Chapter-1

1 Introduction and Literature Review

Functional materials are advanced and engineered materials designed for specific functions with predetermined surface morphology and tailored properties. Semiconductors, crystals, nanomaterials, and polymers are some well-known examples of functional materials with unique physicochemical properties[1]. The synthesis and characterization of functional nanomaterials have gained tremendous attention within the past few decades due to their versatile application in health, energy, and the environment. These functional nanomaterials exhibit advanced physical and optical properties compared to bulk materials. Graphene and its derivatives, such as graphene oxide, nanodiamonds, fullerenes, carbon nanotubes, and carbon quantum dots, are examples of carbon-based functional nanomaterials which were extensively used for many applications[2]. Functional nanomaterials possess excellent properties such as magnetism, highly specific active surface area, catalysis, and electrical and optical properties, making them a crucial material for different applications[3]. In the last decade, a plethora of functionalized nanomaterials having tailored morphologies and tuned surface functionalities have been synthesized using a range of starting precursors varying from inorganic metal salts to organic biomolecules, polymers, and biomass[4]. Yet biomolecules-derived functional nanomaterials gathered special attention for biomedical applications attributed to their biocompatibility, stability and specific affinity with molecules of interest. This thesis majorly revolves around using biomolecules as the starting material for designing and synthesizing functional nanomaterials and further exploring their application in health and biomedical domains.

1.1 Biomolecules

Biomolecules are a class of organic substances produced by cells and living organisms. The four major types of biomolecule are protein, lipids, nucleic acid, and carbohydrate. These molecules perform a vast array of functions in humans and various living organisms and have a wide range of sizes and structures. Among these, proteins are the major structural component of the cells. They are responsible for major cellular activities like transport phenomena and biocatalysis. The building blocks of proteins are amino acids. There are 20 different types of amino acids present in nature with unique functionality. These amino acids undergo condensation reactions and connect each other with peptide bonds. The chain of 30 or greater amino acids is often considered a peptide sequence. Later these peptide chains can form specific spatial conformation through non-covalent interactions such as van der Waals, hydrogen bonding, and hydrophobic packing.

Similarly, nucleic acids (DNA and RNA) have the unique function of storing an organism's genetic information. Nucleotides are the basic unit of nucleic acids, consisting of a sugar molecule attached to a phosphate group and a nitrogen-containing base[5]. Nucleotides link each other with phosphate linkage and form single-strands of RNA and DNA. Further, these single-strands connect with hydrogen bonding to form a double-strand helical structure of DNA. All amino acids, nucleic acid bases, DNA, and proteins can have different morphologies and spatial orientations in different environmental conditions. These molecules can self-assemble into functional nanomaterials that can be utilized for various applications in field of health, energy, and the environment[6].

1.2 Self-assembly of Proteins

The coiling and folding of polypeptide chains into various secondary structures (α-helix and β-sheets) leads to stable tertiary protein structures. These structures vary with the sequence of amino acids present in the polypeptide chain, yield diversity in precursor molecular units, and provide an advantage in constructing different hierarchical assemblies through spatially arranged protein units[7]. These units could be easily used as building blocks for designing nano and micro-scale architectures with diverse functionality. If we adopt a soft condensed matter approach to model the protein, then the interaction between basic protein units can be used to determine their self-assemblies. For example, in the case of globular proteins, most of the hydrophobic residues are buried in the interior part, whereas polar or charged residues are exposed on the surface. Globular proteins can self-assemble into different states such as crystals, dense liquids, gels, fibers, and aggregates. Sometimes even two different states can coexist under specific environmental conditions[8]. Figure 1.1 (a) shows the phase diagram for globular protein, where the boundaries of the phase separation depend on the given protein and solution conditions. The metastability of the states has been explained by modeling the globular protein as a simple colloid. However, this model does not entirely explain the self-assembly of globular protein. Later the anisotropy in the protein-protein attraction was introduced into the system for better understanding which showed more comprehension of the experimental results[9]. Proteins can self-assemble into a wide range of structures with different sizes and symmetries at appropriate physiological conditions to perform diverse applications in living organisms. The protein-protein interactions are the primary driving force for many protein assemblies such as protein cages, viral capsids, crystals, colloidal structures, superstructures, amorphous aggregates, and fibrils, as shown in figure 1.1 (b). Hydrophobicity and van der

Waals interactions combine with an array of ionic and hydrogen bonds and act as a driving force for the self-assemblies of protein. These assemblies construct fine nanostructures to microstructures by fusing different symmetrical subunits[10].

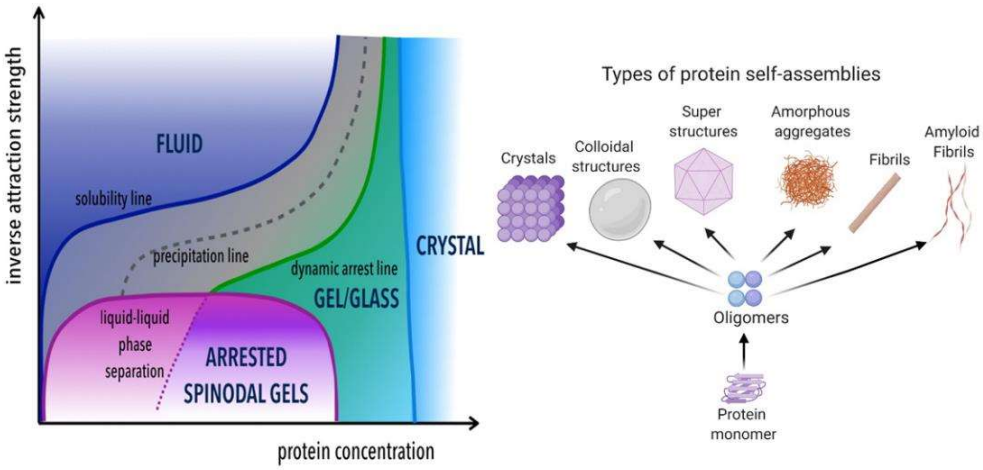

Figure 1.1 (a) Phase diagram for globular protein indicates the different condensed phases it can form[11] (b) Different types of the most observed protein self-assemblies[8]

The rapid development of self-assembled structures provide excellent opportunities for researchers to construct novel structures as functional nanomaterials. Self-assemblies based on proteins have been designed and achieved with a range of nano to microstructures with unique functionality[12]. The supramolecular interactions usually endow the nanostructures with promising properties such as biocompatibility, self-healing, recycling, and responsiveness towards external stimuli. Therefore, using self-assembly to design higher-order structures and utilize them for different applications seems like a powerful method. Thus, we came up with the idea of exploring the fascinating field of self-assembled nanostructures of biomolecules and understanding its applicability to various issues related to biomedicine, such as image-guided drug delivery, biosensing, and skin protection against UVB. Thus, we have synthesized carbon quantum dots and hydrogels using globular proteins such as lysozyme and bovine serum albumin as precursor materials.

