

Recent Advances in Graphene Quantum Dots Nanomaterials for Biomedical Applications: A Literature Review

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ABSTRACT

Background : Graphene Quantum Dots (GQDs) are carbon-based nanomaterials that have attracted considerable attention in recent years due to their unique optical, electronic, and biological properties arising from quantum confinement and edge effects. Their excellent fluorescence, high water solubility, good biocompatibility, and diverse biological activities make them promising candidates for various biomedical applications.

Purpose : To review the current development of Graphene Quantum Dots, including their synthesis methods, physicochemical characteristics, antibacterial activity, cytotoxicity, and potential applications in the biomedical field.

Methods : A literature review was conducted by analyzing published studies on GQDs. The review focused on synthesis approaches (top-down and bottom-up), material characteristics, antibacterial properties, cytotoxicity, and biomedical applications.

Results : The reviewed studies indicate that different synthesis methods significantly influence the size, morphology, and physicochemical properties of GQDs. Furthermore, GQDs exhibit promising antibacterial activity and generally favorable biocompatibility. They have demonstrated considerable potential for use as biosensors, bioimaging agents, drug delivery systems, and multifunctional antibacterial materials. However, further investigations are still required to evaluate their long-term safety and biological effects.

Conclusion : Graphene Quantum Dots represent a promising multifunctional nanomaterial platform for future biomedical applications. Continued research is needed to optimize their synthesis and comprehensively assess their long-term biocompatibility and safety for clinical translation.

Keywords : Graphene Quantum Dots, carbon nanomaterial, characteristics, antibacterial activity, biomedical applications.

INTRODUCTION

Advances in nanotechnology have spurred the exploration of various carbon-based materials with unique physicochemical properties and potential for biomedical applications. One material that has attracted significant attention is Graphene Quantum Dots (GQDs).¹ Graphene Quantum Dots are carbon-based particles at the nanoscale and are part of the graphene family (a zero-dimensional, single-atom-thick crystalline form of carbon). They consist of one or more layers of graphene sheets with lateral dimensions <10 nm and are known as a new type of luminescent nanomaterial.² These carbon nanomaterials exhibit significant

quantum and edge effects. These characteristics result in superior physicochemical, electronic, and biological properties, including good fluorescence, high biocompatibility, and promising biological activity, making them suitable for a wide range of biomedical applications.¹

This material has lateral dimensions larger than its height, consists of one or more layers of graphene, and has chemical groups at its edges that serve as abundant sites for functionalization (Figure 1) illustrates the chemical structure of GQDs.³

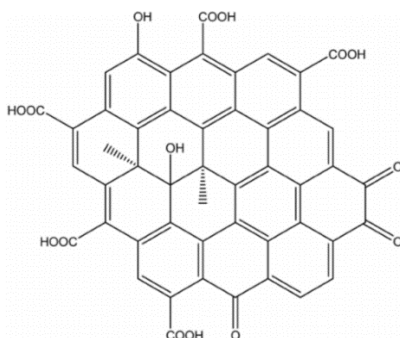


Figure 1. Hexagonal structure of graphene quantum dots ⁴

Figure 1 shows that GQDs have a hexagonal crystal lattice structure resembling a honeycomb, with carbon atoms forming six-sided rings.⁴ This arrangement results in the sp^2 hybridization characteristic of GQDs and allows for electron delocalization in the π orbitals..^{5,6}

RESULT

GQDs SYNTHETIS METHODS

There are two main methods used: the bottom-up and top-down methods, which yield different material characteristics, including particle size and morphology.^{7, 8,9} The bottom-up method involves assembling atoms or molecules (smaller units) to form GQDs, and the particle size is controlled by the reaction conditions during synthesis. The bottom-up method employs common strategies such as the condensation of smaller carbon rings to form larger entities through stepwise solution chemistry, microwave synthesis, pyrolysis, hydrothermal heating, and fullerene cage opening. GQDs produced by these methods generally have well-defined sizes, specific shapes, and interesting properties.¹⁰

