Carbon Quantum Dots for Stem Cell Imaging and Deciding the Fate of Stem Cell Differentiation

Misba Majood,§ Piyush Garg,§ Radhika Chaurasia,∥ Aakanksha Agarwal,∥ Sujata Mohanty, and Monalisa Mukherjee*

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INTRODUCTION

In the domain of medicine, stem cell treatment has enabled us to understand a variety of problems that were before incomprehensible.1−3 Their limitless capacity for self-renewal and ability to differentiate into any cell type sets them apart from other cell types. Their classification is based on a hierarchical pattern. Totipotent stem cells, which are only found in the early embryonic stage, are the most primitive type of stem cells capable of forming a whole embryo. Subsequently, embryonic stem cells reach the pluripotent stage when they may develop entirely into the three germ layers: endoderm, mesoderm, and ectoderm. Subdivisions of these cells progressively destroy their pluripotency, leaving them stationary and restricted. When multipotent stem cells are supplemented with appropriate growth factors and inducers, they differentiate into a specific cell type. These cells are known as adult stem cells (ASCs), and practically, ASCs are found in all specialized tissues in a metabolically quiescent state.4 Embryos are destroyed during the isolation or extraction of embryonic stem cells (ESCs); hence, using ESCs for scientific purposes raises religious concerns and is deemed sometimes immoral. While induced pluripotent stem cells (iPSCs) are formed from somatic cells and may be employed for therapeutic purposes, many laboratories lack the necessary infrastructure and knowledge to manufacture iPSCs, making them an inconvenient source of stem cells. Since both ESCs and iPSCs have a limited number of drawbacks, researchers

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believe ASCs to be the first choice for stem cell-based therapeutics.\(^5\)

Although stem cell treatment has a wide variety of applications and a promising future in the field of regenerative medicine, there are still several constraints connected with the use of stem cells.\(^6−9\) Long-term survival of engrafted cells, limited control over the fate of stem cells, and the irreversibility of the treatment are some of the potential risks associated with this procedure. Other potential risks include the type and proliferative status of the stem cells used in the procedure and the route of administration to deliver them to the intended region.\(^10,11\) Significant risks are the tumor formation ability of stem cells,\(^12\) immune response, and transmission of serendipitous agents.\(^13\) Due to the difficulty of controlling stem cell fate without physical or chemical cues, the existing stem cell therapies are ineffective at entirely replacing injured cells. Due to these limitations, the transplantation productivity and survival rate of ex vivo differentiated stem cells are extremely low.

**Mesenchymal Stem Cells (MSCs).** ASCs, often referred to as MSCs, have demonstrated tremendous potential for tissue engineering and regenerative medicine. Their limitless proliferation and differentiation capabilities have aided researchers in achieving new heights in the medical field. MSCs promote tissue regeneration and repair and are the ideal candidate for culture-based expansion due to their self-renewability, multipotency, ease of isolation and expansion, immunological privilege, genetic stability, and no ethical concerns.\(^14\) Friedenstein and Petrukova discovered MSCs in the stromal layer of bone marrow in 1968.\(^15\) MSCs, like other stem cells, are self-renewing and can differentiate into various cell types such as chondrocytes, neurons, adipocytes, cardiomyocytes, and osteocytes.\(^16\) MSCs can be isolated from various tissues and fluids, including the umbilical cord, adipose tissue, amniotic fluid, and dental pulp.\(^17\) According to the International Society for Cellular Therapy (ISCT) guidelines, MSCs should exhibit the following characteristics: (1) plastic adherence, (2) expression of mesodermal cell surface markers (CD73, CD90, and CD105) but not hematopoietic cell surface markers (CD11b, CD14, CD19, CD34, CD45, CD79, and HLA-DR), and (3) the ability to differentiate into mesodermal lineages such as chondrocytes, adipocytes, and osteocytes.\(^18\) However, MSCs have their own several limitations. Scientists are now searching for a noninvasive and effective method of monitoring MSCs in vivo. Additionally, the high cost associated with chemicals such as inducers to determine the fate of MSCs into appropriate lineage poses a significant financial burden. These impediments limit the utilization of MSCs in clinical settings. Therefore, the development of a cost-effective and reliable approach for steering stem cell differentiation. Significant progress has been made to overcome these obstacles, and a tremendous opportunity has been identified in nanotechnology. Researchers are exploring multidimensional nanomaterials to manipulate the fate and transport of stem cells.\(^19−21\) Due to the small size of nanomaterials, their surface can be modified to imitate the biophysical cues provided by extracellular matrix (ECM).\(^22−24\) Owing to their high surface area to volume ratio, they can be easily used for targeted drug delivery.\(^25\) Additionally, the nanoparticles can effectively direct the differentiation of stem cells into a particular lineage while also allowing for long-term monitoring following implantation.\(^26−28\)