1.3 Carbon Quantum Dots (CQDs)

Carbon-based nanomaterials have attracted widespread attention in recent years due to their ease of synthesis and applicability in different fields such as sensing, nanomedicine, and electro-catalysis[2]. Carbon nanotubes (CNTs), graphene, fullerenes, and nanodiamonds are some examples of 2D and 3D nanomaterials[13]. However, the applicability of fullerenes and graphene is difficult due to their low solubility in a water-based medium. On the other hand, the synthesis and separation of nanodiamonds are complex in comparison to other carbon-based materials. Carbon quantum dots (CQDs) are novel zero-dimensional nanomaterial sizes around 10nm with various functional groups on the surface, such as carboxyl, hydroxyl, and amines. CQDs show extraordinary chemical and physical properties such as tunable photoluminescence, high quantum yields, low cytotoxicity, and photo-stability[14]. The simple synthetic routes and comparable optical properties of CQDs provide an essential application in biomedicine, optoelectronic devices, sensing, and catalysis[15]. In 2004, CQDs were reported by Xu et al. for the first time, which were accidentally obtained from the purification of the single-wall CNTs[16]. Before discovering CQDs, semiconducting quantum dots were thoroughly used for various optoelectronic applications such as solar cells, LEDs, and LASER fabrication[17]. Semiconducting quantum dots are also widely studied for *in vitro* and *in vivo* bioimaging in the past decades. Due to high fluorescence intensity, stability, and resistance to metabolic degradation, quantum dots were preferred over organic dyes and fluorophores[18]. However, these quantum dots are toxic due to heavy metals and are temperature dependent that no quantum dots could internalize at 4°C[19]. They penetrate the cell through endocytosis but cannot reach the nucleus. On the other hand, the use of organic dyes as fluorophore materials was also limited due to their narrow excitation and weak emission spectrum, short fluorescence lifetime, and robust metabolic

degradations *in vivo* studies. The properties of CQDs overcome all these challenges as they have high solubility, biocompatibility, a facile method for synthesis and modification, robust and tunable photoluminescence, and high resistance to photo-bleaching. Therefore, carbon-based quantum dots got considerable attention for bioimaging and labeling for *in-vitro* and *in vivo* studies. Overall, the discovery of CQDs opens up a new class of zero-dimensional fluorescence material that has the potential to work as functional material in the field of health, energy and the environment[20].

1.3.1 Types of CQDs

CQDs can be classified into three major categories depending on their core structure, as shown in Figure 1.2. These categories are graphene quantum dots (GQDs), carbon nanodots (CNDs), and polymer dots (PDs). The basic difference between these CQDs is their carbonous core structures. The core of GQDs comprises a few graphene layers with functional groups on edges however in the case of CNDs the core has a crystalline graphite-like structure. On the other hand, PDs can be either crosslinked or entangled linear polymer or polymer chains aggregated around a spherical carbon core. All these CQDs exhibit similar photoluminescence properties despite their different core structures. The photoluminescence properties like fluorescence lifetime, intensities, and wavelength-dependent excitation and emission depend on their structures, size, and presence of functional group on the core surface[21].

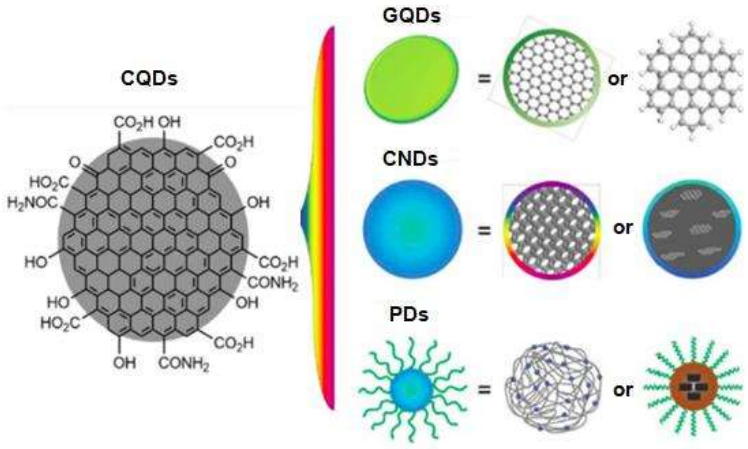

Figure 1.2 Three classes of carbon quantum dots: Graphene quantum dots (GQDs), Carbon nanodots (CNDs), and polymer dots (PDs)[21]

1.3.2 Precursors and Synthesis Methods

In recent years various organic chemicals, biomass, and biomolecules have been used as precursor materials to synthesize CQDs. Mainly nitrogen-rich organic sources were selected to synthesize CQDs to increase quantum yield. The significant carbon and nitrogen-rich compound sources are listed in Table 1-1.

CQDs synthesized from organic chemicals need comparatively high temperature and longer heat duration and are not cost-effective. In contrast, CQDs synthesized from biomass wastes are a cheap carbon source but show meager quantum yields[22]. In comparison, CQDs synthesized from biomolecules and Polymers offer good quantum yield with an abundance of a functional group on the surface. Polymer composites provide the polymeric matrix for CQDs, while the unique PL properties make polymers more applicable in bioimaging, biosensing, and drug delivery. Therefore, polymers have gradually widely permeated every aspect of CQDs.

Table 1-1 List of carbon source materials used for CQDs synthesis

Precursor Material		**PL**	**Ref.**
Organic chemical	Citric Acid, Glucose, Vulcan XC-72 carbon black, Fructose L-glutamic acid, Graphite, O-phenylenediamine, and glycol *p*-phenylenediamine	Green, Blue, Red, Yellow	[22–24]
Biomass	Grass, Coriander leaves, Potato Garlic, Banana, Sugarcane bagasse, Plant leaves, Tea leaves Peanut Shell	Blue, Green, Yellow, Red	[25,26]
Biomolecules	DNA, Amino Acid CQDs, Collagen, L-histidine, or L-arginine, Milk protein casein Egg white	Blue, Green	[27,28]
Polymers	Polyethylenimine, Lignin, Chitosan, Resoles,	Yellow, Green, Blue, Red	[29,30]