The top-down synthesis method starts with carbon-rich materials such as graphene, graphene oxide, graphene nanoribbons (GNRs), graphite, CNTs, carbon fibers, and fullerenes, as well as other biomaterials like cellulose, lignin, and carbon-based polymers. The advantages of this approach include low cost, the use of abundant raw materials, and the production of GQDs with edge functions and water solubility. Furthermore, GQD synthesis is scalable,

produces high-quality, crystalline materials with uniform size and shape, and allows for the tuning of photoluminescence properties through critical process parameters (temperature, time, and energy). However, most methods have several drawbacks, such as low yields, high defect density, and the need for hazardous chemicals, complex synthetic protocols, or expensive equipment.¹⁰

Liquid Peeling Process

The liquid peeling process (LP) one of the best method for producing nanofilms, offering advantages such as low processing costs, ease of use, and minimal environmental impact. Graphite is exfoliated into graphene sheets during the process as a precursor, enabling the LP process to produce GQDs with high crystallinity.¹¹

Hydrothermal Method

The hydrothermal method involves various techniques for crystallizing substances from high-temperature liquids under high vapor pressure.¹¹ The hydrothermal technique has been validated for the synthesis of GQDs using GO powder as the carbon source and H_2O_2 as the reagent. In this process, H_2O_2 decomposes into OH radicals at high temperatures. After the hydrothermal reaction, the graphite plate is thermally fragmented into small pieces. This finding indicates that at high temperatures, graphite is easily broken down.¹¹

Electrochemical Method

Electrochemical techniques can be used to formulate yellow-green emitting GQDs. A NaOH solution in water is prepared as the electrolyte during the GQD synthesis process. GQDs synthesized using the electrochemical oxidation procedure exhibit good stability; however, prior to processing, the preparation of raw materials and the purification of GQDs can be time-consuming, and the quantum yield is very low, making it difficult to achieve large-scale production of GQDs.¹¹

Chemical Oxidation Method

In this method, the carbon bonds of graphene, GO, or carbon nanotubes are typically broken down by H_2SO_4 and HNO_3 .¹² Lu et al. (2017) developed an experimental system using carbon black as a carbon source and nitric acid at a temperature of 135°C to synthesize GQDs. The entire synthesis process takes only 90 minutes and results in GQDs with good light stability, low toxicity, and good biocompatibility. This study found that the GQDs are relatively uniform, with diameters of approximately 3–4.5 nm, and that the presence of oxygen functional groups and hydrophilic groups helps stabilize the GQDs in aqueous solution.¹³ Additionally, Halder et al. (2018) also used GO as a precursor, oxidizing it for 2 hours with H_2O_2 at 180°C to obtain

GQDs, which did not require further purification steps. The synthesized GQDs were then freeze-dried to obtain a solid sample. In this study, the resulting GQDs exhibited hydroxyl, carboxyl, alkene, epoxy, and alkoxy functional groups. The particle size of the resulting GQDs ranged from 4 to 6 nm.¹⁴ elen (2023) synthesized GQDs from graphite, which was converted into GO using the Hummer method at a temperature below 20°C for 30 minutes, then raised to 35°C for 30 minutes; after which the GO was synthesized into GQDs using H₂SO₄ and HNO₃ as oxidants at 100°C, yielding GQDs with sizes of 11–20 nm (40%) and carboxyl functional groups.¹⁵ A comparison of GQD synthesis methods, both top-down and bottom-up, is shown in Table 1.

Table 1. Comparison of top-down and bottom-up methods in the synthesis of GQDs

Comparison Aspect	Top-Down	Bottom-Up
Principle	Breaking down bulk carbon materials into nanoscale particles	Constructing nanoparticles from molecular or atomic precursors
Starting Materials	Graphite, graphene sheets, carbon nanotubes (CNTs), carbon fibers	Citric acid, glucose, organic molecules, biomass, aromatic compounds
Synthesis Methods	Chemical oxidation, electrochemical exfoliation, laser ablation, hydrothermal cutting	Hydrothermal synthesis, pyrolysis, microwave-assisted synthesis, carbonization
Advantages	- Simple preparation process - Suitable for large-scale production - Utilizes readily available carbon materials	- Better control over particle size and morphology - Uniform particle distribution - Tunable surface functional groups and optical properties
Disadvantages	- Broad particle size distribution - Structural defects may be introduced during synthesis - Difficult to precisely control morphology and surface properties	- Scalability remains challenging - Requires careful control of synthesis conditions - Complex synthesis procedures may be involved

