EUROPEAN ACADEMIC RESEARCH
Vol. IX, Issue 7/ October 2021

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Acquisition and Applications of Quantum Carbon Dots in Bioimaging: Use of Fluorescent Probes to Obtain Bioimaging

MARCELO RAMON DA SILVA NUNES[1]
Federal University of Acre, Rodovia BR 364, Km 04 - Distrito Industrial
Rio Branco - AC, Brazil
Federal Institute of Acre, Rua Francisca Sousa da Silva, 318
Getúlio Nunes Sampaio, Sena Madureira - AC, Brazil
FERNANDO SÉRGIO ESCÓCIO DRUMMOND VIANA FARIA
DAWERSON DA PAIXÃO RAMOS
ANSELMO FORTUNATO RUIZ RODRIGUEZ
Federal University of Acre, Rodovia BR 364, Km 04 - Distrito Industrial
Rio Branco – AC, Brazil
MARCELA PATRÍCIA NUNES PEREIRA
Federal University of Alagoas, Av. Lourival Melo Mota, S/N
Tabuleiro do Martins, Maceió - AL, Brazil
KAUÃ GALVÃO DE MACEDO
Federal Institute of Acre, Rua Francisca Sousa da Silva, 318
Getúlio Nunes Sampaio, Sena Madureira - AC, Brazil
PEDRINHO NASCIMENTO DA SILVA
Federal University of Acre, Rodovia BR 364, Km 04 - Distrito Industrial
Rio Branco - AC, Brazil
ROSANNY CHRISTHINNY DA SILVA
ADRIANA SANTOS RIBEIRO
Federal University of Alagoas, Av. Lourival Melo Mota, S/N
Tabuleiro do Martins, Maceió - AL, Brazil

[1] Graduated in Chemistry from the Federal University of Alagoas - UFAL. He is currently a professor of Exclusive Dedication at the Federal Institute of Acre - IFAC and a doctoral candidate at the Legal Amazon Biodiversity and Biotechnology Network - BIONORTE in partnership with the Federal University of Acre - UFAC, under the guidance of Professor Dr Fernando Sérgio Escócio Drummond Viana Faria and co-supervision of Professor Dr Anselmo Fortunato Ruiz Rodriguez. He has research experience in Nanotechnology, Quantum Carbon Dots for cancer diagnosis and treatment, graphene synthesis, production of polymeric nanocomposites (silver, gold, magnetic oxide and nanoclays), production of green plastics, natural pH sensor, analysis of water quality in Amazonian streams and rivers. He has a master's degree in Science Teaching from the Federal University of Acre - UFAC. He has experience in education: higher education, technical, youth and adult education, professional courses, didactic games for teaching chemistry and general basic education. CV: http://lattes.cnpq.br/8424807035559631; ID Lattes: 8424807035559631; email: marcelo.nunes@ifac.edu.br

Abstract

Carbon nanomaterials have different shapes and structures, including carbon nanotubes, graphene, nanodiamonds, fullerenes and carbon dots that have been widely used in biomedical applications in recent decades. However, not all carbon nanomaterials are promising for their applications. Among one of the most promising are the carbon points that have wide prospective applications in several fields, among them the obtaining of Figures. In view of this, this study will present a literature review on the main discoveries related to carbon nanostructures and, in a specific way, describe the processes by which carbon points are obtained, in addition to researching their properties and the main technological applications related to use of carbon points to obtain bio Figures. To this end, a search will be made in periodicals and books that deal with the topic and that may, in some way, assist in the writing of this article. It became evident that only recently have carbon points been used to obtain Figures. The versatility of carbon points offers enormous possibilities in a wide range of in vitro and in vivo imaging applications, including real-time cell tracking, high-resolution multiplexed vascular imaging, intraoperative imaging guidance, among others. In order to expand the functionality of the carbon points it is very important to design multifunctional nano devices that can integrate the Figure, the drug load and even the detection capabilities in a single nanoparticle, but for this to be possible further research is necessary.