Both top-down and bottom-up approaches (Figure 1.3) were used to synthesize CQDs using different techniques. The macromolecule is destroyed using physical and chemical methods to disperse into small-sized CQDs in the top-down process. While in the bottom-up approach, polymerization and carbonization of a series of small organic molecules are used to synthesize CQDs through a chemical reaction. Laser and chemical ablation, electrochemical carbonization, and arch discharge of the carbon resources are some top-down approaches used for the CQDs synthesis[31]. In chemical ablation, strong oxidizing acids carbonize small organic molecules into carbonaceous materials, which can be further cut into small sheets by controlled oxidation. CQDs were also synthesized by laser irradiation of a suspension of carbon materials in organic solvents. By selecting organic solvents, the surface states of the CQDs could be modified to achieve tunable light emission[32]. Electrochemical carbonization is a powerful method to prepare homogenous CQDs;

however, reports showed the limited organic molecules converted into nanodots. In electrochemical carbonization, three-electrode systems were used. Two Platinum sheets were used as the working and auxiliary electrode, and a calomel electrode was used as the reference electrode. The alcohols were transformed into CQDs after electrochemical carbonization under optimal conditions. The applied voltammetry potential can tune CQDs size and the graphitic percentage, and most of the CQDs synthesized by this method show an amorphous nature[33]. In the case of the arc discharge method, the reactor can reach a temperature as high as 4,000 K under an electric current to produce a high-energy plasma. Carbon atoms decomposed from the bulk carbon precursors in the anodic electrode driven by the gas plasma generated in a sealed reactor. In the cathode, the carbon vapor assembly forms CQDs[34]. All these methods help to prepare CQDs with good water solubility. However, synthesized CQDs generally possess a large particle size distribution that extensively decreases the specific active surface area, which may limit the active reaction sites during the electro-catalytic process.

Figure 1.3 Synthesis methods of carbon quantum dots

In bottom-up approaches, hydrothermal/solvothermal, microwave pyrolysis, and ultrasonic methods were used to get quantum size carbon particles[35]. In particular, the hydrothermal method is widely used to synthesize CQDs due to tremendous advantages over other synthesis methods. Hydrothermal is a simple one-pot (teflon-lined stainless steel autoclave), a single-step process in which small organic molecules and/or polymers are dissolved in water or organic solvent to form nanoparticles with homogenous particle size and high quantum yield. The organic molecules and/or polymers merged at relatively high temperatures to form carbon seeding cores and then grow into CQDs with a particle size of less than 10 nm. Current reports suggest that this method yields the highest QY of CQDs, up to about 80%, almost equal to fluorescent dyes[36]. The other frequently used bottom-up technique is microwave-assisted pyrolysis due to the rapid synthesis of CQDs and commercialization. This method is simple, fast, and environment friendly. CQDs synthesized by this method are rich in oxygen-containing functional groups, making them the best candidate for synthesizing carbon-based electrocatalysts by providing sites for metal conjugation[37]. Each of these methods have its own set of advantages and limitations. Most of these methods lack the homogenous particle size after carbonization and can be improved by post-treatment processes such as centrifugation, filtration, dialysis, column chromatography, and gel electrophoresis[38]. The surface passivation of CQDs has also been performed using these methods by changing the experimental parameters such as solvents and temperatures, which can significantly alter the photoluminescence properties.

1.3.3 Optical and Physical Properties

CQDs are quantum confined particles with a size less than 10nm. The small size of the CQDs leads to the quantum confinement effect and is the reason for extraordinary optical properties. The quantum confinement leads to the formation of electron-hole pairs (exciton)

within the material, forming discrete energy levels instead of continuous energy bands[39]. Generally, CQDs shows optical absorption in the UV region ranging from 230 to 300nm with a broad peak and tail extending to the visible range. The absorbance in the UV region is attributed to the π–π* electronic transition of the C=C bonds and the n–π* transition of C=O bonds[40]. Various functional groups on the CQD surface groups contribute to the broad peak and extended tail in the visible region[41].

Photoluminescence (PL) measurements of CQDs show excitation-dependent emission behavior. Different fluorescence emissions of CQDs can be achieved by regulating the environmental conditions such as pH, concentration, heating temperature and duration. All these physical conditions can alter the physical and optical properties of CQDs in pre and post-synthesis conditions. Changes in pH lead to the protonation or deprotonation of the functional group on the CQDs surface and alter the emission properties. Similarly, change in concentration or heating temperature can lead to different surface state emissions or non-radiative decay processes at the surface of CQDs. In one of the studies, various organic carbon sources with different molecular mass such as glucose, sucrose, glucosamine cellulose, and ascorbic acid were taken and converted into CQDs using the hydrothermal method with various solvent conditions (octadecene, octadecene-fatty amine, octadecene-fatty acid, ethylene glycol and water). These conditions promote the rapid nucleation and overgrowth of FCNs (fluorescent carbon nanodots) up to 4nm in size and result in the blue and green color of emission[42]. The addition of H_3PO_4 and H_2SO_4 based carbonizations at lower temperatures (80–120^{0}C) leads the particle sizes between 1 to 10nm and produces PL in blue, green, yellow and red colors, as shown in Figure 1.4.

In addition to conventional PL emission, certain CQDS have an Up Converted Photo Luminescence (UCPL) emission feature. In UCPL, the emission wavelength has lower

energy than the excitation wavelength, which corresponds to the emission wavelength being shorter than the excitation wavelength, opposite to the typical fluorescence mechanism[43]. Reports suggest that the CQDs synthesized by laser ablation or ultra-sonication methods exhibit strong photoluminescence with two-photon excitation near-infrared (800 nm). When these CQDs are excited by long-wavelength light (from 700 to 1000 nm), they show up-converted emissions ranging from 450 to 750 nm[44]. However, some related reports have doubts about the UCPL because they have used monochromatic xenon lamps as the light source without filters, which can have second-order diffraction of the light at half wavelength. Hence, routine PL that always coexists with the excitation wavelength can be misunderstood as fluorescence due to CQDs[45].

