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Fluorescence mechanism and biomedical applications of graphene quantum dots

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Abstract. Graphene quantum dots (GQDs) are fluorescent graphene nanoparticle that have tunable emission wavelength, high emission intensity and biocompatibility. The fluorescence mechanism is still indecisive, yet explanations exist. Surface or edge defects and quantum confinement are two major explanations and they accounted for the florescence properties observed so far. GQDs' quantum confinement is applied to change the emission wavelength. Surface functional groups usually improve biocompatibility and solubility. Under several cases, surface defects are also engineered to tune emission wavelength and intensity to serve specific engineering propose. GQDs have found applications in bioimaging, biosensing, and therapy. GQDs can generate high intensity emission in the NIR-II window which is ideal in in vivo bioimaging. GQDs can be generated from folic acid in a one-step procedure. The GQDs generated this way can track folate receptor and help cancer diagnostics. High intensity emission also enables GQDs to be used in photothermal or photodynamic therapy. Future applications will be promoted by a better understanding of GQDs' fluorescence mechanisms.

Keywords: graphene quantum dots, fluorescence imaging, diagnostics.

1. Introduction

Quantum dots (QD) refer to fluorescent semiconductor nanoparticles. Quantum confinement (QC) effect results in discrete energy state and the energy transitions of electrons moving among these states generate fluorescence. Compared to other fluorescence materials, QDs tend to have high quantum yield (QY), which is the relative intensity of fluorescence emission, and resilient against photobleaching, which means they can retain their photoluminescence after excitation. QD applications include traceable drug delivery, fluorescence-activated cell sorting, and various of biosensors [1].

This diversity makes reviewing them challenging but also indicates high potential in novel applications. QC typically happens if the size of the QD is within the exciton Bohr radius. QC describes the confinement of free electron in QDs. It is usually confined by the energy offset between its states and the surface state. Under this circumstance, a free electron in QD can be treated as the one in the 'particle in the box' model. The energy of ground state in conductive band is size dependent. More specifically, the energy gap is given by:

$$\Delta E = \frac{h^2}{8R^2}\left[\frac{1}{m_e} + \frac{1}{m_h}\right] - \frac{1.8e^2}{\epsilon R} + smaller\ terms \quad (1)$$

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For very small R, the first term dominant, result in a negative relationship between fluorescence cell size and emission energy [1, 2].

In biomedical applications, graphene quantum dot (GQD) is a much more ideal choice for its low toxicity, simple synthesis, and biocompatibility. The synthesis, fluorescence mechanism and optical properties of GQDs are much more complicated than semiconductor QDs. This review will discuss the fluorescence mechanisms of GQDs and their applications in biosensing, bioimaging, and photothermal therapy.

2. Fluorescence mechanism

GQDs are very different from semiconductor QDs by their fluorescence patterns and mechanisms. Although the manufacture strategy is relatively homogeneous, the fluorescence mechanism is not definitive. Two major sources of fluorescence have been identified: quantum confinement and surface/edge states. [3]. These mechanisms are used to explain specify optical phenomenon in various GQDs samples. Combined, they contribute to a wide range of emission and excitation spectrum and tunable fluorescence [3, 4]. Because so far not a single GQD manufacture strategy has shown superiority in conveniency, low cytotoxicity, high QY, resistance against photobleaching, and other desirable prosperities, the mechanisms are iterated on a case-by-case bases.

Quantum confinement, the dominant fluorescence mechanism in metal based QDs, is also applicable in GQDs, characterized by the size dependence of carbon bond structure, instead of the total size [3-5]. Such carbon bond structure is formed when GQD is synthesized from graphene sheet. Graphene sheet is characterized by π-bond network. They can be partitioned by creating sp^2 islands which is achieved either by the reduction of graphene oxides or by oxygen plasma etching [6, 7]. Experimentally, fluorescence from these structures has high absorption but low QY. The low fluorescence was usually attributed to interlayer quenching, but quenching was also observed in GQDs made from single layer sheet [6]. The sp^2 islands contribute to both the high absorption and the low fluorescence. They have high concentrations of electron states, forming deep traps in each other's state distribution and result in gradual relaxation of exciton through these traps [8].

