Research Article

Engineering Quantum Dot (Cadmium Sulfide) on Antibodies for Fluoroimmunoassays

Madeeha Shahzad Lodhi and Zahoor Qadir Samra

Applied Molecular Biotechnology Research Lab (AMBR), Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore 54590, Pakistan

Correspondence should be addressed to Madeeha Shahzad Lodhi; madeehalodhi.phd.ibb@pu.edu.pk

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Antibodies are good escorts for a therapeutic and diagnostic tool against many diseases as well as for targeting drug delivery and in immunohistochemistry. Cysteine-capped cadmium sulfide (quantum dot) nanoparticles have wide applications in immunohistochemistry due to their unique physical, chemical, and fluorescent properties. In this study, polyclonal antibodies against transferrin (as ligand), doxorubicin (anticancer drug), and H. pylori (cancer-causing agent) were developed in rabbits and purified. Purified antibodies were labeled with cadmium sulfide (CdS) quantum dot (QD) and used as a fluorescent-labeled marker for one-step identification in diagnosis and therapeutics. Cysteine-capped CdS QDs were synthesized in the presence of taurine (antioxidant) and characterized by FTIR, VSM, DLS, and TEM. CdS nanoparticles are monodisperse with a narrow size range below 7 nm and showed an increase of almost 30 nm after conjugation to IgG. The binding of QD-labeled antibodies was observed and confirmed by binding on MDA-MB 231 cancer cells, mouse liver tissue, mouse tumor tissue, and H. pylori under a fluorescent microscope. QD-labeled antibodies gave sharp fluorescence after binding with their respective targets. Intensity of fluorescence with quantum dot enhances many folds as compared to that of traditional fluorescent-labeled compounds. Experiments are underway to target transferrin and doxorubicin and to analyze the role of H. pylori in a mouse model of gastric cancer.

1. Introduction

Antibodies are the plasma proteins developed in response to antigen for neutralizing the antigen activity [1]. Antibodies are highly important as a therapeutic and diagnostic tool against cancer, autoimmune diseases, and microbial infections, in targeting drug delivery, and in immunohistochemistry. Pharmaceutical industries and research centers have got attraction for antibodies due to vast applications in all biosciences [2].

The development of polyclonal antibodies is cheaper and time-saving as compared to that of monoclonal antibodies [1]. Many animals are used for antibody production including small animals such as mice, rat, rabbits, and guinea pigs to large mammals like goats, sheep, and horses [3, 4]. Among them, rabbit is the commonly used animal for the polyclonal antibody production due to its manageable size and easy handling and bleeding with adequate amount of serum [5].

Antibodies are tagged with different enzymes and fluorescent organic and inorganic compounds for diagnosis in immunochemistry. In fluorescent immunohistochemistry, quantum dot (QD) nanoparticles have unique physical and chemical properties that have an edge over the fluorescent organic compound for immunochemistry [6]. In contrast to traditional fluorescent compounds, cadmium sulfide quantum dot (CdS QD) has the best properties due to photostability and a wide range of excitation wavelength [7].

The goal of the present study was to produce specific antibodies and their conjugation with CdS QD for the characterization of drug and protein in nanocomposite formulation used for targeting drug delivery against cancer as well as to diagnose infections in animal models of gastric cancer.
In this research model, transferrin was used as ligand and doxorubicin was used as an anticancer drug. H. pylori as a microorganism was used for the induction of inflammation/cancer in a mouse model. Cysteine-capped CdS QD is used as a fluorescent-labeled marker of antibodies.