Keywords: quantum dots of carbon, bioimaging, fluorescence, optical Figure, green synthesis.

INTRODUCTION

Nanomaterials are especially interesting compared to their bulk form due to their adjustable physical, chemical, electronic, thermal, mechanical and optical properties.In 1985 many carbon structures were discovered, the quantum carbon points were only discovered, and

accidentally, in 2004. Since then, a lot of research has been carried out, photo-activated location microscopy due to its unique properties and the potential way they can be used in technological applications.[1-3]

As such, nanomaterials based on carbon fullerene, carbon nanotubes (NTCs), graphene, carbon points (Carbon dots) and nanodiamonds have been and continue to be studied extensively due to their intrinsic properties.

Among these, NTCs, graphene and PC carbon points have attracted enormous attention to other bioanalytical applications due to their ease of functionalization for the detection of analytes of interest.

NTCs and graphene have a similar structure, where NTCs can be seen as seamless cylinders wrapped by sheets of graphene.[3]

It is noteworthy that in recent years, the increase in published articles on the use and application of quantum carbon points in various technological areas has grown exponentially,[4] leading to various areas of knowledge and various uses for carbon points ranging from medicine,[2] to electrical engineering[1] and materials to environmental engineering.[5]

That being said, as biotechnology researchers, we have two basic obligations, according to Ray and Jana: understanding what is happening in the evolution of materials used in different areas and disseminating knowledge so that others can also understand, and finding solutions for many questions still unresolved.[6]

Thus, this work will present a review of the literature related to the main discoveries related to carbon nanostructures and, in a specific way, describe the processes by which the quantum carbon points are obtained, in addition to researching their properties and the main technological applications related to use of quantum carbon dots for bioimaging with applications in medicine and engineering.

A BRIEF REVIEW OF THE MAIN DISCOVERIES OF THE NANO WORLD

Carbon nanotube

Around 1990, while exploring new ways of fullerenes, Smalley suggested the possibility of tubular fullerene: a straight segment of carbon tube capped, perhaps, by two hemispheres of C60 at both ends of the tube. There are also the C70, C76, C84, C92 and C540. Fullerene C20 has only 12 pentagons without hexagons. Fullerene C70, resembles a soccer ball, with more hexagons, but with the same number of pentagons. In general fullerenes are black colored solids and when dissolved in certain solvents they form colored solutions. C60, for example, forms a magenta solution, C70 a wine solution, and C76 a yellow / green solution.[7] Although Smalley's idea may or may not be directly related to the large entry of NTCs into the scientific world, certainly when Sumio Iijima, from NEC (Japan), reported the first observation of multi-walled carbon nanotubes (NTPM) the following year 1991,[3] the world was brought in to witness yet another wave of another new form of carbon.[8]

Iijima was able to clearly demonstrate the presence of concentric nanotubes produced by evaporating graphite by arc discharge, each composed of 2 to 50 layers of carbon sheets. Two years later, in 1993, the single-walled carbon nanotube (NTPU) was first identified in a laboratory by Iijima and Ichihashi and, independently, by Bethune et al..[3] The NTPU is composed of a single layer of carbon in the shape of a leaf arranged in a sp2 configuration, cylindrical and with a diameter ranging from a few angstroms to nanometers, all depending on the particular method and conditions of synthesis. The length usually ranges from hundreds of nanometers to micrometers.[4,6,9]

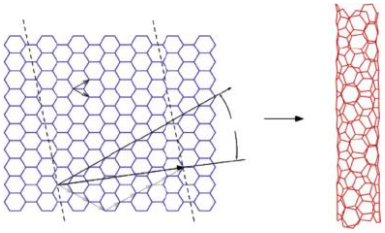

Figure 1. Single-walled carbon nanotube formed from carbon foil[8]

The strong carbon-carbon bonds and the network they formed make NTC 375 times stronger than steel and only 1/16 denser. As a result, NTCs found their commercial use easily in light applications, such as bicycle frames and golf clubs.[6]