The size effect, that emission wavelength increase with respect to size, was experimentally reported. Lee at al. researched the quantum confinement effect on two GO samples with different O:C ratio [9]. They observed increasing binding energy of the excitons and slower relaxation with respect to increasing O:C ratio. The oxidation in GQD generates the structure that unreacted carbon crystalline cores are separated by the oxidized amorphous boundaries. The oxidized regions act as the potential barriers that induce quantum confinement to the separated graphene structure. The higher the O:C ratio, the smaller the partitioned regions, the more dominant the confinement. This coincides with the statement made by Eda et al., who found the fluorescence's dependence on the sp^2 domains [5]. In their attempt to optimize stable, blue fluorescence, they found the bandgaps decrease with the number of unit cells in sp^2 configurations. They also found the intensity of emission increase with increased concentration of sp^2 configurations. This is true in GQD made from graphene sheet. However, in organic synthesized GQDs, Yang et al. argued that, even if size effect exists, they are not the main reason for wavelength variations. They observed the size effect on intrinsic states, but the spectrum shows the dominant fluorescence effect comes from the energy offset between edge states and intrinsic states [10].

Inducing defect is a common approach in synthesizing GQDs. This reflects surface or edge states as a source for fluorescence. The energy gap between surface states and core states results in the wide absorption and emission spectra of GQDs, since different defects contribute to different energy states. In some GQDs, the defects are imbedded in the carbon structure, for example, oxidation partitioned the carbon structure and from hybridizations [9]. Such oxidation may produce redshift on wavelength because these intrinsic defect states are usually continuous and prone to release energy non-radiatively [11]. After top-down synthesis of GQDs, different shapes of edge with different edge state can be obtained. Two edge styles (armchair and zigzag) have been found to shift the wavelength of emission. Specifically, GQDs with zigzag edge have larger emission wavelength than the ones with armchair

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edge and exposure of zigzag edge from any attachments can enhance PL [12, 13]. Besides oxidation, amino group defects also form in GQDs during. Specifically, GQDs fabricated from nitrogen-based precursors (N-doped GQDs) have significantly improved quantum yield. Liu et al treated folic acid, which is chosen because it is rich in nitrogen and promising in cancer diagnosis applications, with hydrothermal method to synthesis GQDs that have the quantum yield up to 94.5%. Choosing the appropriate temperature and pH for hydrothermal method contributes to the high quantum yield by creating single energy states: nearly all excited electrons release their energy as emission [14].

In some cases, the fluorescence may come from the function groups attached to the graphene core. These function groups, usually attached during synthesis, may contribute to the wide emission spectrum of GQDs, reported by An et al. [4] The intensity peaks on the emission spectrum have been identified to be the transition between π–π* bond, electronic-hole pair relaxation from amino (–NH2), carboxyl (–COOH) and carboxylate (–COO−) function groups respectively. Some GQDs reported pH dependence. It may be caused by the attachment of carboxylic acid groups from the acid solvent [15]. Chen et al. attached several function groups to the edge of the GQD samples and observed different degrees of red shift [3]. N-defect GQDs or amino group GQDs are reported to have high QY [16]. CONHR and CNHR function groups eliminate the nonradiative electron-pair relaxation, release energy in emission, and enhance the intrinsic QY of GQDs [17].

So far, GQDs have presented high fluorescence stability. Xu et al. reported slight red shift when their multilayer GQD sample is exposed to air [11]. They experimentally prove that the desorption of water breaks the π-π bond between layers and change the confinement condition. Reapply water can restore the emission wavelength.

Up-conversion fluorescence describe the circumstance where the emission energy is lower than the excitation energy. This optical property is favored by in vivo bioimaging, since it usually involves signals that can penetrate tissues. Up-conversion fluorescence has been observed in QDs synthesized in different ways, yet the origin of up-conversion fluorescence is still indecisive [18].

Cao et al reported up-conversion of GQDs fabricated via laser cutting of a carbon precursor. They observed a 4th order relationship between the excitation energy and the up-conversion emission intensity, which is typical in two-photon process, so they attribute up-conversion to the multiphoton absorption [19]. Shen et al found up-conversion in GQDs synthesized by hydrazine hydrate reduction of GO with polyethylene glycol surface passivation. They observed nearly constant wavelength shifts in up-conversion spectrum with different excitation wavelength. They suggested the energy difference came from the energy gap between π orbital and σ orbital: the excited electrons from π orbital won't fall back, instead it will fall toward the σ orbital, providing extra emission energy [20].