CHARACTERISTICS OF GQDs

Morphology

Morphologically, GQDs are generally quasi-spherical or nanosheet-shaped, measuring less than 20 nm, with a relatively homogeneous size distribution. Their small size and the presence of sp² carbon domains provide a high surface area and result in significant quantum effects. The morphology and particle size of GQDs are strongly influenced by the synthesis method used, which can affect their fluorescence properties, colloidal stability,

biocompatibility, and biological activity. Additionally, the presence of oxygen functional groups on the surface of GQDs enhances water solubility and provides active sites for surface modification, which is important in biosensing, bioimaging, and drug delivery applications.¹⁶ The particle size of GQDs can be observed using TEM. The GQD material exhibits monodispersity with an average diameter of 20 nm.¹⁷ The size distribution plot shows that the average size of the GQDs is approximately 4–6 nm. Furthermore, well-defined lattice edges with a spacing of 0.22 nm were observed. The high resolution of the GQDs, consistent with a hexagonal lattice plane, is a clear indication of the high crystallinity of the synthesized GQDs.

Structure and Composition

The structure and chemical composition of GQDs are typically characterized using Fourier Transform Infrared Spectroscopy (FTIR) to identify the functional groups present on their surfaces. The FTIR spectrum of GQDs indicates the presence of various oxygen functional groups, such as hydroxyl (O–H), carbonyl or carboxyl (C=O), alkene (C=C), epoxy (C–O–C), and alkoxy (C–O). During the synthesis process, the intensity of the epoxy group (C–O–C) may decrease or even disappear due to oxidative reactions that cause the breaking of the epoxy bond in graphene oxide (GO). On the other hand, an increase in the absorption band intensity associated with C–O stretching vibrations indicates the formation of new oxygen-containing functional groups on the GQDs surface. The presence of these oxygen-containing functional groups enhances the material's hydrophilic properties, enabling GQDs to exhibit good dispersibility in water and making them suitable for various biomedical applications.¹⁴

The crystallinity and structural characteristics of GQDs are typically analyzed using X-ray diffraction (XRD). The diffraction peaks observed in the XRD pattern indicate the reflection of X-rays from specific crystal planes within the material.¹⁸ A study synthesized GQDs from graphene oxide (GO); the XRD pattern showed diffraction peaks at 19.11° and 32.09° for GO, and at 28.51° for graphite. These peaks correspond to the data listed in the Joint Committee on Powder Diffraction Standards (JCPDS) database, No. 19-0629. Based on calculations using the Scherrer equation, the crystallite size of the obtained GQDs is estimated to be approximately 17 nm.¹⁹

Similar results were also reported for the synthesis of GQDs from eucalyptus oil ethanol extract, which showed a single, broad diffraction peak centered around 21°. This peak is associated with the (002) crystal plane of the carbon material, which exhibits amorphous characteristics and a graphitic structure. These findings indicate that the carbonization process is capable of producing carbon domains with a graphite-like arrangement.²⁰ In addition, another

study synthesizing GQDs from GO reported a diffraction peak at $2\theta = 25.5^\circ$ associated with the (002) plane and having a d-spacing of 0.366 nm. The peak position, which is close to that of pure graphite at $2\theta = 26.42^\circ$, indicates partial recovery of the carbon atomic lattice following the reduction process. However, the larger d-spacing value compared to graphite suggests that the resulting GQDs still contain oxygen functional groups on their surfaces, even after the reduction process has taken place.²¹

Optical Characteristics

The photoluminescent properties of GQDs in photodynamic therapy (PDT) can generate photoexcitation energy for the bactericidal process. For example, amino-functionalized nitrogen (N)-doped GQDs have been found to serve as efficient photosensitizers. Furthermore, N-doped GQDs can enhance ROS production, thereby increasing the bactericidal rate. As a result, N-doped GQDs caused 100% cell death within 3 minutes of incubation.²² GQDs were capable of inhibiting *Escherichia coli* and *Staphylococcus aureus*, with inhibition zone diameters of 10.40 mm and 9.75 mm, respectively.²³ Another study reported that GQDs exhibited strong antibacterial activity with an inhibition zone of 49.2 mm against *Listeria ivanovi*.²⁴