Transferrin is a carrier glycoprotein that has high affinity with iron and is involved in the depletion of free iron in the blood [8]. The demand of iron by growing cancer cells increases manyfold as compared to normal cells that in turn increase the expression of transferrin receptor in a thousandfold on cancer/tumor cells [9]. Transferrin has proved itself best candidate for therapeutic and diagnostic application. Transferrin also plays an important role in preventing infection because of its iron scavenger action [10]. It is evident that some pathogens develop the ability to express the surface protein that binds the transferrin protein and absorb iron from it [11]. Doxorubicin is used as anticancer medicine that binds the transferrin protein and absorb iron for use in the production of antibodies against transferrin, doxorubicin, and H. pylori. Six New Zealand white rabbits (two for each sample) were housed under standard conditions. Before immunization, preimmune blood was drawn from each rabbit. Serum was isolated and stored at -20°C for using it as control. A total of 80-100 μg of transferrin, 250-300 μg of BSA-conjugated doxorubicin, and 1 × 10⁷ H. pylori cells were suspended in 100 μl of normal saline and mixed with 100 μl of FCA in a 1:1 ratio. Rabbits were injected through subcutaneous route at four different sites (50 μl at each site). Primary injection was followed by four booster injections after 15-day intervals with the same dose (100 μl) of immunogens in 100 μl of IFA (1:1 ratio) at four different subcutaneous sites. Each rabbit was bled from marginal ear vein after a booster dose and tested for the presence of specific antibodies such as anti-transferrin, anti-doxorubicin, and anti-H. pylori.

Immunized blood was collected by cardiac puncture, and rabbits were anesthetized with 35 mg/kg of ketamine chloride and 5 mg/kg of xylazine by intramuscular injection [18]. Serum was separated, aliquoted, and stored at -20°C. Antibodies were further purified from the stored serum samples by using NAB™ spin columns (affinity A/G column) under the given instructions. Purified anti-transferrin, anti-doxorubicin, and anti-H. pylori were characterized by ELISA, immunoslot blot and Western blot analysis under nature and denature conditions. Antibody titer and dilution factors were also calculated by ELISA.

2.2. Antibody Characterization

2.2.1. Enzyme-Linked Immunosorbent Assay. 10 μl of purified antigens (1.0 mg/ml of purified transferrin, dox-BSA conjugates, and 1 × 10⁶ H. pylori) was diluted in 200 μl of 0.05 M carbonate buffer, pH 9.0, and coated on to a microtiter ELISA plate (in triplicate). The plates were incubated for two hours with gentle shaking at 100 rpm. After antigen coating, the nonspecific sites were blocked for one hour at 37°C with 5% skim milk in 1x TBS buffer (50 mM NaCl and 150 mM Tris, pH 7.5). After washing with TBS, an antigen-specific purified primary antibody (1:100 dilutions in 50 mM Tris-Cl buffer, pH 8.5) was added to each antigen-specific well and incubated for one hour at 37°C. After washing the wells, HRP-conjugated secondary antibody (HRP-conjugated goat anti-rabbit IgG 1:5000 dilutions in 50 mM Tris-Cl buffer, pH 8.5) was added in each well and incubated further for one hour at 37°C. After washing, HRPO-specific substrate 1% tetrathyle-benzenide and 0.1% H₂O₂ were added in each well and the results were observed [19].

Antibody titer and dilution factor were determined by ELISA as the substrate reaction was stopped by sulfuric acid and was noted at 405 nm in an ELISA plate reader.

2.2.2. Immunoslot Blot Analysis. The reactivity of antibodies with antigen was also checked on a nitrocellulose membrane (NC) under denature conditions. 50 μl (10 μg/μl) of each antigen (transferrin, dox-doxorubicin conjugates, H. pylori as described in ELISA) was prepared as described above and...
denatured at 70°C for 30 minutes. The denatured antigens were loaded on the NC membrane by a hybrid slot blot apparatus (Bethesda Research Laboratories, USA). After blocking nonspecific sites on the nitrocellulose membrane with 5% skim milk in TBS for 2 hours with gentle shaking, the antigen-specific purified primary antibodies (1:500 dilutions) were allowed to react with antigen onto the nitrocellulose membrane for one hour under gentle shaking. After washing the nitrocellulose membrane with TBS, secondary antibodies (goat anti-rabbit-HRPO-conjugated 1:5000 dilutions) were added and allowed to react with primary antibodies. Again, after washing, the blots were exposed to a HRPO-specific substrate (1% diaminobenzidine and 0.1% H₂O₂) and the results were noted [19].

2.2.3. Western Blot Analysis. Western blot was also conducted to verify the reactivity of antibodies with transferrin. Briefly, transferrin was run on 10% SDS-PAGE and transferred onto the nitrocellulose membrane by a semidy method (Bio-Rad). The presence of protein onto the nitrocellulose membrane was confirmed by Ponceau S stain. After washing, the nitrocellulose membrane was treated with 5% skim milk and then primary and secondary antibodies as described in the immunoslot blot analysis. Results were noted [20].

