J. Mat. NanoSci. 2014, 1(1), 7-11 Review

Journal of
Materials NanoScience

Advances and prospects of sugar capped Quantum Dots

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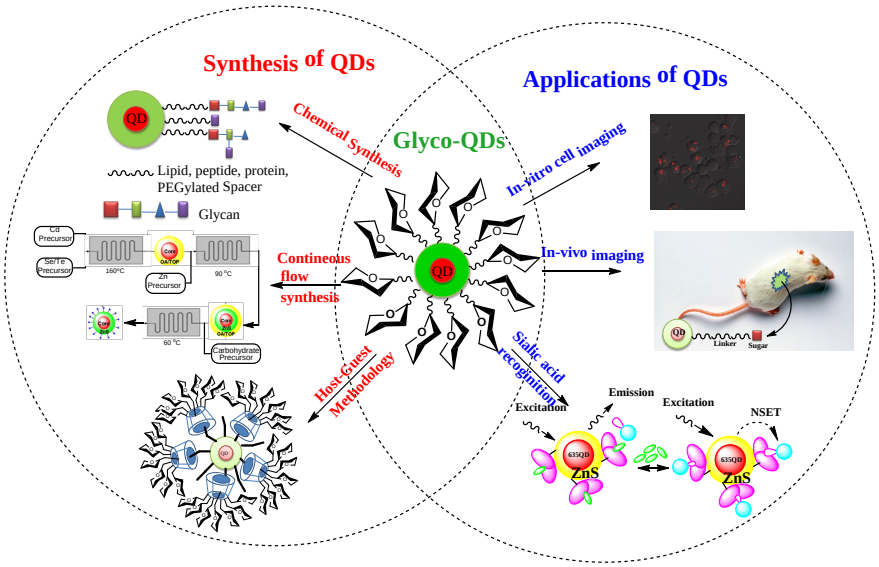
Received Date: 16-June-2014



Over the past few decades, there has been a remarkable progress and development in the field of glyco-nanotechnology, which has brought the glycomic research even more closer to the realization of biomedical applications. This review intends to give an overview of the recent advances and the future prospects of the synthesis of glyco-quantum dots, as well as selected applications of these nanoparticles in biology, biotechnology and diagnostics.

Keywords: Carbohydrate, Nanotechnology, Quantum dots, Imaging, In-vivo

1. INTRODUCTION

Naturally occurring carbohydrates and glyco-conjugates are present on the surface of majority of all the cells in the living system. These carbohydrates play a crucial role in several biological events including cell-cell adhesion, proliferation, bacterial and viral infections.[1] In particular, carbohydrate-protein interactions (CPIs) on normal cells and their malignant counterparts show significant differences.[2] In attempts to understand the role of oligosaccharides/carbohydrates, the tools used encounter serious problems. The difficulties faced in the carbohydrate studies is mainly due to the consequence of weak CPIs and also, the specific difficulties associated with it are the time-consuming synthesis and purification of oligosaccharides. In general the interaction between a protein and a monosaccharide is weak with dissociation constant (K_d) typically in the range of 10^{-4}-10^{-6} M compared with 10^{-6}-10^{-9} M that of antigen-antibody interactions. In recent years, much progress has been made in endowing multivalent glycoclustures with ability to increase the avidity of specific CPIs.[3] Herein, we summerize one particular class of multivalent system, namely glyco-quantum dots and its

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Cite as: *J. Mat. NanoSci., 2014, 1(1), 7-11.*

biomedical applications. First, we illustrate the synthesis of different QDs using conventional and micro-fluid technique and their subsequent functionalization with structurally defined carbohydrates to get multivalent analogues. While the former allows for the synthesis of nanoparticles with well-defined optical electronic and electric properties, the latter is particularly attractive in providing good control over its multivalency. Further, it is shown how the two strategies can be combined to prepare new glycomaterials that would be useful in glyconanotechnology for applications ranging from diagnostics to imaging. The advantages and limitations of the different approaches have been discussed in the context of the applications.