PROPERTIES OF GQD

Fluorescence

GQDs exhibit excellent fluorescent properties, which are influenced by particle size, surface functional groups, and synthesis conditions. These characteristics enable GQDs to be used as sensitive, stable, and biocompatible fluorescent probes in a wide range of applications. Furthermore, the π -conjugation system in GQDs allows for surface functionalization with various organic, inorganic, and biomolecular compounds, enabling the modification of their optical properties to suit specific application needs. These advantages make GQDs a promising material for the development of biosensors and the detection of various biological analytes..²⁵

As one of the fluorescent carbon nanomaterials, GQDs have attracted widespread attention in the fields of biosensing and bioimaging due to their high water solubility, stable photoluminescence, low cytotoxicity, good biocompatibility, and high resistance to photobleaching. Compared to carbon dots (C-dots), GQDs—which have a quasi-zero-dimensional structure consisting of several layers of carbon atoms—are capable of producing strong photoluminescence emission without requiring additional passivation processes. These various characteristics make GQDs a promising platform for biosensing and bioimaging applications, as well as various other biological applications.²⁶

Solubility in water

Water-soluble GQDs hold promise for a wide range of biological applications, such as bioimaging, sensing, and biological therapy. However, the significant heterogeneity in the composition, size, and shape of these water-soluble GQDs poses significant challenges in better understanding their structure-property correlations and their further clinical use, particularly in terms of large-scale manufacturing practices and safety concerns.²⁷ Compared to graphene, GQDs have better solubility in ultrapure water, lower cytotoxicity, and a larger specific surface area, making them more effective drug molecular loading cores.¹²

The synthesis of GQDs via graphite exfoliation yields GQDs that can be stably dispersed in water. The presence of GQDs can inhibit the agglomeration of graphene and facilitate its dispersion in ultrapure water. The presence of oxygen-containing functional groups on the GQDs also contributes to the stable dispersion of graphene in ultrapure water.²⁸ The synthesis of GQDs from graphite flakes yields particles with a size of approximately 20 nm and good solubility in ultrapure water.²⁹ GQDs are a graphene system composed of sp^2 -hybridized carbon atoms, typically terminated at both ends by carboxylic acid groups (-COOH), which confer water solubility. These groups can form hydrogen bonds with water molecules via the oxygen in the hydroxyl (-OH) and carbonyl groups, thereby enhancing the solubility of GQDs in water. The carboxyl groups enable GQDs to interact with water molecules via Van der Waals forces and hydrogen bonding. This interaction. GQDs also possess carbonyl, hydroxyl, and amino groups, providing good solubility in aqueous media and the ability to interact with herbal and inorganic components.³⁰

Biological characteristics

Graphene quantum dots are considered a new type of quantum dot (QD) because they are chemically and physically stable due to the intrinsic inert nature of carbon. In addition, GQDs are environmentally friendly because they are non-toxic and biologically inert.³¹ GQDs are water-soluble or can be dispersed in water and remain stable for 6 months to 1 year due to their numerous hydrophilic groups and small size. The cytotoxicity of GQDs is relatively low.³² Graphene and its derivatives have garnered significant attention due to their unique physical, chemical, and mechanical properties, which can be harnessed for a wide range of applications. Among these, significant research efforts have been dedicated to antimicrobial applications of graphene derivatives. However, the exact mechanism of graphene's cytotoxicity remains a subject of active debate, with oxidative stress, protein dysfunction, cellular damage, and transcriptional inhibition having been proposed to explain its antibacterial activity.³²

Evaluation of the solubility of GQDs in water using a UV-Vis spectrophotometer, which involves measuring the light absorption by GQDs at various wavelengths. The UV-Vis spectrum of the GQDs solution shows absorption bands at 463 and 528 nm, which are identified as the π and α absorption bands. Water-soluble GQDs exhibit dark red fluorescence with three peaks at 650, 694, and 760 nm, where the second peak at 694 nm has the highest intensity. These spectral features confirm the aggregated H-bilayer structure of GQDs without intermolecular π - π stacking in aqueous conditions.²⁷ In another study, GQD dispersions exhibited a pale yellow appearance in daylight, while they emitted a bright green color with high fluorescence intensity under UV (365 nm) irradiation. In the photoluminescence (PL) and photoluminescence excitation (PLE) spectra, a strong emission at 460 nm (black line) can be observed when excited by 347 nm.³³

