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Analysis of folate and curcumin-conjugated cadmium sulfide cystein quantum dots for targeted cancer therapy

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Abstract: The aim of this study was to develop the ranostic nanocomposite by conjugating quantum dots with functional, therapeutic and targeting moieties. The quantum dots nanoparticles were used to diagnose and deliver anti-tumor drugs in a controlled manner to cancerous cells by fusing with tumor cell surfaces. To enhance the attachment of the nano-composite to specific tumor cells without harming neighboring normal cells, folic acid was conjugated with the nano-composite as folate receptors are over expressed in different kinds of tumors. The study was conducted for one year at the University of Punjab. The quantum dots were synthesized by a hydrothermal process using cadmium acetate and sodium sulfide. The response was evaluated on breast tumor samples for binding and nano-composite delivery under a fluorescent microscope. Fourier-transform infrared analysis was performed to confirm CdS conjugation with cysteine, folic acid and curcumin. The results showed that the quantum dot conjugate provides a two-way attack on cancer cells and causes increased cellular apoptosis. Further testing on murine animal models is required to confirm the results of this research study.

Keywords: Quantum dots, Fourier-transform infrared, theranostic nanocomposite, cancer therapy.

INTRODUCTION

The field of science has developed immensely over the past years and has brought several things to light that were once beyond imagination. One of the areas that have benefited from scientific advancements is the medical sector. Innovations in ventilators, medicines and new medical diagnostic techniques have improved patient outcomes. Additionally, a new field known as nanotechnology has emerged in recent years. Nanotechnology allows us to use nano-sized particles for different purposes such as in biomaterials, biosensors and in preparing multifunctional thermostatic, nano medicines. Nano particles range from 1nm to 100nm, which is different from the normal size particles. Nano particles have energy-filtered magnetic and electrical properties and distinctive ocular properties. Specific nanoparticles should have abilities like the conduction of heat and electricity, flipping of orientation in the presence of temperature, which shows their super paramagnetic power, large surface area in comparison to their volume, fluorescence material and optical absorption spectra (Anand *et al*., 2008). In the field of medicine, nanoparticles are essential for the controlled distribution of drugs, which means they should be highly permeable to biological barriers. Today, nanoparticles are of great interest for the precise targeted delivery of cancer drugs. The large surface area of nanoparticles and their physicochemical properties allow them to combine with drugs. This targeted drug delivery only attacks tumor cells and not healthy ones, reducing the risk of healthy cell damage. In photodynamic therapy, tumor cells are destroyed by either apoptosis or necrosis by the action of a photosensitizer drug. This photosensitizer drug produces dioxygen, dioxide and immunogenic response after activating with a laser of a specific wavelength. A reactive oxygen species produced by the drug targets the cancerous cell. In photo dermal therapy, a targeted cancer spot is exposed to near-infrared light that is turned into high temperature, which kills tumor cells. Due to their luminescent qualities, certain nanoparticles have been employed in photodynamic therapy and photosensitizer treatments (Arvizo *et al*., 2010).

The super-paramagnetic charter of nanoparticles such as iron oxide is super-paramagnetic and can bypass the immune response. It can be used in the treatment of hyperthermia cancer. Targeted drug delivery is the delivery of the drug only to its target, which reduces the side effects of drugs. For example, cancer drugs have significant side effects that can affect healthy cells as well, so delivering these drugs through targeted drug delivery will help reduce side effects as they will only attack tumor cells. We can achieve it by using nanoparticles (Bartlomiejczyk *et al*., 2013). Targeted drug delivery is of two types: active targeting and passive targeting. In active targeting, nanoparticles are combined

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with aptamers, tiny molecules, peptides and antibodies. In passive targeting, the effect of retention and increased permeability is used. Other than saving healthy cells from the toxic effects, these targeted therapies also allow for controlled drug release, degradation prevention of the drug, increased half-life, ability of binding and efficient solubility. Quantum dots are nano particles ranging from 1 nm to 10 nm, which lie in group 2 to 4 elements such as cadmium disulfides, ZnSe, CdTe and CdSe. QDs are semiconductor and inorganic. UV or NIR light can excite them. Up to 2 nm quantum dots they produce light of 495 to 515 nm on excitation while 5 nm and larger quantum dots emit 605 to 630 nm wavelength light. Quantum Dots have other characteristics due to which they can be used in biomedical engineering and imaging. These characteristics are high coefficient of absorption, high photo stability against pH change and bleaching and special electrical and optical traits (Bartneck *et al*., 2013).