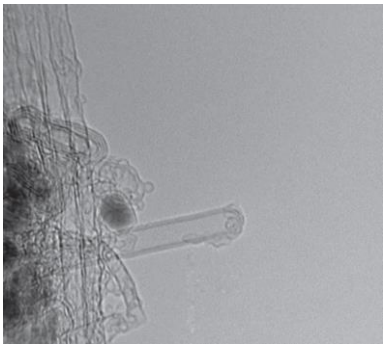

Figure 2. Carbon nanotube presented in a high resolution Figure[10]

With its huge specific surface area,> 1600 m2 / g, NTPU shows an extraordinary capacity for surface adsorption comparable or, in some cases, better than activated carbon as an adsorption material, all because of its highly unusual structure.[4]

Graphene

As far as we are concerned, graphene may have been surrounding us for centuries. However, it was only in 2004 that Konstantin Novoselov and Andre Geim elegantly isolated (using adhesive tape) a single layer and a few layers of highly suspended pyrolytic graphite "suspended" graphene.[3] Graphene is characterized as single stacked

sheets or few layers of sp2 hybridized carbon, where the number of sheets does not exceed 10.[1] The structure of graphene is referred to as infinite polycyclic aromatic hydrocarbons, containing an infinite number of fused benzene rings.[1,11]

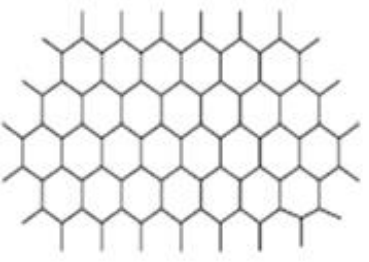

Figure 3. Graphene structure [11]

Graphene is the first two-dimensional nanomaterial known to exist in a suspended form, challenging previous conventional knowledge that the two-dimensional material would be too unstable thermodynamically to exist. In fact, with an intrinsic force of 130 GPa, Young's modulus per layer of 350 N / m and a breaking force of 42 N / m, in its three-dimensional form graphene is one of the strongest materials in the world ever discovered and, therefore, guarantees a title like supercarbon.[3,6,12]

Like a 2D crystalline membrane, graphene has a set of unique properties, collectively surpassing any other material currently known. These properties include an extremely large specific surface area (2630 m2 / g), low density (<1 g / cm3), ultra high charge mobility (> 2 × 105 cm2 / Vs), excellent electrical conductivity (104 S / cm) and thermal conductivity (> 5000 W / mK) compared to copper is 400 W / mK, a uniform broadband optical absorption that ranges from UV ultraviolet to far infrared (FIR) and excellent mechanical resistance and unusual flexibility.[1-]

Carbon dots (CDs)

Also known as carbon quantum dots or graphene quantum dots, having the physical properties and chemical structures similar to

graphene oxides differing graphene in terms of size, almost spherical with a diameter below 10 nm.[13]

Carbon dots demonstrate excellent fluorescence, simple synthesis, low toxicity, good biocompatibility and low costs. Numerous studies have evaluated large-scale synthesis, properties and applications of carbono dits. Comprehensive applications of Carbon dots have been demonstrated, including their use as biosensors, bioimaging probes and catalysts. The favorable fluorescence properties of carbon dots make them useful for application in analytical chemistry, particularly in the detection of environmental pollution and in bioimaging.[5]

Several analytical methods for the detection of toxic compounds based on biosensors or electrochemistry have been proposed;[12] however, most of these methods are cumbersome, imprecise or time-consuming in practical applications.[1]

Carbon dots may be suitable for the detection of toxic compounds. Based on the rich and varied functional groups of the surface and the excellent fluorescence characteristics of Carbon dots, several chemical products / biological sensors have been developed, such as those for the detection of industrial chemical contaminants [p-nitrophenol (p-NP), 4-chlorophenol (4-CP), phenol and 2,4,6-trichlorophenol (2,4, 6-TCP)]. In addition, good photostability and optical properties, as well as the lack of photodegradation, make Carbon dots suitable for the visualization of organisms in vitro and in vivo, as confirmed by many studies.[14-19]