GQDs BIOMEDICAL APPLICATIONS

Bioimaging and Biosensing

Several studies have reported that the presence of certain chemical elements, such as boron and nitrogen, can influence the photoluminescence characteristics of GQDs. This ability to respond optically to environmental changes makes GQDs a promising candidate for use as a sensitive and selective biosensing platform.^{34, 35, 36} Similar to carbon dots, GQDs are capable of producing changes in fluorescence signals in response to interactions with metal ions or specific biomolecules. This characteristic allows GQDs to be used as a sensitive biosensing platform for detecting various biological and chemical analytes. Furthermore, their strong and stable fluorescence, combined with high biocompatibility, makes GQDs a promising bioimaging agent for visualizing and monitoring biological processes at the cellular level.³⁶ The ability of GQDs to produce fluorescence shifts specific to target molecules makes them a promising material for biosensing applications. With characteristics such as strong fluorescence, high biocompatibility, and good photostability, GQDs can be used as nanoprobes to sensitively detect biomolecules and monitor biological processes in real time in living cells.³⁵

Drug delivery System

In addition to their use in biosensing and bioimaging, GQDs also hold potential as drug delivery systems due to their good water solubility, nanoscale size, and high biocompatibility. GQDs can be modified with various targeting ligands to enhance drug accumulation in specific target cells. One example is the conjugation of GQDs with biotin, which can recognize biotin receptors overexpressed on cancer cells, thereby increasing the delivery efficiency of anticancer drugs such as doxorubicin. In addition to exhibiting low toxicity to cells, GQD-based systems

are also capable of releasing drugs in response to the acidic environment within cancer cells, thereby enhancing therapeutic efficacy and minimizing side effects on healthy tissue.³⁷

The development of GQD-based drug delivery systems continues to advance, notably through the integration of GQDs into hydrogel matrices. This combination enhances stability, biocompatibility, and responsiveness to various environmental stimuli. The porous structure of the hydrogel enables controlled drug encapsulation and release, while the large surface area of GQDs increases drug loading capacity and release efficiency. Additionally, the fluorescent properties of GQDs allow for real-time monitoring of drug distribution via bioimaging techniques. Surface functionalization of GQDs with targeting ligands also enables more selective drug delivery to cancer cells, thereby reducing side effects on healthy tissue and improving therapeutic efficacy.³⁸

Antibacterial Properties of GQDs

The antibacterial activity of GQDs has been widely reported against various pathogenic bacteria. This antibacterial effectiveness is influenced by particle size, GQD concentration, and their interaction with microbial cell membranes. Several studies have shown that GQDs can cause cell membrane damage, increase the formation of reactive oxygen species (ROS), and disrupt bacterial metabolic processes. GQDs show promising potential as antibacterial agents because they are capable of inhibiting the growth of various types of bacteria. The integration of GQDs into a lyotropic liquid crystal (LLC)-based nanocolloid system has been reported to produce strong antibacterial activity against *Listeria ivanovii*. This activity is influenced by the GQDs concentration, surfactant concentration, and the LLC phase structure used. Interactions between GQDs and bacterial cell membranes are thought to play a role in disrupting membrane integrity and cellular function, thereby inhibiting bacterial growth. These findings suggest that GQDs have the potential to be developed as antimicrobial components in various pharmaceutical, biomedical, and health product applications.³⁹

The antibacterial activity of GQDs is influenced by the physicochemical characteristics resulting from the synthesis process. Differences in synthesis methods can lead to variations in particle size, surface area, the number of structural defects, and surface functional groups, all of which contribute to the antimicrobial effectiveness of the material. Several studies have reported that composites based on graphene oxide quantum dots (GOQDs) and ZnO exhibit good antibacterial activity against both Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus* and *Escherichia coli*. This enhanced antibacterial activity is attributed to the synergistic effect between GOQDs and ZnO, supported by their large surface area, the

presence of structural defects, and their ability to generate reactive oxygen species capable of damaging bacterial cells.⁴⁰