**Quantum Dots.** If only one dimension of a three-dimensional structure is nanoscale, the structure is referred to as a quantum well; if two dimensions are tiny, the structure is referred to as a quantum wire; and if all three dimensions are nanoscale, the structure is referred to as a quantum dot (QD). In 1998, quantum dots became the first carbon-based nanomaterial used in biological research.\(^39\) QDs can be synthesized in either a top-down or bottom-up fashion. Researchers are concentrating their efforts on the green synthesis of quantum dots, owing to their high quantum yield. The emission of QDs can be tuned in the near-infrared region.
(NIR), enabling deeper tissue imaging. QDs have a unique property of broad excitation range and narrow emission range, which allows multiplexed imaging with a single excitation source.

Additionally, QDs have a high quantum yield of photoluminescence that is proportional to the imaging contrast. Additionally, QDs are resistant to photobleaching and other environmental conditions, allowing for prolonged exposure and outperforming other organic fluorophores. Another distinguishing feature of QDs is their compatibility with biomolecular functionalization and the enhanced permeability and retention (EPR) phenomenon, enabling them to target tumor cells.

These properties of QDs are gathering the attention of biologists as QDs hold the potential to start a new era of fluorescence imaging and can help in unravelling the secrets of biology at a molecular level.

Carbon Quantum Dots. CQDs are attracting considerable attention in the biological area due to their small size, stable photoluminescence, improved surface grafting, biocompatibility, and high-water solubility. Numerous nanoparticles such as magnetic nanoparticles and quantum dots are available for labeling stem cells. However, due to the difficulty of detecting magnetic signals in MRI scanners due to cell division and dilution of magnetic nanoparticles (MNPs), scientists are moving toward quantum dots that are fluorescence-based. CQDs have been observed to be ingested by cells via endocytosis processes. CQDs are also critical for stem cell differentiation into osteocytes, adipocytes, and neurocytes. For instance, CQDs have previously been shown to promote osteogenic differentiation via bone morphogenetic protein (BMP), TGF-β, and Wnt signaling pathways.

CQDs are a novel nanomaterial with numerous biomedical applications such as cell imaging due to their strong fluorescence. They are highly water-soluble, and their fabrication process is economical. They are safe for biological applications due to their greater biocompatibility and minimal cytotoxicity. The exceptional biological features of CQDs, such as hydrophilicity, low toxicity, chemical stability, and strong biocompatibility, ensure that their promised applications in biosensors, drug administration, gene delivery, bioimaging and the manufacture of nanocomposites, which can be employed for a variety of tissue engineering and regenerative medicine applications. CQDs have flourished in a variety of disciplines due to their superior chemical stability, optical characteristics, and renal clearance capabilities. Compared to organic dyes and conventional QDs, the improved photostability of CQDs may be attributed to their structure and composition. CQDs are core–shell composites consisting of a carbon core and surface passivation with various functional groups, such as hydroxyl, carboxyl, and amine, among others. This makes them hydrophilic and allows for a variety of surface functionalization and passivation processes to be carried out. Due to the π–π stacking, they are useful as carriers for various aromatic anticancer medicines. The cells take up these nanoscale quantum dots via energy-dependent endocytosis pathways.