In 2020 has established the viability of Carbon dots for in vivo Figures in mice, with detectable emission of bright fluorescence.[20] In the same year a team of researchers revealed that, under excitation at 405, 488 and 543 nm, Carbon dots cultured with cells emit strong blue, green and red fluorescence, respectively. Therefore, Carbon dots show great potential for cell imaging, bacterial imaging and labeling as biocompatible fluorescent nanomaterials.[21]

History

The first reported discovery of carbon dots (PC) was the result of an accidental appearance of these fluorescent nanoparticles as a by-product during the purification of single-walled carbon nanotubes (NTCPU) by Xu and colleagues in 2004.[3] This fluorescent fraction separated during purification was identified as a mere impurity in the soot of the crude nanotube, which in later analyzes showed fluorescence in UV light (Figure 4).

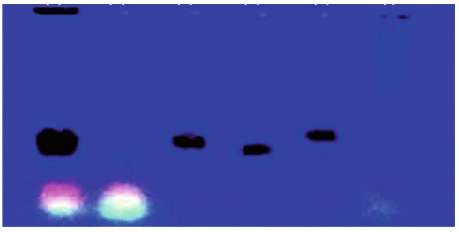

Figure 4. Identified fluorescent carbon nanoparticles by Xu et al. in 2004[3]

Other related analyzes, such as Fourier transform infrared spectroscopy (FTIR) and X-ray energy dispersive analysis (EDAX), were carried out by the researchers motivated by their curiosity to understand these fluorescent particles (as it was called during the discovery) , which showed the presence of portions of carbon on the surface and no metallic residue from the catalysts used in the synthesis of NTCs.

In addition, elementary analysis showed the highest carbon content followed by oxygen, hydrogen and nitrogen.[3] The discovery of Carbon dots, which was the realization of a new form of fluorescent carbon nanostructure, attracted researchers to explore these newly found nanoparticles and understand their synthesis and properties.[1,2,8]

Subsequently, these carbon nanoparticles were called Quantum Carbon Points or Carbon Points due to their striking similarities with the traditional semiconductor PQ, such as size, properties and photoactivity.[3] Compared to inorganic semiconductor PQs, Carbon dots show enhanced properties of no toxicity

(biocompatibility), resistance to photographic lightening, water solubility and comparatively cheap synthesis protocol.[1]

CARBON DOT SYNTHESIS METHODS

Carbon points are the most important zero-dimension carbon nanostructures. Researchers are particularly interested in them due to their numerous and attractive functionality, including size and wavelength-dependent luminescence emission, photodegradation resistance, bioconjugation facility, quantum confinement and many other properties.[3]

A consensus has been reached that, for comprehensive applications, it is necessary to prepare high-quality carbon points at low cost, easily and on a large scale.[6] Since 2004, after the identification of these carbon points by Xu et al., a variety of highly effective physical and chemical methods have been introduced to prepare fluorescent carbon points in the past decade, including synthesis methods such as microwaves, assisted methods, hydrothermal carbonization, electrochemical oxidation, chemical oxidation, ultrasound, plasma treatment, laser ablation, supported and thermal routes and so on.[10]

The demand for these carbon points has increased and several strategies have been developed to meet these demands. Typically, in 2006, Sun et al., Developed the laser ablation method for the production of carbon spots. In this method, a type of carbon target, prepared by hot pressing a mixture of graphite powder and cement, was first eliminated by an Nd: YAG laser with Q switching (1064 nm, 10Hz) in an argon gas stream that carries water vapor at 900 ° C and 75 kPa. Subsequently, the treated carbon target was further heated to reflux in 2.6 M HNO3 for up to 12 h to produce carbon spots with strong fluorescence.[4] In the following years, soot derived from the combustion of unscented candles or natural gas burners was also explored as an efficient and simple source of carbon dots.