2. QUANTUM DOTS

Quantum dots (QDs) are spherical semiconductor nanoparticles with a diameter of 2-10 nm. Changes in the particle size of quantum dots sowed drastic differences in their characteristics such as optical absorption, excitation energies and electron-hole pair recombination. These different characteristics can be controlled during their synthesis, as they are depend on different factors, such as size, shape, defect, impurities and crystallinity.[4] QDs absorbs white light and then reemitting a specific color a few nanoseconds later depending on the bandgap of the material, which gives unique wide and continuous absorption spectra and narrow emission spectra.[4] The semiconducting nature and the size-dependent fluorescence of these nanoparticles have made them an attractive target for biological applications.[5] In comparison with organic dyes, QDs are about 10-100 fold brighter mainly due to their large absorption cross-section and being 100-1000 folds more stable against photo-bleaching.[5] However QDs are macromolecules that are in an order of larger magnitude than organic dyes, which may limit their application in size dependent studies. Yet, these macromolecular structures allow a large surface area for multivalent display of biological molecules to improve the avidity of specific interactions.[6]

3. MOTIVATION FOR RESEARCH IN GLYCO-NANOTECHNOLOGY

Despite knowing that carbohydrates are first line of contact for any biological interactions on cell surfaces, the glycomic research was not immediately obvious, mainly due to the extreme complexity and variability of the glycans structure and weak chemical tools used for their interactions studies. Nanomaterials can provide a formidable platform for multivalent ligand presentation through its large surface to volume ratio, increasing the avidity by several orders of magnitude. QDs are constituted with highly toxic substrates, such as cadmium and selenium and shows native inability to disperse within biological compatible solutions. However, fuctionalization of QDs with different glycans confer biological activity to QDs and along with that it improves their solubility and stability in water.

4. SYNTHESIS OF QUANTUM DOTS

Several routes have been used to synthesize quantum dots[7] The synthesis of CdTe QDs is based on the reaction of principle precursors (Cd(II) and Te(II) ions) in the presence of mercapto succinic acid or mercaptopropionic acid.[8] The size of the QDs can be controlled using the growth time between a few minutes to several hours in an autoclave at 120 °C. In order to stabilize the surface dangling bonds, CdS or ZnS shell was made over the CdTe core. The CdS shell was easily made by adding an excessive amount of thiourea (for the S precursor) in the solution containg CdTe QDs and annealed at 120^{o}C for certain periods of time depending on the required sizes of CdTe/CdS. The synthesis of CdSe QDs is based on the mixing of cadmium oxide (CdO) and selenium precursors in presence of dodecanoic acid and tri-n-octylphosphine oxide (TOPO) and tri-n-octylphosphine (TOP) surfactants.[9] Both homogeneous and heterogeneous nucleation of the CdSe seed have been carried out by the researchers using fast mixing of Cd and Se precursors followed by heating of solvent at 250 °C –300 °C under N_2 gas atmosphere. For the passivation of surface dangling bonds; CdSe/ZnS, CdSe/CdS/ZnS and CdSe/ZnSe/ZnS core/shell(s) structures have beensynthesized using similar conditions as described in CdTe/ZnS core/shell synthesis.[4]

5. SYNTHESIS OF GLYCO-QUANTUM DOTS

The most difficult challenging task of glyco-QDs is the synthesis of glycoconjugates with reduced toxicity and non-specific interactions. The last decade has seen a number of innovative approaches to synthesize glyco-conjugations, with much higher efficiency, with good control over critical parameters such as carbohydrate distribution, linkage chemistry and controlled cytotoxicity.

5.1. COUPLING CHEMISTRIES FOR GLYCOMATERIALS SYNTHESIS

Basic challenge of glyco-QDs research is the sequence specific carbohydrate conjugations on quantum dots. Novel platforms are being developed with the aim to create glycomaterials to perform mechanistic studies of carbohydrates interaction as well as for medical applications (diagnostics, drugs).

Two categories of glycomaterials based on the source of carbohydrates immobilized on QD surfaces can be found. Naturally isolated glycolipids, glycoproteins, proteoglycans and lipopolysaccharides have been directly conjugated on QDs using self-assembly[10] or synthetic conjugation techniques (Figure 1).[11] For synthetic conjugation technique, mono and oligosaccharides have been synthesized with suitable linkage system to immobilize them on the surfaces. Various immobilization strategies to present carbohydrate on solid surfaces for multivalent display have been developed for studying high-throughput carbohydrate-lectin interactions. For example, Wang *et. al.*, immobilized carbohydrates non-covalently on QDs by physical adsorption of naturally occurring oligosaccharides and polysaccharides, glycoproteins and glycolipids (chemically unconjugated) *via* hydrophobic interactions.[12] The advantage of this method is that carbohydrates are not modified before immobilization. But the disadvantage is that the molecular weight of the polysaccharides have to be high enough to guarantee sufficient immobilization and oligosaccharides cannot be densely and stably immobilized on the surface unless they are first modified by coupling to another larger moiety such as, in particular, the use of 2-[2-(2-mercaptoethoxy)ethoxyl]ethanol proved to be successful in view of the ease of temporarily masking thiol with a protecting group.