MATERIALS AND METHODS

The cancer cells are either isolated from tumor tissue specimens or obtained from reputable cell repositories. These cancer cells are then cultured in appropriate growth media that provide the necessary nutrients, pH and temperature conditions for their survival and proliferation. Techniques such as enzymatic digestion, mechanical dissociation, or tissue explant culture may be used to obtain viable cancer cells. Subsequently, the cancer cell line is characterized to confirm its identity, purity and relevant features. This may involve using techniques such as DNA fingerprinting, PCR-based assays, immunohistochemistry, or flow cytometry to verify the origin and characteristics of the cancer cells. It is important to validate the cancer cell line to ensure that it accurately represents the intended cancer type and is free from contamination or misidentification. Cells obtained from the tissue specimen are cultured in various forms, such as monolayer or three-dimensional cultures including multi cellular spheroids, scaffold-based cultures (such as organoids), or matrix-embedded cultures. It is essential to characterize each primary cell line to determine its source, purity and important characteristics. Characterization should involve the use of specific markers, both extra cellular and intracellular, through various assays. The prepared cell lines were taken for the study from University of Punjab.

Cadmium sulfide quantum dots preparation

The synthesis of cadmium sulfide quantum dots and their conjugation with cysteine was achieved using a hydrothermal process. Cadmium acetate was used as a precursor and sodium sulfide was added with constant stirring in basic pH of 10.5 at high heat (121^{o}C) and pressure to liberate cadmium sulfide quantum dots. The resulting mixture was subjected to centrifugation at 10,000 rpm and 24^{o}C for 20 minutes and the pellet was washed with distilled water and suspended in 550ml of distilled water. Next, 12 ml of 0.01M cysteine was added at 100 degree Celsius and high pressure with constant stirring, followed by incubation for 5.4 hours at 100^{o}C. The superfine particles present in the supernatant were sedimented using the freeze-thaw method and dried overnight in an incubator at 46^{o}C. The characterization of the particles was done using spectrophotometric absorption analysis. To confirm the binding of cysteine to the quantum dots, the Ninhydrin test was carried out. This involved adding 0.6ml of 1% Ninhydrin in 10ml of acetone solution to a few quantum dot particles in a test tube and adding the same solution without particles to a separate test tube as a control (Bataller *et al*., 2003).

Binding of cysteine coated quantum dots with tumor

The glutaraldehyde method was employed in this study. A coupling buffer was prepared by combining 10 milligrams of cysteine-coated cadmium sulfide with 24 milliliters of 0.01M pyridine. Subsequently, 5.5% glutaraldehyde was added to the reaction mixture, which was then shaken for 3 hours at room temperature on a shaking incubator set at 100 rpm. After 3.5 hours, 11 milligrams of folic acid was added and the reaction mixture was further incubated on the shaking incubator at 100 rpm for 25 hours at room temperature. Following the 25-hour incubation period, 3 milliliters of the pre-coupling solution was separated into another Falcon tube and the pellet was washed with 2 milliliters of Tris washing buffer containing 0.01M Tris and 0.14M NaCl. To assess the binding of folic acid-cysteine-CdS on breast tumor samples, the synthesized cadmium sulfide particles coated with cysteine and conjugated with folic acid were collected as a pellet in an Eppendorf tube. Fluorescent microscopy was employed using normal white light without any spectrum for analysis. Slides for immunohistochemistry were prepared by embedding the breast tumor sample in a compound, sectioning the tumor sample and placing it on a slide. The particles were then added to the slide and incubated for 10 minutes. After incubation, the slide was washed with normal saline and fixed with formalin before being visualized under a fluorescent microscope (Bednarski *et al*., 2015).

RESULTS

The CdS quantum dots were dried after synthesis, overnight at 46°C in an incubator. The dried CdS quantum dots were obtained as a result. Spectrophotometric absorption analysis was performed on the cysteine-coated CdS particles at various wavelengths to determine their absorption spectra and identify the wavelengths at which they illuminate (Carnovale *et al*., 2016).

The binding of CdS with Cysteine determined by the Ninhydrin test is as follows: No color is produced by the

Ninhydrin reagent in the control sample due to the absence of cysteine amino acid. Conversely, in another test tube containing CdS particles coated with cysteine and Ninhydrin reagent, the presence of cysteine is indicated by a visible color change. A color shift confirms the successful coating of cysteine on CdS. One sample serves as the control, while the other is CdS coated with cysteine. To analyze cadmium sulfide, quantum dots were examined under white light, and they produced green fluorescence. Microscopy was used to study CdS at a wavelength of 510 nm.

Table 1: Comparison of spectrophotometer absorption of CdS particles

Absorption	
450	0.213
460	0.192
470	0.188
480	0.187
490	0.183
500	0.181
510	0.193
520	0.180
530	0.162

The binding of particles was evaluated using a fluorescent microscope and both the breast tumor sample and normal sample were examined. The positive results obtained from the fixed tumor sample on the slide demonstrate the successful binding of the composite, which produces fluorescence under normal white light when observed using a fluorescent microscope. These positive results indicate the binding of folic acid, which is present in CdS, with the folate receptor located on the surface of the tumor cells (Galon *et al*., 2012).