For example, to synthesize carbon dots Mao et al., collected soot by placing a piece of aluminum foil or a glass plate on top of a lit

candle, mixing the soot with 5 M HNO3 and then refluxing it for 12 h to oxidize the particle on the surface and, eventually, produce carbon dots with sizes <2 nm by centrifugation or dialysis and additional fractionation of polyacrylamide gel electrophoresis.[3]

Pang and colleagues synthesized fluorescent carbon spots by electrochemically oxidizing a 3 V graphitic column electrode against a calomel electrode saturated with a wire counter electrode in 0.1 M aqueous NaH2PO4 solution.[10] In addition to the top-down method mentioned above, bottom-up methods can also provide carbon points using carbonaceous molecular precursors.[3]

In 2008, Giannelis et al. employed a one-stage thermal decomposition (calcination of the precursor at 300 ° C for 2 h) of molecular precursors of low temperature fusion (ammonium citrate salt or 4-aminoantipyrine) to form surface passivation of carbon points, whose geometry and physical properties could be designed and precisely controlled.[22] In 2009, Yang's group employed an easy microwave pyrolysis approach to prepare the carbon spots, in which an aqueous solution containing PEG200 and a saccharide (eg, glucose, fructose) was heated in a microwave oven. 500 W waves for 2 to 10 minutes to produce carbon dots with controllable emission wavelengths.[23] In the same year, Li and colleagues used silica spheres modified with surfactant (F127) as a support to locate the growth of carbon spots during high temperature treatment (900 ° C in argon for 2 h).[24] In this case, the carboxylic groups can be easily introduced to the surface of the carbon spots by refluxing in 3 M HNO3 for 24 hours, followed by passivating the surface with Polyethylene Glycol 1500. Note that, for all of these methods mentioned, it is necessary to modify the surface necessary to allow carbon dots to exhibit superior photoluminescent properties, stability and biocompatibility, which generally involves additional and relatively complicated procedures.

Therefore, easier and greener synthetic methods that require cheaper and accessible carbon sources (for example, proteins and small molecular carbohydrates) have been extensively developed in recent years.

Examples include, but are not limited to, natural silk, bovine serum albumin, hair fiber and gelatin, glycerol, chitosan and sucrose.[3,10] In addition, food products (for example, caramels, honey and peppers) and biomass (such as grass, plant leaves and paper ashes) were also explored as several reaction precursors for the synthesis of carbon points.[12] In order to use all these sources of biodiversity in the preparation of carbon spots, the most frequently selected methods are hydrothermal and microwave assisted strategies because of their simultaneous, homogeneous and fast heating, simple handling, low energy consumption and good selectivity, resulting in photoluminescent carbon spots without the need for further functionalization or passivation.[3]

FLUORESCENT PROBES FOR BIOFIGURE APPLICATIONS

Fluorescence imaging techniques are being used more and more by many scientists, thanks to their distinct benefits, such as the availability of biocompatible imaging agents, maneuverable instruments and high temporal resolution with good sensitivity.

Undoubtedly, fluorescence imaging has taken a highly significant step from basic life science research to clinical applications, since it is non-invasive or toxic, fast, highly sensitive and inexpensive.[3,10]

Aiming in creating an accurate representation of biological objects or processes, a number of state-of-the-art imaging technologies have been developed to improve resolution of the optical Figure, including stimulated emission depletion microscopy, stochastic optical reconstruction microscopy, photoactivated location microscopy and total internal reflection fluorescence microscopy, all with greater sensitivity and higher resolution.[11]

These technologies are more advantageous when used to observe cellular structures, to visualize dynamic events that occur in a living cell, there is another factor that requires additional improvements, that is, temporal resolution. Fluorescent probes with high brightness and photostability are in great need of all imaging

modalities. Fluorescent probes are essential for bioimaging applications, such as labeling target molecules, investigating in vitro and in vivo behavior of chemical and biological species, diagnosing diseases.[2]

It is agreed that the fluorescent probe largely determines the quality of the fluorescence Figure.[3] As a result, high quality fluorescent probes are basically essential and must be highly fluorescent, dispersible in water, chemically and photostable and biocompatible.[1]