However, Wen et al believes the reported up-conversions indeed came from the second order diffracted light from xenon lamp grating which was used to generate monochromatic excitation light. When beam passes through the grafting, phase and amplitude changes will be introduced depending on the wavelength of the lights. The grafting 'filters out' all the wavelength but the target wavelength by reducing their amplitudes. However, the signal with 1/2 the target wavelength will still be detectable, and the authors believe this signal triggers the emission. They tested five CQDs and GQDs that have up-conversion behavior and repeated the tests with filters that explicitly block light with 1/2 the excitation wavelength. Consequently, the up-conversion emission disappears in all five samples. The linear dependence of fluorescence intensity and emission intensity excludes two-photon process (The relationship would have been quadratic) [21].

3. Applications

GQDs' preserved many desirable optical proprieties of conventional semiconductor QDs, including viable emission wavelength, high QY and high photostability. They also process distinct optical proprieties such as wide emission band, excitation-dependent emission, and pH-dependent emission. With some surface modification, QD's fluorescence make them good candidates in biomedical applications. GQD has found applications in biosensor, photothermal therapy, and MRI contrast agent [22].

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A major advantage of GQDs is its plasticity. The size effect of GQDs can be manipulated to generate emission of ideal wavelength. GQDs with different visible emission can be used to distinguish different organ cell in vitro bioimaging; in vivo imaging, emission within NIR band is preferred because it have less scattering by organ, so it has deeper tissue penetration. GQDs' surface groups can be easily engineered to fit specific design requirement. For example, in vivo medical applications, water solubility, cytotoxicity and internalization ability are generally required. OH function groups are known to provide solubility; surface coating can reduced cytotoxicity and improve photostability; target specific functional groups can bind with target receptors [22]. Biosensor applications based on GQDs include the detection of folate receptors and H_2S, both are helpful in cancer diagnosis.

GQDs synthesized form folic acid have been used to detect folate receptor in organ cells. Folic acid is important in cell regeneration and growth and its concentration will increase in the presence of some cancer cells, so folate receptor detection can be applied in cancer diagnosis. Liu et al fabricate GQDs without any deliberate coating from folic acid [14]. Under X-ray photoelectron spectroscopy analysis, distinctive signal peaks indicating the existence of C–N, C=N and C=N–C molecular structures, which represent the residual folic acid on GQDs. The GQDs were incubated into three samples to test their biocapacity: Hela cells, overexpressing folate receptor; A549 cells, which have few folate receptors; and Hela cells pretreated with excess folic acid. Bright fluorescence was only observed in the untreated Hela cells, which indicates a sufficient binding between GQDs and folate receptors.

Zhang et al. synthesized similar folic acid GQD and analyze the mechanism of its properties [23]. They first confirmed the emission spectrum, high QY (77%), cellular internalization, and low cytotoxicity of GQDs. The selectivity of GQDs was tested by SKOV3 cells pretreated with folic acid. Increased concentration of folic acid resulted in reduced emission intensity of GQDs, meaning the combination of GQDs with folate receptor is suppressed by folic acid. They further look into the mechanism of the high QY, photostability and low cytotoxicity of GQDs. The high temperature during synthesis broke the pterin moiety in folic acid but the selectivity and high QY are retained. The QY of the folic acid GQDs is attributed to the suppression of nonradiative relaxation by the surface amide group. This coincided with the previous finding that. The photostability od GQDs are usually degraded by the formation of reactive oxygen species. By comparing GQD to another photocatalyst (TiO_2), they confirmed the low reactive oxygen species production rate. Based on previous research, they believed the three hydroxymethyl groups near the fluorescent source reduce the reactive oxygen species formation. The cancer targeting mechanism may not fully come from the pterin group, which is part of the surface folic acid, as previous research had indicated, because they were proven to be broken during the thermal process. The research group point to the function groups of C-N, N-C-O, and N-C-N: the residue of folic acid. They were found on the surface of the GQDs and can bond with folate receptors.