2.3. Synthesis of Cysteine-Capped Cadmium Sulfide Quantum Dot (Cys-CdS QD). Cysteine-capped cadmium sulfide QDs were synthesized in the presence of taurine as an antioxidant. A 0.01 M cadmium acetate solution (pH: 8) and 0.01 M L-cysteine solution (pH: 8) were refluxed in 200 ml reaction volume for 30 minutes in the presence of a final concentration of 0.1 M taurine. Then, 0.01 M sodium sulfide in 10 ml deionized water was added dropwise in a reaction mixture and refluxed further for 12 hours. Addition of taurine as an antioxidant prevents the surface oxidation of QD synthesized in the presence of air and improves the size and fluorescent quality of QDs. After the addition of Na₂S, a yellow color of suspended particles was observed that indicated the onset of reaction.

2.4. Conjugation of Cysteine-Capped CdS QD Nanoparticles with Rabbit Polyclonal IgG. Purified and characterized rabbit polyclonal IgG was cross-linked with cysteine by a 1-Ethyl-3-[3-dimethylamino propyl]carbodiimide (EDC) method. 0.01 g of cysteine-capped CdS QD was taken in one ml of deionized water and a pH of 8 was adjusted with NaOH. One ml of EDC (20 mg/ml of deionized water) was added for carboxylic group activation, and a pH of 6.4 was adjusted with 1 M HCl. The reaction mixture was incubated for 30 minutes in the dark with continuous shaking at 100 rpm. After 30-minute incubation, one ml of carbodiimide (10 mg/ml of deionized water) along with 100 μl of purified rabbit IgG (anti-transferrin, anti-doxorubicin and anti-H. pylori) (1 μg/μl) was added separately and incubated further for 2 hours at 37°C in the dark with continuous shaking at 100 rpm. The conjugated particles were separated by centrifuging at 14000 g for 10 minutes at 4°C and washed twice with PBS. The washed nanocomposites were suspended in 1x TBS buffer for further use or stored at -20°C.

2.5. Characterization of Nanocomposites. Synthesized cysteine-capped CdS and its conjugates with antibodies were characterized by UV-Vis spectroscopy (PG instrument, Model T90+), excitation and emission spectra (PerkinElmer fluoroscopic spectrophotometer, LS45), fluorescent microscopy under different filters (Olympus BX51), Fourier-Transform Infrared spectroscopy (FTIR, Agilent Technology Cary 630), Transmission Electron Microscopy (TEM, JEOL JEM-1010), and dynamic laser scattering analyzer (HORIBA Scientific, nanopartica SZ-100) techniques under standard conditions.

2.6. Receptor Binding Studies

2.6.1. Quantum Dot-Linked Immunosorbent Assay. 10 μl of purified antigens (1.0 mg/ml of purified transferrin, Dox-OVA conjugates, and 1 × 10⁶ H. pylori cells) was diluted separately in 200 μl of 0.05 M carbonate buffer, pH 9.0, and coated onto a microtiter ELISA plate and incubated for two hours with gentle shaking at 100 rpm (in triplicate). The non-specific sites were blocked for one hour at 37°C with 5% skim milk in 1x TBS. After washing with TBS, an antigen-specific quantum dot-linked primary antibody (1:100 dilutions in 50 mM Tris-Cl buffer, pH 8.5) was added to each antigen-specific well for one hour at 37°C. Fluorescence was observed on a UV plate in the presence of antibodies and then washed three times with TBS buffer. After washing, wells were filled with deionized water and again, fluorescence was observed on a UV plate.

2.6.2. Immunohistochemical Studies

(1) Binding Efficiency of Rabbit Anti-Transferrin Antibody. An anti-transferrin antibody binding with transferrin, bound with its receptor, was checked on MDA MB 231 cancer cells and mouse liver tissue section. MDA MB 231 cells were grown under microaerophilic conditions in a humidified chamber on gelatin-coated coverslips. The growing adhered cells on the coverslip were fixed with 4% paraformaldehyde for 15 minutes and covered with 5 μg of purified transferrin and incubated for 20-25 minutes at 37°C. The cells were washed with PBS for 5 minutes to remove the unbound transferrin and then covered with 5% skim milk. After washing, cells were incubated with QD-conjugated rabbit anti-transferrin (1:100 dilutions) antibodies for 25-30 minutes. After washing, stained cells were observed under a fluorescent microscope.