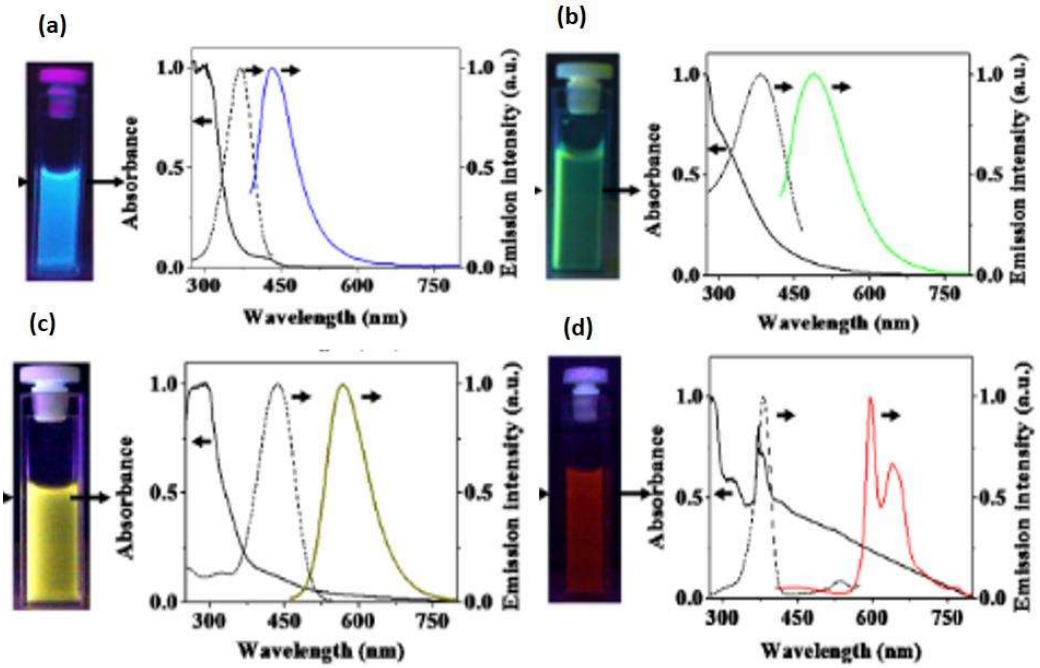

Figure 1.4 Images of CQDs solutions under appropriate excitations and their absorption (solid black lines), excitation (dashed black lines) and emission (color lines) spectra. Emission spectra were measured by excitation at 370 nm, 400 nm, 425 nm, and 385 nm for ((a) CQD – blue, (b)CQD – green, (c)CQD – yellow, and (d) CQD – red, respectively. All excitation spectra were recorded in respective emission maxima [42].

The fluorescence of CQDs can be quenched efficiently through either electron acceptors or electron donors by adding analytes to solutions. These substantial photo-induced electron transfer properties open up new opportunities for potential uses in metal detection, biosensing, and redox processes like light energy conversion and related applications. It was reported that photo-excited CQDs have a redox-active nature, which reduces metal ions in an aqueous solution. Specifically, the irradiation of CQD solution with a noble metal (silver, gold, or platinum) salt results in the formation and deposition of the noble metal on the surface of CQDs and leads the fluorescence quenching[46]. In some cases, interaction with the analyte can facilitate the electronic transfer, and instead of quenching, enhanced fluorescence has been observed. In addition to the above stated properties, CQDs show longer decay time, chemical stability and negligible photo-bleaching. The optical properties of CQDs make them a potential tool for bioimaging and sensing application.

1.3.4 Mechanism of Fluorescence

The exact mechanism for wavelength-dependent fluorescence of CQDs is still debatable. Unlike semiconducting quantum dots, the photo-physical properties of carbon quantum dots are not directly correlated with the size of the particle[47]. Various studies have been done to understand the mechanism of origin of these behaviors. Still, there is ambiguity if this occurs because of optical selection of differently sized nanoparticles (quantum effect) and/or different functional groups present on the CQDs surface act as emissive traps[48]. The exact mechanism for wavelength-dependent emission is currently unresolved and a topic of active research.

Moreover, the reason for surface passivation is only partially understood but appears to be linked to the synthesis methods. However, as the particle size distribution narrows down and particles have similar surface functionally, more and more cases have emerged with an

excitation-independent emission position. Some reports also show that the HOMO–LUMO gap depends on the size of the graphene fragments and as the size of the carbonous core increases, the HOMO–LUMO gap decreases gradually and changes the excitation energy of the electrones[49]. However, some studies have also shown that CQDs derived from different alkyl gallates[50] with different sizes display steady-state wavelength-independent wavelength, which implies that PL emission of the CQDs is size-independent[51].

Many studies suggest that the origin of photoluminescence has been related to the percentage of Sp^2 and Sp^3 hybridized species present on the CQDs surface. The Sp^2 domain size and the abundance of oxidized surface defects play an important role in deciding the fluorescence emission range. Multiple fluorescence intensity levels have been observed with single CQDs, suggesting the presence of numerous chromophoric units within the core and emissive surface states. However, direct imaging of surface-accessible spatially localized oxidized defects is still lacking[46]. Recent reports claim that CQDs synthesized from solvothermal methods are rich in spatially localized oxidized defects, suggesting that presence of carboxylates and phenolic hydroxyls are responsible for their wavelength-dependent fluorescence properties. Various models were prepared to study the redshift in the fluorescence spectrum of CQDs based on degrees of sp2 hybridization of carbon surface domains. Results suggest that emission from oxygen-rich surface defects is the major reason due to direct imaging of localized optically active defects and the sensitivity to pH of the CQDs. The abundance of such defects increases the probability of a smaller energy gap and produces red fluorescence emission. SMA−STM reveals that the emissive defects are not necessarily the ones with the smallest electronic bandgap, although smaller gaps correlate with emission wavelength[52].

1.3.5 Electrochemical Properties

CQDs possess exceptional charge transfer properties, enhanced electroconductivity, and a large effective surface area with minimal toxicity compared to other carbon-based nanomaterials or semiconducting quantum dots. The cost-effectiveness and ease of synthesis routes make them a promising candidate for electrochemistry and electrocatalysis. The large functional groups such as hydroxyl, carboxyl, and amine on the surface provide many sites for surface modification and enhance the intermolecular conductivity and electrocatalytic activity. The surface functionality of the CQDs can be tuned by pre and post-synthesis treatments. The doping of heteroatoms and metal ions can significantly improve the electronic properties of CQDs by changing the intramolecular charge transfer properties. All these properties make them ideal electrocatalytic agents to serve desired electrochemical applications[53].

1.3.6 Biological Application

The physical and optical properties of CQDs make them a promising candidate for many applications. CQDs are being used in various applications such as bioimaging, medical diagnosis, biosensing, chemical sensing, photocatalysis, and photovoltaic devices. In past decades, remarkable advancements have been made in synthesizing bio-probes with strong PL and stable CQDs. Nevertheless, the biocompatibility of CQDs is still a concern for any further applications in living cells and tissues. A comparative study showed that CQDs are more biocompatible and luminescent with a QY of 20% than CdSe/ZnS QDs. The typical size of the CQDs is less than 10nm, whereas semiconducting QDs are more than 20nm. The smaller size gives an advantage to CQDs to be used as probes in small biological structures, and minimum volume can be used in *in vivo* injections. During the synthesis reaction or post-treatment, surface passivation can also be done by a different mechanism

that produces high luminescence intensity with a two-photon excitation mechanism in the NIR region. The CQDs passivated with functional groups, such as PEG, PEI, and PAA (polyacrylic acid), were also synthesized and injected into mice for 28 days and evaluated for toxicity cytotoxicity assays[54]. Results confirm that functionalized CQDs are highly biocompatible, and fluorescence intensity does not alter with modifications. CQDs passivated with polymers were not toxic up to a very high concentration than the required concentration needed for bioimaging and other associated applications[55].