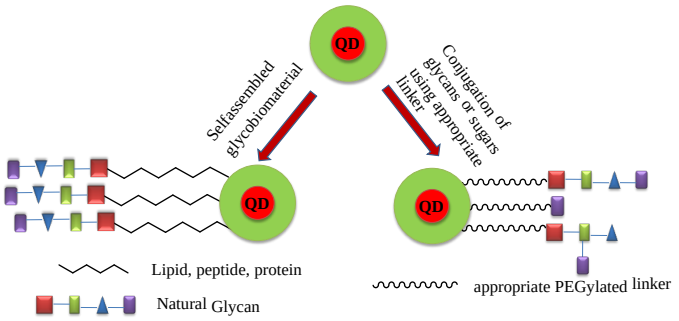

Figure 1. Schematic representation of the synthesis of glyco-quantum dots

Another example of powerful and versatile coupling reaction for the production of glyco-QDs include maleimido-thio coupling,[13] carboxylic acid-amine coupling.[14] Yang and co-workers used the phase transfer chemistry to immobilize short thio linker or anomeric thio group containing mono- and oligo-saccharides to QDs.[15]

The disadvantage of the approach is the non-specific interactions between quantum dot surfaces and biomolecules, which might be overcome by incorporation of specific length of PEG in between carbohydrate and QD surfaces.[16] Using the same strategy mannose-tagged glyco-QDs were synthesized.[17] This might be a useful future approach to prepare a wide range of macromolecular and nano objects mimicking glycoproteins. In an another development, Ohyanagi *et. al.*, reported the protocol for the synthesis of phosphorylcholine self-assembled monolayers $(SAMs)_{12}$-coated QDs displaying various glycans such as Lewis^x, sialyl lewis^x (glyco-PC-QDs). Here they have used enzymatic modification of simple sugar glyco-PC-QDs in the presence of suitable glycosyl transferase and sugar nucleotides to obtain complicated glycan-PC-QDs.[18]

5.2. Continuous Flow Synthesis of Glyco-QDs

The widespread application of quantum dots (QDs) in electronic and life science research has resulted in the need for the large scale production of these nanocrystals with high monodisperse particle size. Traditional batch processes show inherent limitations for the large scale production, due to the limited temperature control and lack of homogeneous mixing.[19,20] Microreactors are valuable tools for reaction scale up, enabling a degree of control over reaction conditions. The high surface-to-volume ratio of the microreactor channels provides the precise control of temperature as well as efficient mixing, allowing the preparation of QDs with narrow size distribution as showm in Figure 2 and 3. Glyco-QDs have been prepared by using microreactor with emission ranging between 480 to 598 nm.[21] The QDs were prepared at lower reaction temperatures, allowing for a narrow size distribution from commercially available starting materials in microreactor. Specific carbohydrate-lectin interaction was established between carbohydrate coated QDs and ConA.

5.3. Synthesis of Glyco-Nanoparticles by Host-Guest Strategy

A number of glyco-nanoparticles with multivalency have been

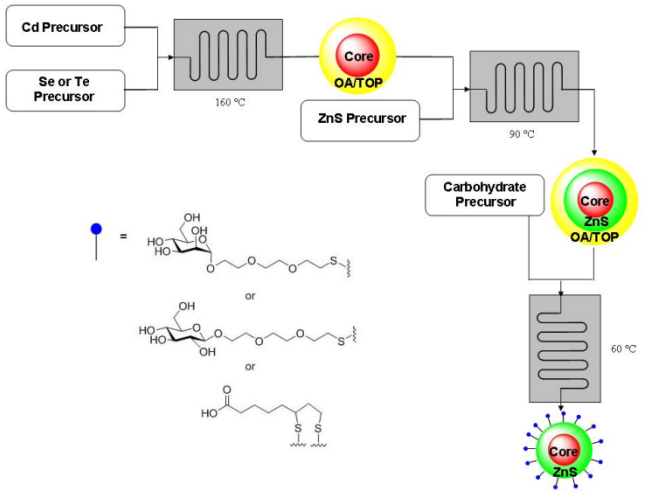

Figure 2. Experimental set-up for the synthesis of functionalized QDs. Adapted from reference 21 with permission from Wiley Publication.