Paraformaldehyde-lysine-periodate (PLP) fixed liver tissue was obtained from AMBR and cut into small 5 mm cubes. Tissue dehydration was done by ascending series of sucrose gradient (10%, 15%, 20%, and 25%) and then embedded in an OCT compound. Sections of 6-8 μm size were cut in cryostat machine (Leica, Germany), collected on albumin-coated slides, and proceeded further for staining with QD-labeled antibodies as described above. Sections were observed under
a fluorescent microscope using a blue excitation filter (Olympus BX51).

(2) Binding Efficiency of Rabbit Anti-Doxorubicin Antibody. Binding efficiency of QD-conjugated anti-doxorubicin antibodies with doxorubicin was checked on MDA MB 231 cancer cells and on a mammary tumor tissue section of mouse. MDA MB 231 cells were incubated with doxorubicin and fixed as described above. Fixed cells were washed with TBS and treated with 5% skim milk for 30 minutes. The cells were exposed to anti-doxorubicin antibodies for one hour, and after washing, cells were observed under a fluorescent microscope. Mouse with mammary gland tumor was obtained from the animal house of AMBR, and doxorubicin (50 μg/saline) was injected near the tumor. After one hour, the mouse was dissected by following the standard procedure and tumor was collected. Tumor tissue was fixed in paraformaldehyde-lysine-periodate (PLP) fixative, and immunohistology was completed as described above using anti-doxorubicin antibodies. Stained tissue sections were observed under a fluorescent microscope.

(3) Binding Efficiency of Rabbit Anti-H. pylori Antibody. H. pylori culture suspension (20 μl) was taken on albumin-coated glass slides, and smear was prepared. 100 μl (1 : 100 dilutions) of QD-conjugated anti-H. pylori antibody was added onto the smear. After one hour of incubation, the slide was washed with PBS and observed under a fluorescent microscope.

3. Results

3.1. Characterization of Antibodies. Polyclonal antibodies were generated against transferrin protein, doxorubicin drug, and H. pylori microorganisms. The titer of the antibodies was also checked during immunization process (Figure 1).

The specificity and reactivity of affinity-purified antibodies towards their specific antigens were checked under non-denaturing and denaturing conditions. Greenish blue color formation in ELISA and appearance of brown precipitates in immunoslot blots (Figure 2(a)) confirmed the specific reactivity of antibodies with their respective antigens. Anti-transferrin reactivity and specificity were further confirmed by Western blot under denature conditions. Transferrin band was appeared at ~78 KDa after Coomassie staining (Figure 2(b)) and brown precipitates at 78 KDa on the nitrocellulose membrane (Figure 2(c)) confirmed the reactivity of anti-transferrin with transferrin.

3.2. Characterization of Cysteine-Capped CdS QD and Labeling with Antibodies

3.2.1. Optical Characterization. UV-VIS spectra of cysteine-capped and IgG-conjugated CdS QD are shown in Figure 3. The UV-VIS spectra showed a clear blue shift in wavelengths as compared to CdS salt (512 nm) that was the indication of the QD nanoparticle synthesis. Cysteine-capped CdS QD synthesized in the presence of taurine showed a greater blue shift in wavelength and broad absorption peak (Figure 3(a)). A stable absorption spectrum of IgG-conjugated cysteine-capped CdS QD was observed (Figure 3(b)). Excitation and emission spectra were collected by fluoroscopic analysis (Figure 4). A single excitation and emission spectrum peak of cysteine-capped CdS QD is shown in (Figure 4(a)) while IgG-conjugated CdS QD showed two emission peaks (Figure 4(b)). Different intensities of fluorescence were observed for cysteine-capped CdS QD and IgG-
conjugated cysteine-capped CdS QD. Fluorescent properties of nanoparticles were further observed under UV and blue filters of a fluorescent microscope, and particles appeared blue and green, respectively (Figures 4(c) and 4(d)).