1.3.6.1 Bioimaging

CQD are fluorescent materials with higher quantum yields than conventional dyes and quantum dots. CQDs synthesized from hydrothermal or microwave methods can enter the cells and emit green fluorescence. Surface functionalized CQDs were also synthesized using different molar ratios of alkali lignin, citric acid, and ethylenediamine, which changed their optical properties for bioimaging, and cell viability remained unaffected. They displayed 90% cell viability when incubated with HeLa cells at the concentration of 0.62 mg ml^{-1}, even after 24 h. fluorescence microscope images confirm that many CQDs entered the cells through endocytosis and emitted fluorescence[56,57]. They mainly accumulated in the cytoplasm, and weak emissions were observed from the nucleus. These CQDs show stable fluorescence behavior compared to the commercially available fluorophore 4′,6-diamidino-2-phenylindole (DAPI) used to stain the cell's nucleus, even under changing temperatures from 4°C to 60°C and when undergoing photo-bleaching treatment. When these CQDs were excited at different wavelengths, they emitted red, green, and blue fluorescence. Due to their biocompatibility and inherent ability to emit multicolor fluorescence, CQDs bring great opportunities in bioimaging and biolabeling[58].

1.3.6.2 Fluorescent Biosensing

CQDs were also used to fabricate biosensors of their aqueous solubility, high photo-stability, excitation-dependent multicolor emission, non-toxic nature, and cell permeability. CQDs detected a series of biomolecules, analytes, neurotransmitters, and genetic materials at very low concentrations in vivo and in vitro studies. The CQDs-based biosensors can visually monitor glucose, phosphate, cellular iron, potassium iron, and pH[59]. Many studies showed that nucleic acid with selective single-base mismatch could be observed by CQDs using the fluorescence on-off-on mechanism[60]. Reactive oxygen species (ROS) are important biomarkers for many diseases, including cancer, arthritis, neurodegenerative disorders, DNA damage, infections or inflammations, and chemotherapeutic drug screening. Functionalized CQDs were encapsulated with polymers and hydrogels to develop ROS sensors to check the drug's efficacy by measuring ROS levels after administering chemotherapeutic compounds[61]. The inner filter effect of CQDs was used to detection of β-glucuronidase (GLU) inhibitor, a biomarker for early-stage cancer diagnosis and many physiological diseases, because of its inhibition activity to control the proliferation of cancer cells. The other method to detect cancer at its early stages is checking the cell's environment. Generally, pH values of the normal tissues are in the range of 7.2–7.5. However, the pH of tumor cells is mildly acidic and ranges from 6.4–to 7.0[62]. CQDs synthesized by the electrochemical method were used to develop a two-photon fluorescent probe to observe pH changes from 6 to 8.5 with high sensitivity and specificity. The synthesized CQDs were effectively used in two-photon biosensing and bioimaging to monitor the pH changes in living cells and tissues. Adsorption–desorption of H^+ ions on CQDs surface induces a shift in fluorescence intensity by forming covalent bonds with terpyridine-based receptor molecule. In real-time, the imaging and biosensing of pH were

accomplished in *vitro* in lung cancer A549 cells and mouse LLC-MK2 cells and *in vivo* tumor tissues by inducing tumor cells in nude mice through two-photon microscopy[63]. Recently, multiple studies have shown that CQDs fabricated by biomasses and waste carbon products can show selective and sensitive detection of various ions, metals, and molecules in actual samples.

The ability of CQDs to detect metal ions is the inevitable sensing application, including biological and chemical scenarios. Metal ions get attracted and align themselves in a certain way due to the presence of negatively charged ions on the aromatic functional group CQDs surface. Even a very minute concentration of analytes or metal ions can cause luminescence quenching and can be detected. CQDs were also conjugated with other dyes and active molecules to synthesize dual emission nanoparticles; for example, red fluorescence of rhodamine and blue fluorescence of CQDs was emitted under a single excitation wavelength. After adding Cu^{2+} metal ions, blue fluorescence intensity decreased significantly compared to red. This phenomenon can be used for the selective detection of metals in vivo and in vitro studies[64]. All these findings suggest that CQDs can act as non-invasive detectors that are inexpensive and sensitive and help improve human health.

1.3.6.3 Electrochemical Biosensing

CQDs have high stability, ease of surface functionalization, and good electrical conductivity, making them potential candidates for biosensing, chemical sensors, optical sensors, photovoltaics, and electrocatalysis. CQDs have attracted significant attention in developing electrochemical biosensors to detect biomolecules, neurotransmitters, analysts, vitamins, antigens, bacteria, etc[65]. CQDs have been conjugated with other materials such as metallic nanoparticles, conductive thin layers, and dendrites to form stable and electrochemically active electrodes for selective detection and enhanced electrochemical

activity[66]. Graphene composite CQDs show good catalytic activity in electrochemical bio-sensing of damaged DNA markers. Another electrochemical biosensor has been developed to identify glucose with glucose oxidase/GQDs/carbon-based ceramic electrodes. These bio-conjugated/CQDs, graphene/CQDs, and Ceramic/CQDs electrodes have shown high biocompatibility, a hydrophobic carbon frame, hydrophilic edges, and more absorption of the enzymes on electrodes[67]. In one study, CQDs nano-composites PtNPs-CDs/IL-GO was used to detect H_2O_2. Due to their unique chemical structure and presence of multiple active sites, they are responsible for enhanced catalysis of H_2O_2 using electrochemical oxidation and reduction reactions. This hybrid system showed significant selectivity ranging from 1 to 900 μM and a low detection limit of 0.1 μM concerning the reduction of H_2O_2, which are the characteristics of suitable electrochemical sensors having high conductivity and electrocatalytic activity. The electron transport phenomenon involved in the reduction process increases the method's selectivity and sensitivity[68]. Many studies have been done to selective detection of pharmaceutical formulations, hormones, neurotransmitters, and other active molecules responsible for the essential signaling pathways in the human body using electrochemical methods.