CQDs can be synthesized via various physical, chemical, and biological ways but top-down and bottom-up approaches are the two most widely used methods. While any method can be used to synthesize CQDs, researchers favor the biological approach since it is cost-effective, cleaner, greener, and environmentally friendly.

The top-down strategy involves the chemical or physical breakdown of bulk material into nanostructures. Although this technique is straightforward and easily adjustable to meet our requirements, there are disadvantages, such as imperfections on the surface of the nanostructures. On the other hand, the bottom-up method may result in less waste and, therefore, can be more cost-effective. Bottom-up approaches entail assem-
blending material atom by atom, then molecule by molecule or cluster by cluster, ultimately producing nanostructures.\textsuperscript{55} Various techniques involved in the bottom-up and top-down approaches have been illustrated in Figure 1.

**CQDs: Role in Bioimaging.** Numerous organic fluorophores are commercially available; however, QDs are favored due to their excellent resistance to photobleaching.\textsuperscript{3,5} CQDs made from natural products exhibit a greater luminescence, biocompatibility, and solubility than those synthesized from other sources.\textsuperscript{58} Compared to organic dyes and other cadmium-based QDs, CQDs feature emission peaks that are more clearly defined and broader excitation spectra. So, by varying the size of CQDs and the excitation wavelength, the same CQDs can give different fluorescence.\textsuperscript{54} CQDs made with citric acid monohydrate and diethylene glycol BIS ether was extremely fluorescent, and their synthesis was also straightforward, environmentally friendly, and scalable.\textsuperscript{59} Mukherjee et al. demonstrated the green production of sulfur-doped GQDs using sugar cane molasses. The GQDs were extremely fluorescent, biocompatible, and had a quantum yield of 47%.\textsuperscript{60} Shen et al. manufactured nitrogen-doped carbon quantum dots (N-CQDs) for bioimaging applications using glucose and m-phenylamine. The quenching of Fe\textsuperscript{3+} and CrO\textsubscript{4}\textsuperscript{2−} was found to be static.\textsuperscript{61} CQDs were produced for multicolor imaging utilizing citric acid and the doping ions YB\textsuperscript{3+} and Nd\textsuperscript{3+}. The synthesized CQDs also emit in the near-infrared range, indicating their suitability for visible and infrared bioimaging.\textsuperscript{62} CQDs have been shown to attach to DNA and RNA in Caenorhabditis elegans live cells and emit distinct fluorescence, allowing for detecting DNA and RNA localization.\textsuperscript{63} The quantum yield of CQDs produced with alanine as the carbon source and ethylenediamine as the passivation was 46.2%. CQDs tagged MCF-7 cells and were suitable for bioimaging. The addition of dihydroxycinnamidic adenine dinucleotide (NADH) into the as-synthesized CQDs quenched their fluorescence, resulting in the generation of a new protocol for NADH determination inside the cells.\textsuperscript{64} CQDs not only mark cells but also have the capacity to target other elements. CQDs were coupled with amino TPEA (N-(2-aminoethyl)-N,N,N′,N′-tris(pyridine-2-yl-methyl)ethane-1,2-diamine) to enable imaging of Cu\textsuperscript{2+} inside cells. The compound aided in the production of Cu\textsuperscript{2+} and exhibited extremely minimal cytotoxicity.\textsuperscript{65} Because autofluorescence might cause interference with CQDs, Wei et al. produced boron and nitrogen codoped CQDs (BN-CQDs) utilizing o-phenylenediamine as the carbon and nitrogen source and boric acid as the source of boron and used them for bioimaging of HeLa cells.\textsuperscript{66} In greater quantities, CQDs have been reported to be hazardous. Extended exposure to CQDs may be detrimental to certain cell types. Another group reported that CQDs synthesized from waste cotton linter were economical, easy to synthesize, and had high fluorescence. On the other hand, when these CQDs were incubated for a longer duration or at higher concentrations, they proved to be toxic for HUVEC and H12452 cells.\textsuperscript{67}