3.2.2. Structural Analysis. FTIR analysis of nanocomposites is shown in Figure 5. The FTIR spectrum of cysteine with characteristic SH peak (2549.7 cm⁻¹) and associated carboxylic (1575.5 cm⁻¹ and 1387.0 cm⁻¹) and amine (3166.1 cm⁻¹) peaks is given in Figure 5(a). The spinal structure of nascent CdS QD FTIR spectrum is shown in Figure 5(b) and cysteine-capped CdS QD is given in Figure 5(c). Characteristic SH peak of cysteine disappeared due to covalent attachment of cysteine to CdS, and all cysteine-associated peaks were observed on the spinal structure of CdS QD. Primary amine NH₂-associated peaks were clearly visible between 3280 and 3340 cm⁻¹ and carboxylic COO-associated peaks in region 1540-1600 cm⁻¹ and 1390-1410 cm⁻¹. Figure 5(d) shows visible changes in peak patterns and intensities that confirmed the successful conjugation of IgG with cysteine-capped CdS QD.

The schematic presentation of cadmium sulfide quantum dot engineering with antibodies is shown in Figure 6. The model presents the different orientation possibilities of antibodies on cysteine-capped cadmium sulfide quantum dot.

3.2.3. Morphology and Size Characterization. Dynamic light scattering (DLS) analysis of particles showed the size and dispersity of CdS QD nanoparticles (Figure 7). All nanoparticles showed monodispersity and a narrow size range. DLS analysis of cysteine-capped CdS QD showed nanoparticle
distribution in a size range of 0.4 to 3 nm and fewer in range of 10 to 60 nm with an overall mean size of 6 nm and a Polydispersity index (PI) of 0.214 (Figure 7(a)). The DLS analysis of IgG-conjugated nanocomposites with an increase of approximately 30 nm in size was observed (Figure 7(b)). IgG-conjugated cysteine-capped CdS QDs were fallen in a size range of 10 to 100 nm with a mean size of 30 nm and a PI value of 0.334. TEM micrographs of nanoparticles are

**Figure 4:** Fluorescent study and excitation and emission spectra of nanocomposites. (a) Cysteine-capped CdS QD in taurine presence with a maximum excitation of 361.5 and maximum emission of 362.8. (b) Rabbit polyclonal IgG-conjugated cysteine-capped CdS QD with a maximum excitation of 363.6 and maximum emission of 365.2. (c, d) The fluorescence observation of nanoparticles under UV excitation and blue excitation filters.

**Figure 5:** FTIR analysis. (a) This shows the FTIR spectrum of L-cysteine. (b) Spectrum of bare CdS QD shows the specific spinal structure of CdS QD with associated peaks. (c) Spectrum of cysteine-capped CdS QD confirmed the successful capping of CdS with cysteine because of the absence of SH peak and appearance of amine and carboxylic peaks. (d) Spectrum of rabbit polyclonal IgG-conjugated cysteine-capped CdS QD showed successful binding of proteins because of the change in peak patterns and intensities.
shown in Figures 7(c) and 7(d) which further confirmed the DLS analysis. Micrograph of cysteine-capped CdS QD showed spherical-shaped monodisperse particles of a size below 7 nm (Figure 7(c)). After conjugation with IgG, an increase of almost 30 nm was observed but particles retain the spherical shape, as shown in Figure 7(d).

3.3. Quantum Dot-Linked Immunosorbent Assay (QD-LISA). Fluorescent analysis of antigen binding with QD-conjugated rabbit IgG was conducted. Fluorescence in the wells was observed on a UV lamp tray in the presence of QD-labeled rabbit IgG was conducted. Fluorescence in the wells was observed in both cases. QD-conjugated antibody binding with antigen were also observed under a fluorescent microscope. Nanoparticles were clearly observed (Figures 8(c) and 8(d)) that confirmed the binding of antibodies with their respective antigens on microtiter wells.

3.4. Immunohistochemical Studies. Histochemical studies about the binding of CdS-conjugated rabbit anti-transferrin, anti-doxorubicin, and anti-\textit{H. pylori} with their respective antigens were further studied.

3.4.1. Anti-Transferrin Antibody Binding Studies. Observation of the stained MDA-MB 231 cells (Figure 9(a)) and mouse liver tissue section (Figure 9(b)) with QD-conjugated anti-transferrin antibody under blue excitation confirmed the binding at specific sites. The cells and tissue exposed to nonconjugated anti-transferrin antibodies were devoid of any fluorescence.