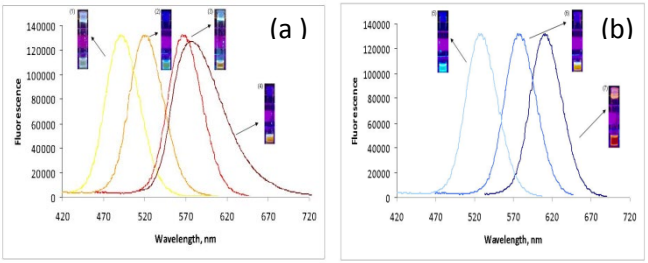

Figure 3. Normalized luminescence spectra of CdSe nanoparticles in chloroform after 3, 10, 20, 30 mins respectively (a) and CdTe nanoparticles in chloroform after 3, 10, 20 mins respectively (b). Adapted from reference 21 with permission from Wiley Publication.

synthesized by decorating the surface of QDs with different sugars and glycans. Another approach for achieving the multivalency is by host-guest strategy. This strategy is thought from the view point of providing a versatile synthetic platform for glyco-QDs displaying multivalent sugars, and a reduction in nonspecific interactions without the loss of quantum yield of the original QDs. Main idea behind the synthesis is hydrophobic and hydrophilic interactions between sugar capped β-cyclodextrins (β-CD) and TOPO coated QDs. We have reported host-guest approach to prepare water soluble glyco-QDs using O-α-manno- and O-β-galacto-pyranoside capped β-CD (Figure 4).[22]

6. Applications

6.1. Glyco-QDs for In-Vitro and In-Vivo Imaging

Glyco-QDs have been found to be powerful imaging agents for specific lectin recognition on living cells (Figure 5). Betanzos *et. al.*, have coupled QDs with E.*coli* lipopolysaccharide and studied their binding onto the surface of the cells,[23] after addition of these conjugates to cultured mouse monocytes as a model. The flow cytometry and confocal imaging studies clearly showed efficient staining of the monocytes, while the control QD-PEG 20K did not. LPS–QD conjugation is ideally suited for studying interactions of the polysaccharide moiety of LPS in micellar presentation.[23] Similarly, Yang *et. al.*, synthesized lactose conjugated QDs.[17] The biological assay showed that oligosaccha-

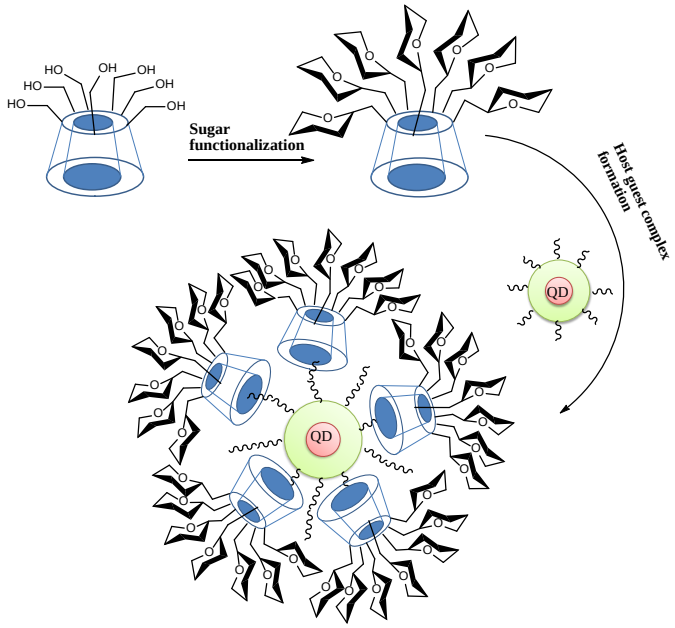

Figure 4. Synthesis of glyco-QDs using host-guest strategy.

-rides coated on QDs surface can dramatically enhance their binding activity through cluster effect. Kim *et. al.*, developed similar quantum dots with hyaluronic acid (HA), and confocal images of B16F1 cells showed HA receptor mediated endocytosis of HA-QDs.[24] whereas HEK 293 cells without HA receptor clearly showed no uptake. *In vivo* assays have shown the accumulation of HA-QDs in liver 5 minutes after intravenous injection. We have used galactose-QDs to show preferential binding and uptake by HepG2 cells that express asiaglycopotein receptors.[22] *In vivo* assay after 2 h showed both mannose and galactosamine-QDs uptake in the liver.