1.3.6.4 Drug Delivery

Drug delivery is one of the significant applications of CQDs. CQDs can do targeted drug delivery to the active site to achieve maximum therapeutic outcomes in an organism. In the last few years, CQDs emerged as promising materials to develop new therapies and improve the efficacy of drugs due to their excellent bioavailability, increased circulation times, reduced toxicity, and controlled particle and binding sites[69]. Traditionally available nano-based drug delivery systems such as polymeric missiles, liposomes, and microspheres succumb to biological systems. These methods use the passive methods for drug release,

where effectiveness was directly related to circulation time[70]. Therefore, the development of nanoparticle-based drug delivery, which could support the systemic and intracellular evaluation of the drug and beholds its original properties with high resolution and high sensitivity, is highly required. CQDs have attracted researchers as an excellent drug carrier over the past decades due to their small size, versatile surface chemistry, semiconducting nature, and luminescent properties that allow real-time monitoring of CQDs vehicle transport and drug release cellular levels. Especially during cancer treatment, conventional methods such as chemotherapy and radiotherapy typically lack cellular precision and cause additional drug toxicity and multi-drug resistance problems. Therefore, targeted drug delivery of biocompatible carriers of drugs can be preferred over conventional methods to improve efficacy and reduce side effects. Functionalization of the CQDs avoids the leakage of the drug in normal cells. Different mechanisms for targeted drug release were used, such as receptor binding, MRI imaging pH-responsive, and reduction potential-based release. The report shows that conjugated systems of CQDs with theranostic doxorubicin (DOX) were incorporated in targeted drug release in the nucleus, mitochondria, and apoptotic cancer cells[71]. Fluorescence of CQDs helps to locate the difference between living cells and cancerous cells and enables imaging-guided drug delivery. Many biological studies confirm that drug-loaded CQDs were more localized than DOX alone, drastically affecting the drug's dosing amount and efficacy. CQDs can work as efficient dual targeted delivery agents comprising bioimaging and specific drug delivery with minimum cytotoxicity.

1.3.6.5 Crossing Blood-Brain Barriers

One of the significant features of CQDs is their small size which ranges from 2-10nm is very important for the biomedical application related to crossing the blood-brain barrier (BBB). BBB is a tight junction (TJs) of endothelial cells that serve as a strong barrier for

substances entering the brain. Recent studies show that CQDs and their conjugated nanoparticles were used to deliver drugs to targeted sites in the brain. Still, the sizes of these nanoparticles should be less than the size of the capillaries to cross the BBB[72]. In the case of a brain tumor, the delivery of a drug or the imaging probes to the cancerous site is difficult because of the selectivity and small pore size of BBB. The successful insertion drug or imaging probe depends on the size and topography. CQDs were injected into the different organs of mice, and bioimaging was done after 6 hours of incubation bright fluorescent has been observed, which confirms that CQDs can enter the brain by crossing the BBB. Many studies showed similar results and later used these CQDs for bioimaging and targeted drug delivery. Fluorescent CQDs were coated with other materials such as magnetic nanoparticles, hydrophilic polymers, and nano-drug for specific applications like *In vivo* fluorescence imaging of glioma cells and sustained and targeted release of DOX in brain tumors[73]. In addition to all these applications, CQDs were used for many other biomedical applications such as photodynamic therapy[74], gene delivery[75], synthesis of pharmaceutical formulations[76], and treatment of infection and inflammation[77]. The specific bioapplication of modified CQDs is mentioned in Table 1-2.

Table 1-2 List of biomolecules based CQDs with different emission wavelengths used for biomedical applications

CQDs	**Emission wavelength**	**Biomedical application**	**Ref.**
PEG CQD, PCQD	455-790 nm, 460 nm	Bioimaging	55,56
L-CQD,CQD-Tpy	454-535 nm,495 nm	Biosensor	57,78
CQDs/Silica	450nm	Drug delivery	79

T-tages CQD, Dox conjugated CQDs, CQDs PEI	460-365 nm, 300-500 nm, 365nm	Gene delivery	22,71
SWCNTs-PEG-FE_3O_4@CQDs	330-390 nm	Photodynamic therapy	80
FeN@CQDs	470 nm	Photothermal therapy	81
CQDs/H2O2, PtNPs-CQDs/IL-GO, CQDs/GQDs	440 nm, 462 nm, 440-625 nm	Electrochemical biosensor	82,83
CQDPAs, Quaternary ammonium CQDs, Cur-CQDs	300-450 nm 497 nm	Antibacterial/Antiviral effect for treatment of cancer	84
NHF-CDs	500-580 nm 424 nm	Pharmaceutical formulations	85

1.4 Hydrogels

Hydrogels refer to the three-dimensional hydrophilic crosslinked networks that absorb high amounts of water but resist dissolution when placed in water or other biological fluid. Hydrogels can be synthesized in various physical forms, from nanofibers to microstructures such as thin films, slabs, and coatings[86]. In swollen states, hydrogels resemble soft and rubbery consistency like polymer structures resembling natural living tissues, making them promising and ideal biomedical material[87]. The crosslinked network of the hydrogels contains a large amount of hydrophilic functional groups such as hydroxyl, carboxylic, primary amidic, and sulfonic amidic present at the backbone and forms physical or chemical bonds between polymer networks. The nature of the crosslinks between the polymers chains of hydrogels decides the physical behavior of hydrogels, which varies from pure elastic material to viscoelastic. Hydrogen bonding, van der Waals interactions, covalent bonds, or physical entanglements are the major interaction present in crosslinked

backbone and the reason for the insolubility of gels in an aqueous environment[88]. The biodegradability and biocompatibility confirm the presence of labile bonds in the network structure. These labile bonds tend to dissolve under altered environmental conditions either through the action of enzymes or harsh physiological conditions such as temperature, pH, or electric field or through chemical reactions, mainly through hydrolysis. The structure and thermodynamics of the precursors and crosslinking agents are vital in deciding the molecular mesh size and diffusional performance of hydrogels. Numerous preparation methods are reported in the literature where natural polymers or synthetic sources were used for hydrogel fabrication[89]. Generally, hydrogels are synthesized by chemical crosslinking or physical interaction between the polymer chains. For the preparation of chemical crosslinked hydrogels, crosslinking agents are used to develop crosslinks between the polymer chains connected by covalent or ionic bonds. Some methods to prepare chemically crosslinked hydrogel are suspension polymerization, copolymerization, and chemical reaction of corresponding functional groups, enzymatic crosslinking, and polymerization through irradiation. On the other hand, Physical hydrogels are formed by molecular entanglements and/or secondary forces, including hydrogen bonding, crystallization, protein interaction, ionic interaction, and hydrophobic interaction. These bonding are formed via different routes, such as bulk polymerization, radiation method, solution casting, solution mixing, and interpenetrating network formation[90].