3.4.2. Anti-\textit{H. pylori} Antibody Binding Studies. The binding of rabbit anti-\textit{H. pylori} antibody to Anti-\textit{H. pylori} surface proteins in immunohistochemistry is shown in Figures 9(c) and 9(d).

3.4.3. Anti-Doxorubicin Antibody Binding Studies. Doxorubicin carried MDA-MB 231 cells, and tumor tissues expressed to doxorubicin were incubated with anti-doxorubicin antibodies and observed under a fluorescent microscope. Fluorescence observation of unstained MDA-MB 231 cancer cells and tumor tissue sections under green filters confirmed the uptake of doxorubicin by cells (Figures 10(a) and 10(c)), while stained MDA-MB 231 cells (Figure 10(b)) and tumor tissue section (Figure 10(d)) with QD-conjugated anti-doxorubicin antibody were observed under a blue excitation filter, and it confirmed the targeted binding at specific sites.

4. Discussion

Antibodies are not only known as therapeutic escorts against cancer, autoimmune diseases, and microbial infections but also act as an essential tool for diagnosis. Three different types of molecular moieties were selected for the development of polyclonal antibodies used in immunohistochemical studies. In this study, transferrin as a protein, doxorubicin as a hapten, and \textit{H. pylori} as a microorganism were selected due to their applications as important clinical tools for targeting, prognosis, and diagnosis, respectively.

Transferrin is an iron-binding glycoprotein, and the transferrin-specific receptor expression increased on cancer cells. Iron is an essential nutritional element required for many biological functions, and correlation is reported between cancer cells and transferrin receptor expression [21, 22]. That can be justified due to high iron demand of abruptly growing cells for their DNA manipulation and energy cycles involved in cell cycle progression [23, 24]. This relationship makes transferrin receptor an important target for targeting drug delivery by using either transferrin protein [25, 26] or anti-transferrin receptor antibody [27]. Other than transferrin applications for targeting drug delivery, transferrin also has free-iron sequestering, antioxidative, and antimicrobial actions that enhance its importance in several therapeutic applications including reperfusion injury, ischemia, organ transplantation, and cardiovascular diseases [28–31].

Doxorubicin is an anthracycline antibiotic used in chemotherapy and treatment of solid tumor. Many studies were focused on the use of doxorubicin widely as a chemotherapeutic agent loaded on or conjugated to nanomaterials [12, 32] or peptides [33–35] for targeted drug delivery. Therefore, anti-doxorubicin antibodies may be useful in characterization of designed nanomedicines or nanocomposites.

\textit{H. pylori} is a gram-negative bacterium that colonizes the gastric epithelial cells and infects almost half of the world population. Gastric \textit{H. pylori} infection causes gastric ulceration [36], and prolonged colonization leads to site-specific diseases including dreadful gastric cancer [37, 38]. Early diagnosis and eradication of \textit{H. pylori} can prevent the onset of gastric cancer [39]. Application of QD conjugated with anti \textit{H. pylori} antibodies for diagnosis of \textit{H. pylori} infection in biopsy samples will help in the diagnosis at the early stages of infection.
Figure 7: DLS and TEM analysis. (a) The DLS analysis of cysteine-capped CdS QD shows particles in a size range of 0.4 to 3 nm and fewer in 10 to 60 nm with a mean size of 6 nm. (b) The DLS analysis of IgG-conjugated cysteine-capped CdS QD shows particles in a size range of 10 to 100 nm with an increase of 30 nm in size with a mean size of approximately 30 nm. (c) TEM image of cysteine-capped CdS QD synthesized in taurine presence (25 nm scale) shows the monodisperse spherical particles in a size below 7 nm. (d) TEM image (50 nm) shows the uniform distribution of IgG-conjugated cysteine-capped CdS QD with a spherical shape and mean size of 30 nm.

Figure 8: Quantum dot-linked immunosorbent assay. (a) The fluorescent observation of microtiter wells with QD-linked antibodies on a UV lamp tray. (b) The fluorescent observation of microtiter wells after washing with TBS and filling with deionized water on the UV lamp tray. (c) Observation under a UV filter of a fluorescent microscope gives a blue color. (d) Observation under a blue excitation filter gives a green color and confirmed the binding of antibodies with antigen.
Rabbits were selected for antibody production because of many factors. Rabbits offer many advantages for the development of polyclonal antibodies over other laboratory animals due to body size, easy housing, and easy collection of immunization blood to check antibody titer \[5, 40, 41\]. After the completion of the immunization process, blood was collected by cardiac puncture and antibodies were purified using A/G affinity columns.