**CQDs: Effect on Cancer Cells.** Cancer is one of the most lethal diseases, and there is ongoing research to unravel a potential cure for it. To this end, nanoparticles can be utilized in combating cancer. Nanoparticles internalize into cells via the endocytosis mechanism. Furthermore, CQDs are biocompatible and are employed exclusively for bioimaging; however, a few CQDs may also generate excessive reactive oxygen species (ROS) and can be toxic to cells. By inhibiting the expression of F-actin, VEGF, MMP-2, and MMP-9, CQDs/Cu,O produced by the Jiaqiang group preferentially destroyed SKOV3 ovarian cells. Additionally, these CQDs destroyed the cells via apoptosis by halting them in the S phase.\textsuperscript{68}

CQDs synthesized with o-phenylenediamine were readily taken up by HeLa cells, and when activated with blue light, they generated a substantial amount of ROS, leading to cell death.\textsuperscript{69}

Highly fluorescent quantum dots were synthesized from tea leaf extract and were apoptotic for lung cancer cells (A549).\textsuperscript{70} Cancer CSCs are a subtype of stem-like cells seen within malignant tumors. These cells exhibit characteristics of both stem cells and malignant cells.\textsuperscript{71,72} They are regarded as the architect of tumor spread and relapse. Their capacity for self-renewal and resistance to chemotherapeutic drugs jeopardizes cancer treatment.\textsuperscript{73,74} As a result, CSCs may be a therapeutic target for tumor metastasis and relapse prevention. Su et al. demonstrated that doxorubicin-loaded red emission CQDs enter the nucleus of CSCs, exhibiting antitumor activity, hence eradicating the possibility of metastasis.\textsuperscript{75} There are various ways via which CQDs could be used for the elimination of CSCs (Figure 2).

**CQDs: Role in Stem Cell Differentiation.** A range of diverse stem cell characteristics, such as pluripotency, differentiation, and reprogramming, may be regulated by several different small molecules. However, because most small molecules are hydrophobic, organic solvents like dimethyl sulfoxide (DMSO) are frequently used to dissolve the small molecules for cell culture supplementation. This practice can cause cytotoxicity and impair the differentiation of treated stem cells. The therapeutic use of small molecule medications is also hampered by their low water solubility. CQDs, on the other hand, are soluble in water, and the surface of these particles may be altered to facilitate targeted distribution.\textsuperscript{76} Even though positively charged CQDs are more cytotoxic and have lower photoluminescence (PL), in comparison to negatively charged

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**Figure 2.** Potential role of CQDs in cancer stem cells targeting.
CQDs, they nonetheless have a greater labeling efficiency due to their higher absorption capacity. Differentiation of stem cells into adipocytes and osteocytes was disturbed due to positively charged CQDs. Relatively weak positively charged CQDs could have high biocompatibility and labeling efficiency in human stem cells. CQDs, by their nanostructure, also play a part in the differentiation of stem cells. (Figure 3). The healing of bone is a complex physiological phenomenon; during this process, the stem cells replace the damaged bone tissues with new bones. CQDs have been successfully used to promote osteogenic differentiation of MSCs and their bioimaging. An increase in the expression of specific markers indicates the differentiation of MSCs into a distinct lineage.

Han et al. reported that adenosine aspirin-based CQDs upregulated the osteogenic markers alkaline phosphatase (ALP), RUNX2, OCN, and BSP in the presence of osteogenic inductive factors such as dexamethasone, ascorbic acid, and \( \beta \)-glycerophosphate, indicating osteogenic differentiation of bone marrow mesenchymal stem cells. Bu et al. synthesized CQDs using ascorbic acid as a precursor and demonstrated bioimaging and osteogenic properties. The osteogenic impact was further boosted by miR-2861, a promoter of osteoblast differentiation. Wang et al. synthesized carbon dots with \( \text{Zn}^{2+} \) passivation (Zn-CDs). \( \text{Zn}^{2+} \) is included due to its osteogenic activity. Zn-CDs promote wound healing by generating ROS. The ROS generated triggered oxidative stress,
which activated the MAPK signaling pathway, resulting in osteogenic differentiation. However, further research is needed to elucidate the precise mechanism behind the upregulation of these components and, in the pathways involved (see Figure 4).\(^{28}\) Ghafary et al. successfully absorbed the covalently bound pDNA complexed quantum dots into the stem cell nucleus.\(^{84}\)

