistry:

Figure 5.4: Cell immunohistochemistry:

a. CdS Final Composite With Hela Cells
b. Fixed Hela Cells With CdS
c. Hela Cells + CdS Composite+ Taxus Red Labeled Anti Dacarbazine Antibody

Figure 5.4 a: shows attachment of Composite to HeLa cells showing positive binding of folate present in Composite to folate receptor present on cell surface.

Figure 5.4.b: shows gluteraldehyde fixed HeLa cells bound to nanoComposite showing positive bindng of folate to folate receptor present on cell surface.

Figure 5.4 c: shows that Samples showed positive result for Composite binding and appearance on taxus red indicated the presecnce of drug in the Composite

DISCUSSION

Remarkable advances are being made in the field of nanotechnology every day. Bread fields of life and industry are now utilizing nanotecnological advancements to obtain better and specific results and products. Construction of nanomaterials tat are precise and accurate requires years of research and data comparisons to get good quality product. It may be possible that in future nanorobots may be injected into human bloodstream that can locate and cure any problem in human body without the problem of disease aggrevation. These theoretical robots may seem futuristic; however, currently researchers are working on individual nanoparticles which have the potential to alter the way cancer is detected and treated.(Freitas, 1999)

The nanoparticles can be designed to diagnose and trewat the cancer cells silmultaneously. This is done so by encapsulation or coating of the anticancer frug and detection molecule onto the

nanoparticels is such a way that the composite binds specifically to the cancer cells and does not impact on the normal healthy cells of the patient. Once the particles are in the tumor, they slowly release medication, destroying the cancer over a period of hours or days. (Farokhzad & Langer, 2006)

One of the advantages of the use of nanoparticels is that these utilize a comparatively low dose of the chemotherapeutic medicine as compared to conventional chemotherapeutic methods. This results in specific rug delivery and rapid treatment of cancerous cells which is normally prolonged in normal chemotherapy treatment. Further the side effects of chemotherapy treatment can be minimized by this approach.(Frieler, et.al, 2021)

Nanoparticles become entrapped in the tumor, evading the healthy tissue, due to improper formation of blood vessels in tumor. Another approach for drug delivery utilizes encapsulation; these capsules slowly release drugs over a specific time period. Ligand-targeted nanoparticles can also be used for cancer treatment, which carry medication on the outside of the particles, then bind with cancerous cells, and finally release the drugs once inside the cancer. (Haolin Chen, et.al, 2021)

Nanoparticles have the ability to make medical images brighter thus the treatment can be directed according to the imaging of the nanoparticles and their effect on the tumor. This type of screening can be done for days and can reduce the cost of the treatment and the treatment can be directed according to the imaging results of the previous nano dose. (Jain & Stylianopoulos, 2010)

Mulder et al.,(2006) said that one of the negative aspects of the use of quantum dots is that they have the ability to form clumps and aggregate at a specific site. This can be avoided by the processs of encapsulation of the quantum dots in a surfactant polymer. The charge on the polymer will cause the particles to repel each other and therefore no clumping will be observed.

Quantum dots have a great deal of scope in cancer diagnosis as well dur to their fluorescent nature. These particles can specifically bind to the cancer cells by use of a cancer specific ligand and the fluorescence of the particles in the biopsy sample will indicate the presence of cancer cells. This method can also be used to check through imaging if the treatment is effecting the tumor or not. This makes them an excellent candidate for cancer biomarker synthesis.

quantum dots are mostly being studied for toxic analysis and imaging purposes not by conjugation of specific ligands there is reported delivery of drugs into brain tumors by quantum dots that can cross blood brain barrier. Cadmium telenium quantum dots are also conjugated with antibodies for antibody mediated cellular cytotoxicity in cancer cells. Serrone, et al., (2000)

CONCLUSION

Quantum dots provide a promising platform for both diagnosis and treatment of cancer. By specifically binding to cancer cells and giving green fluorescence in normal white light, cadmium sulfide quantum dots conjugated with cysteine, folic acid and dacarbazine can be used for treatment of various cancer types including cervical cancer, malignant melanoma, non Hodgkins's lymphoma, sarcoma and islet cell carcinoma of pancreas. This method of treatment is highly economical as it reduces the cost of treatment involving various combinations of chemotherapeutic drugs and radiotherapy and has less side effects as compared to conventional cancer therapeutics methods**.**