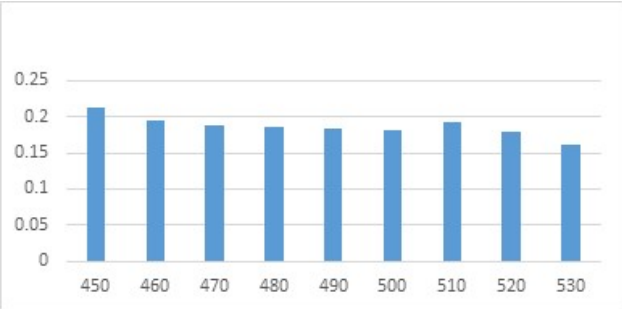

Fig. 1: Comparison of absorption spectrum of CDs particles (X-axis shows wavelenghth and –axis shows the absorption reading)

The pellet containing CdS coated with cysteine was subjected to FTIR analysis. During the FTIR analysis, three distinct peaks were observed on the graph: peak 3500 indicated the presence of the visible carbon-hydrogen bond of cysteine, peak 1400 indicated the presence of the visible C=O bond and peak 1000 cm-1 indicated the presence of the visible carbon-carbon bond of cysteine. These findings demonstrate the successful coating of CdS on cysteine.

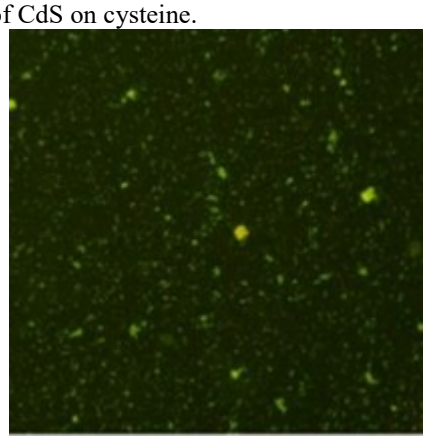

Fig. 2: Cds analysis under microscope

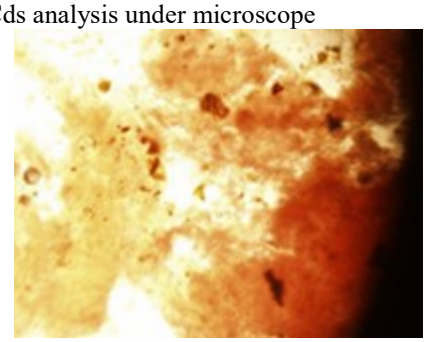

Fig. 3: Binding of nano-composit particles with Tumor cells

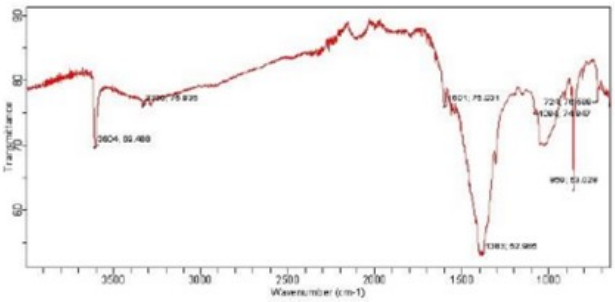

Fig. 4: Graph showing FTIR analysis of CdS coated with Cysteine

DISCUSSION

Nanotechnology is becoming increasingly important in the treatment of chronic human diseases such as cancer, diabetes, infectious diseases, and blood and brain-related illnesses. Nano-based targeted drug delivery is emerging as a superior therapy for cancer treatment, delivering drugs specifically to cancerous cells without harming neighboring cells, which reduces toxic effects (Zhang *et al*., 2019). In a study, polymeric nanoparticles were conjugated with asparaginase and paclitaxel micelles of polymeric particles to treat cancer (Ali *et al*., 2021). In another study, green cadmium sulfide quantum dots made from leaf tea extract from Camellia sinensis showed

potential therapeutic effects against antibacterial activity and demonstrated a toxic response in A549 cancer cells. When compared to conventional therapy, conjugating the synthesized green quantum dots with a standard drug cisplatin enhanced the efficacy of drug delivery and action. Their Quantum Dots produced high-contrast fluorescence images proofing the binding of nano-composite with tumor cells, thus demonstrating both diagnostic and therapeutic abilities against cancer cells (Wang *et al*., 2020).