H_2S generation is involved in many human physiological processes, so H_2S detection can provide information on these processes, include cancer metabolism. Li et al. presented a rather innovative approach to detect H_2S in living systems by using surface quenching in GQDs [24]. H_2S can cleave down the function group attached on the edge of the GQDs during synthesis, which reduce quenching and increase QY. By intentionally keeping the function groups, specifically (2,4-dinitrophenoxy) tyrosine (DNPTYR), they can attribute the increase of QY to detection of H_2S because it must be present to expose the fluorescence states. DNPTYR are chosen as quenching function group for several reasons. It contains amino group which binds well with the carboxyl group in GQDs; it induces significant (76.5%) photoluminescence quenching caused by its electron-withdrawing dinitrophenoxyl moiety; the oxygen-containing function groups also prevents aggregation. GQD-DNPTYR is not cytotoxic under the concentration of 0.5 mg/mL, which makes it suitable for bioimaging. The sensitivity of the GQDs is tested by NaHS which mimic the condition of dissolved H_2S in human body. QY recovery (from. 5.88 to 21.15%) is observed in GQD-DNPTYR response to 10 μM NaHS and as little as 2nM NaHS can be sensed under a signal-to-noise ratio of 4.3. Experiments have shown compatible sensitivity of GQD-DNPTYR to low concentration of H_2S in

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human body. The specificity of GQD-DNPTYR is also tested by various interference species including CO 2- and SO 2- anions, Ca2+ and Mg2+ metal ions, reactive oxygen species, and reducing agents. All of them triggered little to none PL recovery. Thusly, GQD-DNPTYR can be used to accurately and selectively detect H_2S in complex intracellular environment.

Nitrogen and boron graphene quantum dots can be tuned to emit light in NIR-II region, reported by Wang et al. [25]. The present NIR-II fluorescence use semiconductor QDs and rare-earth metal nanoparticles. These materials have higher toxicity than GQDs. The toxicity of GQDs was tested on three cell lines (SF763, 4T1, and B16F10). 94% survive rate after 72 hours showed no obvious toxicity. The N-B-GQDs had emission range in the NIR-II window. The photoluminescence intensity was reported to be 1.0%, higher than the previously reported QYs of NIR-II semiconductor QD and fluorescence dye. This value seems to be significantly lower than the QYs of the GQDs mentioned earlier, but it reasonable because the GQDs emission QY was measured from living mice's inner organ and some excitation light might be dispersed by mice tissue. N-B-GQDs was injected into mice vessels and produced NIR-II bioimaging photograph that show clear edge in arteries, liver, and kidney. The reported N-B-GQDs have shown great potential in in vivo bioimaging.

Another interesting application for GQD is photothermal therapy. The N-B-GQDs mentioned previously mentioned have been tested on its potential in photothermal therapy [25]. Because N-B-GQDs have high NIR absorption, they are also viable for photothermal therapy. They experiment show temperature increase with water containing N-B-GQDs (26.6 ◦ C, 200 μg/mL) compared to the control (3.2 ◦ C). The photothermal effect of N-B-GQDs promotes applications in photothermal theory against cancer. The SF-763 cells were injected with N-B-GQDs incubation and NIR radiation for 2 hours and only 4% still have cell viability. Four C6 glioblastoma sample was respectively administrated with N-B-GQDs and 5 min radiation, saline and the same radiation, GQDs only, and saline only. All sample shows similar rate of tumor growth, expect the one treated with both N-B-GQDs and radiation, which have almost no tumor growth. This substantially provide the success of photothermal therapy by N-B-GQDs.

4. Conclusion

The florescence properties of variously synthesized GQDs are presented. The fluorescence mechanisms of GQDs, although currently inconclusive, were discussed. Surface or edge defects and quantum confinement are two major explanations and they accounted for the florescence properties observed so far. The size effect as the result of quantum confinement is actively applied to change the emission energy by change the size of carbon structure size. Surface functional groups usually improve biocompatibility and solubility, enabling GQDs in many in vivo and in vitro applications. Under several cases, surface defects are also engineered to tune emission wavelength and intensity to serve specific engineering propose. GQDs with surface functional groups displayed high selectively and resistance to photobleaching, which makes them ideal as fluorescent indicators. Several recent biomedical applications were considered based on how they apply GQDs' the optical and chemical advantages. GQDs have found applications in bioimaging, biosensing, and therapy and have potentials in both in vivo and in vitro biomedical. Size effect means tunable emission wavelength and various defects result in excitation dependent emissions. Emissions of different wavelength can serve as fluorescence dye. GQDs can generate high intensity emission in the NIR-II window which is ideal in in vivo bioimaging. GQDs can be generated from folic acid in a one-step procedure. The GQDs generated this way can track folate receptor and help cancer diagnostics. They also contain N-defects which are experimentally related to high QY. The quenching effect of surface groups promotes an innovative application on detection of H_2S. High intensity emission also enables GQDs to be used in photothermal or photodynamic therapy. A more comprehensive understanding of GQDs, their synthesis method, and properties will encourage more promising biomedical applications in the future.

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