Xiangzho *et. al.*, developed cyclodextrin functionalized CdTe QD systems containing an adamantyl guest, and showed the endocytosis and the drug delivery into the cell.[25]

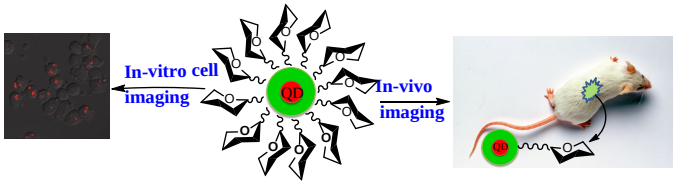

Figure 5. Applications of glyco-QDs

6.2. QDs FOR BIOSENSING SIALIC ACID COMPOSITION

QDs also provide a versatile platform to develop sensitive biosensors. Most of the sugar sensors exploit the fluorescence resonance energy transformation (FRET) principle between QD-lectin and sugar conjugated gold nanoparticles.[26] Recently, semiconductor quantum dots-confined nanometal surface energy transfer (NSET) technique has been developed to detect compositions of different sialic acid forms.[27] In brief, NSET between QDs and gold nanoparticles (AuNPs) is propagated by specific sialic acid-binding protein-carbohydrate interactions and biosensing is based on the switching-on NSET by adding sialic acid that competes for binding to the Sia-binding protein (SBP) (Figure 6).

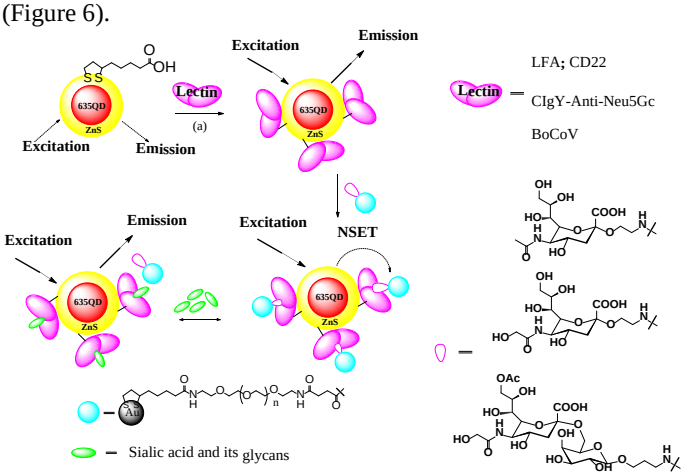

Figure 6. Biosensing of sialic acid using QDs and NSET assay.Adapted from reference 27 with permission from ACS Publication

In order to quantify the sialic acid composition in the biological samples, gold nanoparticles carrying PEGylated Neu5Ac/Neu5Gc sugar moieties were reacted with QDs immobilized with four distinct SBP's. Limax *flavus* agglutinin (LFA), CD22 (Siglec-2), bovine corona virus HE^0 (BoCoV) and Chicken-IgY anti-Neu5Gc (anti-Neu5Gc-IgY) were selected as SBPs. LFA is a lectin that binds to all the common sialic acids, while human CD22-Fc, BoCoV and chicken anti-Neu5Gc-IgY are specific to Neu5Ac/Gcα2-6-linked sialic acids, 9-*O*-acetylated-sialic acids and non-human sialic acid Neu5Gc respectively. Using LFA, we have detected total concentration of sialic acid in a given sample.[27] CD22, anti-Neu5Gc-IgY and BoCoV were able to detect different linkages and forms of sialic acids. An array containing all these four SBP NSET mixture would render high-throughput detection of different forms of sialic acid composition on a single platform.

4. OUTLOOK

It has been more than two decades after the inception of the first quantum dots, that these nanoparticles have found applications in various areas, including the bioimaging, biosensors, solar cells, etc. With the development of glyco-quantum dots, the approach has been further extended to understand the very important roles of glycans on the cell surfaces. We believe that the emergence of glyco-nanotechnology and the imaging concepts have opened up a new sensing process, which the existing biomarkers find it difficult to address.

ACKNOWLEDGMENTS

We thank IISER, Pune, Indo-German (DST-MPG) program and DAE (Grant No.2011/37C/20/BRNS) and CSIR-RA, India for financial support.

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