QDs are smaller size, unique semiconductor nanoparticles, and their photoluminescence and chemical properties depend on their sizes. Cysteine-capped CdS QDs were synthesized in the presence of taurine and characterized. Cysteine-capped CdS QD showed smaller and narrow size distribution as compared to bare CdS QD. Bare CdS QD showed higher coalescence, and due to this property, it is necessary to cap them with some organic cover to protect the reactivity of particles with air oxygen and moisture \[42–44\]. QD nanoparticles are synthesized in inert atmosphere to avoid the damage of precursors and formation of metal oxide as a side product and also to protect the luminescence properties of QD \[42\]. Here in this research work, a novel method is reported for the synthesis of CdS QD in the presence of taurine (antioxidant) to get a smaller size good-quality CdS QD even in the presence of air and moisture. Cysteine-capped CdS QD synthesized in the presence of taurine is superior over other cysteine-capped CdS QDs synthesized in the absence of taurine (data not shown).

QDs are photostable and bright and significantly used in biological imaging and diagnostic methods \[45–47\]. Antibody-conjugated cysteine-capped CdS QD was further applied in QD-linked immunosorbent assays and immunohistochemistry in order to check its binding efficacy and fluorescent ability.

In QD-LISA, microtiter wells processed for immune assays gave sharp fluorescence after exposure to UV light. Observation under a fluorescent microscope confirmed the binding of antibodies with their respective targets. In immunohistochemistry, binding of QD-conjugated anti-transferrin antibodies with transferrin bound to transferrin receptor was checked on MDA-MB 231 cancer cells because receptor expression is increased to a thousandfold on cancer.

Figure 9: Immunohistochemical study of anti-transferrin and anti \( H. \text{ pylori} \) binding. (a, b) Binding of anti-transferrin antibody to transferrin present on transferrin receptors on MDA-MB 231 cancer cells and mouse liver tissue sections is confirmed. (c, d) Observation under a brightfield and fluorescent microscope confirms the anti-\( H. \text{ pylori} \) binding with \( H. \text{ pylori} \) surface proteins.
cells as compared to normal cells [26, 47, 48] as well as on liver tissue as it is actively involved in the upregulation of iron load for rapid metabolism [49, 50]. Results showed the successful binding of anti-transferrin antibodies with transferrin receptors. Anti-doxorubicin antibody binding study was done on MDA-MB 231 cells and on mouse mammary gland tumor tissues. Fluorescent studies further confirmed the binding of anti-doxorubicin to doxorubicin that resides on the cells. The binding of anti-*H. pylori* antibodies with *H. pylori* surface proteins showed fluorescence that confirmed the bindings. This QD-conjugated anti-*H. pylori* may be a good diagnostic tool for *H. pylori* in gastric biopsy samples and laboratory diagnostic tests.

This one-step identification of transferrin receptor or drug in the tissue will be a fruitful pathway in the diagnosis and therapeutic studies. The final size of the nanocomposites was below 45 nm which can be used for intravenous injection. It is a general observation that the size of nanocomposites in a range of 60 nm to 100 nm is considered to be good for targeting drug delivery. Experiments are underway to detect the transferrin receptor expression and the role of *H. pylori* in gastric inflammation in a mouse model.

5. Conclusion

This research article summarizes the production of rabbit polyclonal antibodies against different immunogens and discusses their applications after conjugation with cysteine-capped CdS QD as diagnostic and characteristic tools in laboratories and researches, respectively. A new approach for the synthesis of cysteine-capped CdS QD is reported in the presence of taurine (antioxidant). CdS has intrinsic redox properties, and prone toward air oxidation during their growth, an antioxidant not only keeps the particle size to a lower range but also gives high luminescence, best-quality QD nanoparticles. Conjugation of these highly fluorescent particles with produced polyclonal antibodies gives the best diagnostic and characteristic approach in our research laboratory.
Data Availability

The Experimental data used to support the findings of this study are included within the article. However, we are here to provide any additional related data on request.

Ethical Approval

This manuscript complies with the ethical rules applicable for this journal.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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