REFRENCES

1. Avril, M., Aamdal, S., Grob, J., Hauschild, A., Mohr, P., Bonerandi, J., Gilde, K. (2004). Fotemustine compared with dacarbazine in patients with disseminated malignant melanoma: a phase III study. *Journal of Clinical Oncology, 22*(6), 1118-1125.
2. Bailey, R. E., & Nie, S. (2003). Alloyed semiconductor quantum dots: tuning the optical properties without changing the particle size. *Journal of the American Chemical Society, 125*(23), 7100-7106.
3. Bartholomä, M., Valliant, J., Maresca, K. P., Babich, J., & Zubieta, J. (2009). Single amino acid cHeLates (SAAC): a strategy for the design of technetium and rhenium radiopharmaceuticals. *Chemical Communications*(5), 493-512.
4. Ballou, B., Lagerholm, B. C., Ernst, L. A., Bruchez, M. P., & Waggoner, A. S. (2004). Noninvasive imaging of quantum dots in mice. *Bioconjugate chemistry, 15*(1), 79-86.
5. Bowen, M. L., & Orvig, C. (2008). 99m-Technetium carbohydrate conjugates as potential agents in molecular imaging. *Chemical Communications*(41), 5077-5091.
6. Cai, W., Shin, D.-W., Chen, K., Gheysens, O., Cao, Q., Wang, S. X., . . . Chen, X. (2006). Peptide-labeled near-infrared quantum dots for imaging tumor vasculature in living subjects. *Nano letters, 6*(4), 669-676.
7. Crespo, L., Sanclimens, G., Pons, M., Giralt, E., Royo, M., & Albericio, F. (2005). Peptide and amide bond-containing dendrimers. *Chemical reviews, 105*(5), 1663-1682.
8. Davis, M. E., & Shin, D. M. (2008). Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nature reviews Drug discovery, 7*(9), 771-782.
9. Dubertret, B., Skourides, P., Norris, D. J., Noireaux, V., Brivanlou, A. H., & Libchaber, A. (2002). In vivo imaging of quantum dots encapsulated in phospholipid micelles. *science, 298*(5599), 1759-1762.
10. Duncan, R., & Spreafico, F. (1994). Polymer conjugates. Pharmacokinetic considerations for design and development. *Clinical pharmacokinetics, 27*(4), 290-306.
11. Farokhzad, O. C., & Langer, R. (2006). Nanomedicine: developing smarter therapeutic and diagnostic modalities. *Advanced drug delivery reviews, 58*(14), 1456-1459.
12. Freitas, R. A. (1999). *Nanomedicine, volume I: basic capabilities*: Landes Bioscience Georgetown, TX.
13. Frieler M, Pho C, Lee BH, Dobrovolny H, Akkaraju GR, Naumov AV. Effects of Doxorubicin Delivery by Nitrogen-Doped Graphene Quantum Dots on Cancer Cell Growth: Experimental Study and Mathematical Modeling. *Nanomaterials*. 2021; 11(1):140. https://doi.org/10.3390/nano11010140
14. Gao, X., Cui, Y., Levenson, R. M., Chung, L. W., & Nie, S. (2004). In vivo cancer targeting and imaging with semiconductor quantum dots. *Nature biotechnology, 22*(8), 969-976.
15. Greenwald, R. B., Choe, Y. H., McGuire, J., & Conover, C. D. (2003). Effective drug delivery by PEGylated drug conjugates. *Advanced drug delivery reviews, 55*(2), 217-250.
16. Haolin Chen, Zhiming Liu, Bo Wei, Jun Huang, Xinru You, Jingyang Zhang, Zhiling Yuan, Zhilie Tang, Zhouyi Guo, Jun Wu, Redox responsive nanoparticle encapsulating black phosphorus quantum dots for cancer theranostics, Bioactive Materials, Volume 6, Issue 3, 2021, Pages 655-665, ISSN 2452-199X, https://doi.org/10.1016/j.bioactmat.2020.08.034.
17. Hilgenbrink, A. R., & Low, P. S. (2005). Folate receptor-mediated drug targeting: From therapeutics to diagnostics. *Journal of pharmaceutical sciences, 94*(10), 2135-2146.
18. Hoshino, A., Fujioka, K., Oku, T., Suga, M., Sasaki, Y. F., Ohta, T., Yamamoto, K. (2004). Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. *Nano letters, 4*(11), 2163-2169.
19. Iannazzo D, Espro C, Celesti C, Ferlazzo A, Neri G. Smart Biosensors for Cancer Diagnosis Based on Graphene Quantum Dots. *Cancers*. 2021; 13(13):3194. https://doi.org/10.3390/cancers13133194
20. Jain, R. K., & Stylianopoulos, T. (2010). Delivering nanomedicine to solid tumors. *Nature reviews clinical oncology, 7*(11), 653-664.
21. Jun, Y. w., Lee, J. H., & Cheon, J. (2008). Chemical Design of Nanoparticle Probes for High-Performance Magnetic Resonance Imaging. *Angewandte Chemie International Edition, 47*(28), 5122-5135.
22. Kim, S., Lim, Y. T., Soltesz, E. G., De Grand, A. M., Lee, J., Nakayama, A., Dor, D. M. (2004). Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nature biotechnology, 22*(1), 93-97.
23. Lee, R. J., & Low, P. S. (1994). Delivery of liposomes into cultured KB cells via folate receptor-mediated endocytosis. *Journal of Biological Chemistry, 269*(5), 3198-3204.
24. Lu, Y., & Low, P. S. (2012). Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Advanced drug delivery reviews, 64*, 342-352.
25. Madeeha Shahzad Lodhi & Zahoor Qadir Samra (2019) Purification of transferrin by magnetic nanoparticles and conjugation with cysteine capped gold nanoparticles for targeting diagnostic probes, Preparative Biochemistry & Biotechnology, 49:10, 961-973, DOI: 10.1080/10826068.2019.1643736

26. Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., . . . Weiss, S. (2005). Quantum dots for live cells, in vivo imaging, and diagnostics. *science, 307*(5709), 538-544.
27. Mulder, W. J., Koole, R., Brandwijk, R. J., Storm, G., Chin, P. T., Strijkers, G. J., . . . Griffioen, A. W. (2006). Quantum dots with a paramagnetic coating as a bimodal molecular imaging probe. *Nano letters, 6*(1), 1-6.
28. Murali G., Binhee Kwon, Hansol Kang, Jeevan Kumar Reddy Modigunta, Seongmin Park, Seongeun Lee, Hwiyoung Lee, Young Ho Park, Juhea Kim, Sung Young Park, Young-Jin Kim, and Insik In *ACS Applied Nano Materials* **2022** *5* (3), 4376-4385 DOI: 10.1021/acsanm.2c00443
29. Nandy, P., Periclou, A., & Avramis, V. (1997). The synergism of 6-mercaptopurine plus cytosine arabinoside followed by PEG-asparaginase in human leukemia cell lines (CCRF/CEM/0 and (CCRF/CEM/ara-C/7A) is due to increased cellular apoptosis. *Anticancer research, 18*(2A), 727-737.
30. Rhyner, M. N., Smith, A. M., Gao, X., Mao, H., Yang, L., & Nie, S. (2006). Quantum dots and multifunctional nanoparticles: new contrast agents for tumor imaging.
31. Robert, C., Thomas, L., Bondarenko, I., O'Day, S., Weber, J., Garbe, C., . . . Grob, J.-J. (2011). Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *New England Journal of Medicine, 364*(26), 2517-2526.
32. Schoemaker, N., Van Kesteren, C., Rosing, H., Jansen, S., Swart, M., Lieverst, J., . . . Spinelli, R. (2002). A phase I and pharmacokinetic study of MAG-CPT, a water-soluble polymer conjugate of camptothecin. *British journal of cancer, 87*(6), 608-614.
33. Serrone, L., Zeuli, M., Sega, F., & Cognetti, F. (2000). Dacarbazine-based chemotherapy for metastatic melanoma: thirty-year experience overview. *Journal of experimental & clinical cancer research: CR, 19*(1), 21-34.
34. Smith, A. M., Duan, H., Mohs, A. M., & Nie, S. (2008). Bioconjugated quantum dots for in vivo molecular and cellular imaging. *Advanced drug delivery reviews, 60*(11), 1226-1240.
35. Šprincl, L., Exner, J., Štěrba, O., & Kopeček, J. (1976). New types of synthetic infusion solutions. III. Elimination and retention of poly-[N-(2-hydroxypropyl) methacrylamide] in a test organism. *Journal of biomedical materials research, 10*(6), 953-963.
36. Sudimack, J., & Lee, R. J. (2000). Targeted drug delivery via the folate receptor. *Advanced drug delivery reviews, 41*(2), 147-162.
37. Weissleder, R., & Pittet, M. J. (2008). Imaging in the era of molecular oncology. *Nature, 452*(7187), 580-589.
38. Woggon, U. (1997). *Optical properties of semiconductor quantum dots*: Springer.
39. Yoo, H. S., & Park, T. G. (2004). Folate-receptor-targeted delivery of doxorubicin nano-aggregates stabilized by doxorubicin–PEG–folate conjugate. *Journal of Controlled Release, 100*(2), 247-256.
40. Zahoor Qadir Samra, Nadia Dar & Muhammad Amin Athar (2012) Biocatalytic Activity Of Recombinant Human B-Mannosidase Immobilized Onto Magnetic Nanoparticles For Bioprocess, Preparative Biochemistry & Biotechnology, 42:1, 97-112, Doi: 10.1080/10826068.2011.575910