Stem cell gene therapy may be used to treat various inherited genetic disorders, including severe combined immunodeficiency caused by adenosine deaminase, adrenoleukodystrophy, and infantile blindness. Among others, there is Leber Congenital Amaurosis (LCA).\(^{85,86}\) The treatment entails genetic material transferred into stem cells to mend and eradicate genetic abnormalities. Thus, the simultaneous delivery of genes and tracking of CQDs boosts their utility in stem cell therapy.

## CONCLUSION

CQDs are promising agents for stem cell bioimaging due to their unique physicochemical features and biocompatibility. Moreover, the green synthesis of CQDs is an eco-friendly method for their fabrication. Importantly, CQDs promote the expression of many regulatory factors involved in determining the fate of stem cells. They can also be employed as an alternative to expensive induction factors, owing to their ability to direct stem cell differentiation and tracking in vivo. Depending on the size of the cargo to be internalized, many energy-dependent mechanisms exist to allow the extracellular cargo inside the cell. Caveolae mechanisms usually internalize smaller cargo, intermediate cargo internalizes by the clathrin mechanism, and large cargos are internalized by macropinocytosis. Depending upon the size of CQD, the photoluminescence and light emission color vary. This property of CQD can be leveraged for drug delivery and bioimaging for patients on multidrug therapy. Bestowed with robust and multifaceted nature, functionalized CQDs can also act as a drug carrier for targeted stem cell therapy. The possibility for CQDs to build up in organs and cause unwanted side effects can be a concern that should be factored in for their clinical applications. The recent development of hybrid CQDs with other biomolecules that can synergistically uplift the drug delivery and bioimaging properties of CQDs holds promise for future development. To date, there are only a handful of reports that describe some signaling transduction pathways in the determination of stem cell fate. However, further research is needed to elucidate the precise mechanisms of how CQDs direct stem cell lineage. While the theranostic properties of CQDs have made them an ideal candidate in the biological sector, it is critical to understand the mechanisms of their action to minimize side effects. We envision that rigorous investigation into the molecular basis for CQDs in directing stem cell lineage and other biological applications will open new avenues for translational research.

## ASSOCIATED CONTENT

**Supporting Information**

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Author biographies (PDF)

## AUTHOR INFORMATION

**Corresponding Author**

Monalisa Mukherjee — Amity Institute of Click Chemistry Research and Studies, Amity University Uttar Pradesh, Noida 201313, India; orcid.org/0000-0002-7493-0409; Email: mmukherjee@amity.edu

**Authors**

Misba Majood — Amity Institute of Click Chemistry Research and Studies, Amity University Uttar Pradesh, Noida 201313, India

Piyush Garg — Amity Institute of Click Chemistry Research and Studies, Amity University Uttar Pradesh, Noida 201313, India

Radhika Chaurasia — Amity Institute of Click Chemistry Research and Studies, Amity University Uttar Pradesh, Noida 201313, India

Aakanksha Agarwal — Amity Institute of Click Chemistry Research and Studies, Amity University Uttar Pradesh, Noida 201313, India

Sujata Mohanty — Stem Cells Facility, DBT-Centre of Excellence, All India Institute of Medical Sciences, New Delhi 110029, India

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03285

**Author Contributions**

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**Author Contributions**

\(^{2}\)M.M. and P.G. have contributed equally as first author.

**Author Contributions**

\(^{1}\)R.C. and A.A. contributed equally as second authors.

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## ABBREVIATIONS

QDs, quantum dots; CQDs, carbon quantum dots; MSCs, mesenchymal stem cells; CSC, cancer stem cells

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