The biocompatible and degradable matrix of hydrogels provides perfect starting points for neo-tissue growth, making them potential candidates for biomedical applications such as organ transplants, superabsorbent of shock and heat, and wound dressings, TE scaffolds, diagnostic devices, drug delivery carriers, and biosensors. In recent years, hydrogels were synthesized using different precursors and cross-linkers via physical and chemical

techniques. They can be formulated by either natural polymers or synthetic sources. Numerous methods for preparing biomedical hydrogels have been reported in the literature. The scope of this thesis is limited to hydrogels synthesized from proteins using the hydrothermal method[91].

Proteins are natural polymers that can be used for the fabrication of hydrogels. The structural and functional characteristics such as biodegradability, biocompatibility, tunability and wide range of pH stability make proteins one of the best candidates for hydrogel synthesis. Indeed, all the proteins can be crosslinked through amine, and carboxylic acid functional groups, particularly aggregated proteins converge into a gel network, known as the protein gelation process by physical cross-linking. These gel networks hold water molecules within their structure. They can be stabilized through non-covalent cross-links, such as hydrophobic, van der Waals, electrostatic interactions, and hydrogen bonds. Physically crosslinked hydrogels show improved stability, controllable degradation rate, and mechanical properties in physiological circumstances. Still, so many studies confirm that the addition of cross-linking agents can drastically enhance the physical properties of hydrogels[92,93].

1.4.1 Protein-based Hydrogels (PBH)

All proteins are composed of peptide chains amino acids known as a primary structure which forms secondary structures of the proteins such as α-helix, β-sheet, turn, and loop contents. These secondary structures arrange themselves by different interactions to form protein's 3D conformation known as tertiary structures[94]. The presence of carboxyl and amino functional groups on protein helps form hydrogels via physical, chemical, and enzymatic cross-linking approaches. Fabrication of PBH via the physical crosslinking

method offers two significant advantages. First, this is the most simple and nontoxic method, and second, this process does not change the native properties of the protein[95].

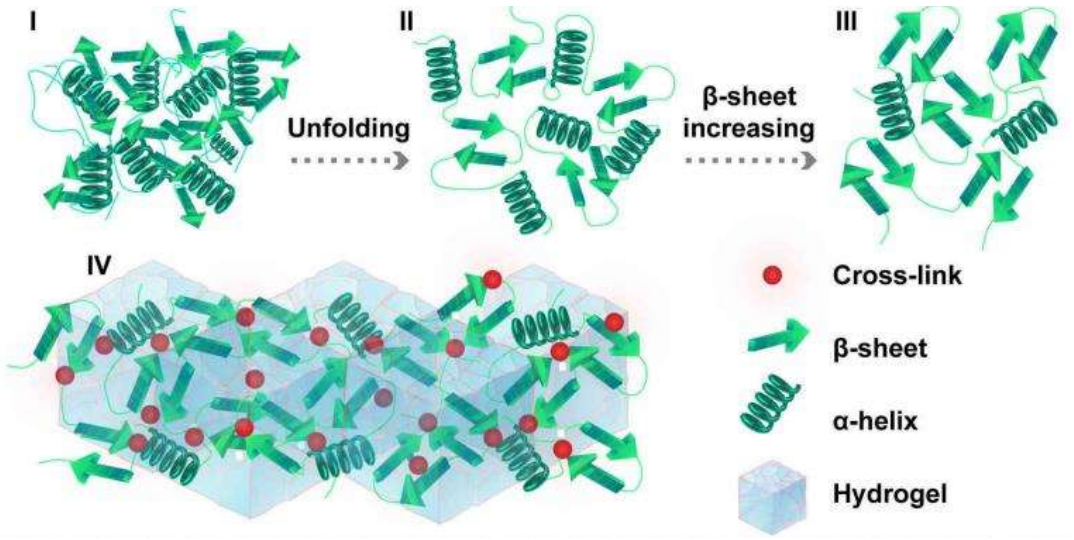

Figure 1.5. The protein's unfolding and structural changes and interaction within the hydrogel matrix. (A) (I, II) Conformational changes from the third to second structure. (III, IV) Increasing the β-sheet content, which forms the desired gel matrix[96].

Figure 1.5 represent the most common and standard process of protein gelation in which protein starts to unfold and accumulate in a gel matrix. An external stimulus such as pH or temperature changes the 3-D conformation of the protein into a secondary structure, leading to the formation of random coil content, α-helix, and β sheet, which provides unlimited flexibility within the protein structure and favors the gelation process. The structural alteration by the unfolding and change in the secondary structure content offers a template for cross-linking between the amino and carboxyl protein groups, as shown in Figure 1.5 (I, II). Exposure of amino and carboxyl groups on the surface of unfolded proteins creates a hydrophilic environment leading to the emergence of high swelling property, an inherent feature of PBHs[97].

Several studies show that proteins containing amino acid cysteine can form better hydrogen than other proteins due to the presence of the –SH functional group in the cysteine structure, which facilitates the water holding and absorption capacity. Additionally, parameters like protein and polymer concentration, pH, and other hydrophilic functional groups impact the swelling ratio, which can be adjusted based on the application[98]. During the physical crosslinking process, protein should unfold and move back to the secondary structure such that the β-sheet content should increase and helps to form the gel matrix, as shown in Figure 1.5 (III. IV). Several studies suggest that solvents and ultra-sonication can exchange and induce the β-sheet content into protein molecules, directly assembling a hydrogel using hyaluronic acid[99]. The other major factor for gelation is the protein concentration, at a higher concentration physically cross-links itself to form hydrogels. In addition to these single network wrapping or multiple networks superposition, some other common strategies exist to form hydrogel using a physical crosslinking method[100]. In these methods, the matrix was prepared by unfolding protein using heat, which causes denaturation, aggregation, and gelation. After that second layer of polymer matrix was added to the system. These superimposed double matrix PBHs possesses improved mechanical features with instanced adhesion properties. Freeze thawing, crystallization, amphiphilic graft block copolymers, ionic interaction, complex coacervation, and polar electrostatic reactions are other well-defined methods to synthesize hydrogels from proteins. In these methods, hydrophobic and weak interactions, such as electrostatic interactions or hydrogen bonds, drive forces to form protein-based double matrix hydrogels. Hydrogels synthesized by these methods showed thermos-plasticity, recyclability, self-healing ability, and reusability, which can benefit many biomedical applications. Physical crosslinking enhances the stability of hydrogels, shows a controllable degradation rate, and has excellent

mechanical properties in physiological conditions. According to the hydrogel's desirable properties, suitable strategies can be chosen to execute the biomedical application of protein-based hydrogels. Table 1-3 shows the list of proteins used for hydrogel using physical crosslinking with other hydrogel components.