The researchers synthesized green carbon dots having luminescence property using active dry yeast and further conjugated them with folic acid. As folic acid can bind to folate receptors highly expressed on cancer surface, it has an optical probe ability and will identify cancerous cells (Usman *et al*., 2023. Another study used graphene quantum dots coated with sulfur in situ synthesis for the first time. The researchers synthesized their nano-composite by decomposing citric acid, 3-mercaptopropionic acid, and folic acid at high temperature. This nano-composite excited at 370nm and showed emission spectra at 455 nm by giving blue fluorescence. They targeted and bound with folate receptor in types of cancer with high FRs and showed fluorescence, thus separating out those types of cancers that don't have folate receptors. In this way, they are effective biomolecules for fluorescent nano-probes, providing early diagnosis of cancer cells by targeted imaging (Abdelghany *et al*., 2023).

In one study, graphene nanoparticles were conjugated with glucosamine against breast cancer having a high expression of glucosamine receptor (GlcN), delivering curcumin at the target site (Zhang *et al*., 2023). They were quite versatile, sensitive to pH, biocompatible, and had luminescence properties. The study revealed that the nano-composite was up-taken by MCF-7 breast cell at a higher rate by carrying out GlcN receptor-mediated endocytosis. The curcumin as an anti-cancerous agent showed a high toxicity and cell death rate in MCF-7 cells confirming targeting delivery and potential treatment. Another study used carbon dots conjugated with folic acid to provide increased imaging by fluorescent and targeted therapy against liver cancer. The researchers conjugated their nano-composite with doxorubicin (DOX) for delivery and targeted hepatoma cells by attaching folic acid to the composite. So, the entire CDs-DOX-FA demonstrated good biocompatibility and carried out targeted and selective delivery of the drug, providing potential treatment against liver cancer (Ghosh *et al*., 2023). Quantum dots are nanoscale particles that possess unique optical and electronic properties, making them promising candidates for various biomedical applications, including cancer therapy. In this study, the researchers investigated the effects of quantum dot conjugates on cancer cells and their mechanism of action.

The results of the study demonstrated that the quantum dot conjugate exhibited a two-way attack on cancer cells. Firstly, it induced increased cellular apoptosis. Apoptosis, or programmed cell death, is a crucial cellular process that regulates cell growth and eliminates damaged or abnormal cells. In cancer cells, apoptosis is often dysregulated, leading to uncontrolled cell proliferation and tumor growth. The quantum dot conjugate was found to enhance apoptosis in cancer cells, triggering a cascade of intracellular events that resulted in cell death. This suggests that the quantum dot conjugate has the potential to effectively target cancer cells and promote apoptosis, which could be a promising strategy for cancer therapy.

Secondly, the quantum dot conjugate was found to possess an additional mechanism of action against cancer cells. The exact mechanism was not clearly elucidated in the study, but it is hypothesized that the quantum dots may disrupt cellular processes critical for cancer cell survival, such as cell signaling pathways or energy production, leading to cell death. Further research is warranted to fully understand the underlying mechanism(s) by which the quantum dot conjugate exerts its anticancer effects.

The dual effects of the quantum dot conjugate on cancer cells, namely increased cellular apoptosis and the additional unidentified mechanism, highlight its potential as a multifaceted approach for cancer treatment. This dual attack on cancer cells could be advantageous, as it may address the heterogeneity and adaptability of cancer cells, which often develop resistance to single-target therapies. The use of quantum dot conjugates could potentially overcome this limitation and provide a more effective treatment strategy for cancer patients.

It should be noted that further studies are needed to validate these findings and explore the safety and efficacy of quantum dot conjugates in preclinical and clinical settings. Additionally, the potential off-target effects of quantum dot conjugates on normal healthy cells and long-term safety considerations need to be carefully evaluated.

In conclusion, the results of this study suggest that the quantum dot conjugate exhibits a two-way attack on cancer cells by promoting increased cellular apoptosis and potentially disrupting critical cellular processes. This dual effect makes quantum dot conjugates a promising approach for cancer therapy. However, further research is warranted to fully understand the underlying mechanisms and evaluate the safety and efficacy of quantum dot conjugates as a potential therapeutic strategy for cancer treatment.

CONCLUSION

Nanotechnology is increasingly important in the treatment of chronic human diseases such as cancer, diabetes, viral

diseases, blood and brain-related illnesses. Targeted drug delivery using nanotechnology is a promising therapy for cancer, as it delivers medication specifically to cancerous cells without harming neighboring cells, resulting in less toxicity. In a study, polymeric nanoparticles were conjugated with asparaginase and paclitaxel micelles for cancer treatment.

Quantum dots are used as the ragnostic agents for controlled delivery of therapeutic agents to target areas for cancer treatment. These dots exhibit green fluorescence when exposed to white light and can attach to tumor cells. Cadmium sulphide quantum dots coated with cysteine, conjugated with folic acid and curcumin, caused cell death in a breast tumor sample. Compared to conventional chemotherapy and radiotherapy, this approach is more cost-effective and has fewer side effects.

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