Modifying GQDs with polymers can enhance antibacterial activity because the polymers improve the binding of GQDs to bacterial cell membranes. PEGylated polymers and GQDs demonstrated 100% growth inhibition against *S. aureus* (25 µg/mL) and *P. aeruginosa* (50 µg/mL) after 8 hours of incubation¹⁰¹.²² Furthermore, polyvinylidene fluoride (PVDF)-modified GOQDs (GODQs-PVDF) can be fabricated as antibacterial membranes. The GOQD-coated PVDF layer effectively inactivates *E. coli* and *S. aureus* cells, resulting in excellent antimicrobial activity (an inhibition rate of 88.9% within 1 hour) and anti-biofouling capabilities. These significant antimicrobial and anti-biofouling properties are attributed to strong interactions between the particles and the thiol groups of the cell membrane's phospholipid bilayer, which subsequently induce oxidative stress.²²

GQDs ANTIBACTERIAL MECHANISM

The antibacterial mechanism of GQDs against microbial organisms occurs through three mechanisms: (1) destruction of the cell wall or cell membrane; (2) production of reactive oxygen species (ROS) to destroy cells; (3) binding to nucleic acids (DNA/RNA) to inhibit cell proliferation (Figure 2).²²

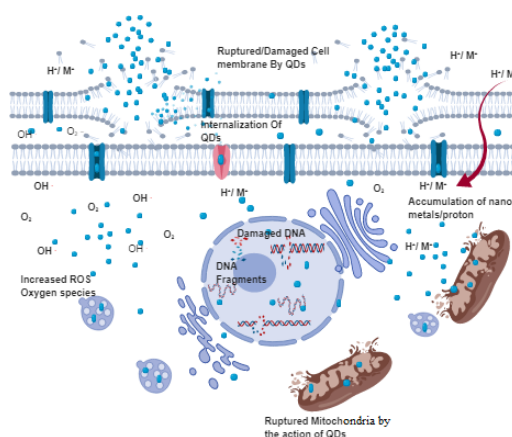


Figure 2. General antibacterial mechanisms of quantum dots (QDs). QDs produce antimicrobial effects by destroying cell walls or membranes, inducing free radicals, binding to genetic material, and inhibiting cell proliferation.

Figure 2 illustrates the antibacterial mechanism of GQDs, which involves damaging the cell membrane and DNA, and generating ROS. The interaction of GQDs with the phospholipid bilayer causes the cell membrane structure to become irregular and shrink. Consequently, the cell undergoes lysis, leading to the release of cellular components. The electrostatic interaction between the positive charge of GQDs and the negative charge of cellular components creates

pressure on the cell wall, increases intracellular toxicity, and results in cell death.²² Furthermore, photoactivated GQDs can generate excessive free radicals, including hydroxide anions (OH^-), superoxide, singlet oxygen (O_2^-), triplet oxygen, and perhydroxyl anions. The accumulation of ROS within cells can inhibit respiration and replication, ultimately leading to microbial death.²²

Some GQDs can also enter and accumulate within the nucleus, interacting with its components. These interactions can inhibit cellular respiration, division, replication, and the production of adenosine triphosphate (ATP). Damage to the nucleus can ultimately lead to cell apoptosis.²² Antibacterial efficacy can be enhanced by functionalizing GQDs with polymers and photosensitizers to induce more ROS and improve their binding to bacterial components. The inhibitory effectiveness of functionalized GQDs depends on their ligands, size, shape, zeta potential, and charge. Functionalized GQDs have proven to be effective antibacterial agents. The large π -conjugated system of GQDs readily attaches to bacterial cell walls via electron transfer. GQDs induce membrane stress by increasing oxidative stress, and ROS damage the cell membrane, leading to leakage of cellular contents and cell death.²²

CONCLUSION

Graphene quantum dots are zero-dimensional carbon nanomaterials with intriguing physicochemical, optical, and biological properties for various biomedical applications. The synthesis method used plays a crucial role in determining the size, morphology, and properties of the resulting material. Their excellent fluorescence properties, surface functionalization capabilities, relatively high biocompatibility, and antibacterial activity make GQDs a promising candidate for use in biosensing, bioimaging, drug delivery, and the development of antimicrobial materials. Although various studies have shown promising results, further research is still needed regarding synthesis standardization, biological interaction mechanisms, and long-term safety before GQDs can be more widely applied in clinical settings. With the continuous advancement of research, GQDs are expected to become one of the nanomaterials playing a significant role in the development of biomedical technology in the future.

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