1.4.2 Biological Application of PBH

Tissue engineering has an essential contribution to regenerative medicines. In the last decade, extraordinary research has been done to synthesize soft hydrogels for tissue engineering applications. Traditional materials used for tissue engineering were deficient in hemostasis, repair, and regeneration. Hydrogels fabricated from protein show various properties such as wound healing, tissue repair, and regeneration with excellent biocompatibility. Proteins were crosslinked with other biomolecules to enhance properties and showed extraordinary results. Collagen and alginate were used to fabricate a temperature-sensitive hydrogel bed that allows the blood to clot rapidly and helps wound healing[101]. BSA-polycaprolactone bio-collagen was also developed for wound healing which can be injected into wounds to allow them to heal and accelerate tissue regeneration at room temperature. These hydrogels are used to recover lesions of conventional injuries and treat cancerous lesions[102]. Protein-polymer hydrogels were encapsulated with metal nanoparticles to treat tongue cancer without damaging the healthy side tissue. Generally, hydrogels used in tissue engineering are responsive to temperature, redox reactions, mechanical stress, magnetic field, or gas via free radical polymerization[113].

Table 1-3 lists of proteins used for the synthesis of hydrogels using different active components

Protein	**Hydrogel components**	**Effect of protein Component**	**Ref.**
Collagen	Alginate	Showed rapid mouse myoblast cells' infiltration and micro-vascularization, Increased chondrocyte cell viability (up to 90%), Formed a lattice pattern for cornea structure, Displayed significant osteogenic differentiation	103
Gelatin	GelMA, Alginate, PEI-Ppy,	Promoted mouse chondrocytes' adhesion, viability, and proliferation, Developed antibacterial properties Aided keratinocytes' proliferation and differentiation	104,105
Serum Albumin	PEG-SS2– bioglass, Sodium alginate Hydroxyapatite, Fibroin	Accelerated the wound healing process, Significantly increased osteogenesis differentiation, Affected the differentiation and proliferation of human bone marrow-derived mesenchymal stem cells, Created contractile heart tissue	106,107
Elastin	Collagen, Plasma	Accelerated the heart valve endothelial cells' growth, Improved mechanical characteristics and biological capabilities, Tackled bacterial infection, Controlled angiogenesis	108
Keratin	Konjac glucomannan, Oat	Showed rapid penetration, propagation, and differentiation of MSCs, Aided collagen formation, Decreased gel formation time, Developed hydrogel biocompatibility	109,110
Silk	Fibroin, Glycidyl methacrylate, Chitosan	Improved rat cardiomyocytes cells' attachment and activities, Provided the repair of osteochondral tissue, Displayed proliferation and viability of chondrocytes cell after four weeks, Positively impacted MC3T3-E1 cells' adhesion and proliferation	111,112

Protein-based hydrogels are a water-based porous non-toxic, biodegradable network that can encapsulate materials and release them slowly and continuously by diffusion and permeation at a stable and controlled rate. Protein hydrogels have high drug loading and releasing capacity compared to conventional hydrogels, making them the best prospect materials in biomedicine. The composite material of collagen, BSA, gelatin, and silk proteins was fabricated and used as a controlled drug delivery platform for cardiovascular and renal diseases. The dense matrix of protein hydrogels slows down the speed of water entering the internal structure of the drug. Besides that, drug encapsulation by protein hydrogels enhances the short-life time of the drug in physiological conditions and is degraded in vivo within six weeks[114]. Hydrogels have fabricated dual drug loading systems that help treat tumor chemotherapy. In-short proteins hydrogels are a potential candidate for drug delivery and encapsulation in therapeutic applications. In addition to above mentioned biomedical applications, protein gels are frequently used for wearable sensors, absorption, and other biomedical purposes and 3D printing[115]. Conduction protein hydrogel materials emerge as a potential and promising material for flexible electronic sensor applications. Wearable medical devices are getting more attention in medical electronics and should be skin-friendly, biocompatible, high sensitivity, and low hysteresis. Presently conductive protein hydrogels were synthesized by mixing silk protein, PAAm, graphene oxide and in the same ratio of poly (3, 4-ethylene dioxythiophene) and poly (styrene sulfonate). Protein hydrogels were also used for 3D printing, where the complex scaffold geometries can be synthesized, and patient-specific implants can be provided. Conventional hydrogels have low mechanical strength and viscosity, leading to rapid gel degradation, and cannot be used for printing applications. Instead, protein-based hydrogels show viscoelastic properties, making them ink material for 3D printing. The mechanical strength

of hydrogels was increased by incorporating gelatin, PACG, and carbon nanofibers, which facilitate a robust framework, growth, and better therapeutic application.

Overall, we can state that protein-based hydrogels retain protein's physical and chemical properties at the molecular level and possess advanced properties after gelation. Crosslinking these protein hydrogels with other biomolecules and polymers increases mechanical strength and adds various properties. Depending on the application, physical and chemical properties like adhesion, responsiveness, swelling, degradability, and mechanical strength can be altered. They can be used for 3D printing, tissue engineering, drug delivery, wound healing, etc.

1.5 Scope of the Thesis

Overall, the current literature survey confirms the applicability of carbon quantum dots and hydrogels for various biomedical applications. Selecting the correct precursor materials makes it possible to synthesize homogenous CQDs with high quantum yields. Later, the physical and optical properties of CQDs can be enhanced by surface modification and used for desirable applications. Similarly, hydrogel showed versatile application in biomedicine where the physical and mechanical properties can be easily tuned by external stimulates and further applied in various biomedical applications. The scope of this thesis includes the synthesis and characterization of nanomaterial synthesized by biomolecules for biomedical application. Globular proteins, bovine serum albumin, and lysozymes were used to synthesize protein-based hydrogel and nanodots. Later, these materials were applied for their best-suited applications like sensing, drug delivery, bioimaging, and UV-protection applications. Chapter 3 deals with temperature-dependent self-assembled phases of bovine serum albumin via hydrothermal process to synthesize novel functional hydrogel for skin

protection against UVB. In the fourth chapter, synthesized novel protein dots have been used for the drug delivery of melatonin as drug delivery carriers with enhanced biological efficacy of melatonin in Breast Cancer Cells. In chapter five, synthesized novel protein dots have been used for the drug delivery of melatonin as drug delivery carriers with enhanced biological efficacy of melatonin in breast cancer cells.