In order to obtain a favorable signal output, a wide range of fluorescent probes based on various types of colloidal molecules and nanoparticles were investigated.[3] Specifically, the small molecules (for example, fluorescent proteins and organic dyes) have excellent biocompatibility and relatively small sizes, which are more suitable for intracellular targeting.[1,5,6]

For a long time, fluorescent proteins and organic dyes, served as fluorescent biosonds and were widely used for biological and biomedical research.[6,11] However, its severe photobleaching has severely limited its applications, especially for long-term bioimaging.
In contrast to fluorescent proteins and organic dyes, inorganic quantum dots have excellent photostability with narrow emission spectra and wide photoexcitation, high fluorescence and size-adjustable emission wavelengths, making them ideal for multiplexed figures for long periods.[11]

Its only disadvantage is that most quantum dots contain heavy metal ions (for example, Cd2 +, Te2− etc.), which presents a major safety concern, despite a series of surface modification strategies (for example, coating ZnS / silica / polymer coating) available that reduce toxicity to some extent.[3]

It is important to note that upward converting nanophosphors, composed of nanocrystals doped with lanthanides, can convert several near-infrared photons into a visible light photon through energy transfer processes, which are resistant to photodegradation when compared to fluorescent and organic proteins.[11]

With the advantages of minimal auto-fluorescence (background noise), low toxicity, high quantum yields, crisp absorption and emission lines and long life, uplink nanophores are opening new doors for a next generation of possible bioimaging modalities , as the use of near infrared excitation leads to Figures of deep penetration.[6,11] Recently, silicon nanoparticles have been shown to be biodegradable and can be easily excreted from the body through renal clearance. Without toxicity detectable in vivo, fluorescent silicon nanoparticles are characterized by favorable biocompatibility and their unique optical properties, such as high fluorescence and robust photostability. Fluorescent silicon nanoparticles have also been extensively investigated as a new type of high quality nanosound for bioimaging applications.[10]

Meanwhile, there is another new generation of promising probes that inspire great interest due to their long fluorescence life, prolonged emission of distant red (600-800 nm), excellent photostability (ie, without photodegradation and without photodetection) and ease in surface functionalization: fluorescent nano-diamonds containing negatively charged nitrogen vacancy centers such as fluorophores.[10]

Similar to nanodiamonds, the carbon dot as the closest form of nanocarbon is attracting world interest on its own. In comparison with fluorescent nanoprobes based on well-studied quantum dots, fluorescent carbon dots, which are made of biocompatible carbon element without toxic heavy metals, are considered a fascinating alternative.[19-21, 25-27]

These carbonaceous quantum dots contain several favorable characteristics of traditional semiconductor-based quantum dots, including ease of bioconjugation, photodegradation resistance, wavelength-dependent luminescence emission and absence of intrinsic toxicity.[1,5,6]

Therefore, in the last decade, carbon spots have been intensively explored as a new type of fluorescent nanoprobes, taking advantage of their unique optical properties, their favorable

biocompatibility and have been widely used for in vitro and in vivo bioimaging applications.[19-21, 25-27]

IN VITRO FIGURE OBTATION

Sun et al., were the pioneers in the use of carbon points for bioimaging applications. They indicated the possibility of carbon points functionalized with poly (propionyl ethyleneimine-co-ethyleneimine) (PPEI-EI) for two-photon luminescence microscopy using human breast cancer MCF-7 cells as a cell model.[25,28]

When incubated with carbon dots for 2 h at room temperature, the cells showed bright green luminescence in the cell membrane and cytoplasm regions after being washed to remove any extracellular carbon dots and observed by a fluorescence microscope with 800 nm excitation.[25]

His work inspired scientists around the world to use carbon dots as fluorescent probes in bioimaging. In 2014, Kong and colleagues synthesized reflective fluorescent carbon spots in Polyethylene glycol (PEG), which exhibited excellent biocompatibility, strong photoluminescence and stable fluorescence properties, even when exposed to different temperatures, ionic forces and times (Figure 5).[29]

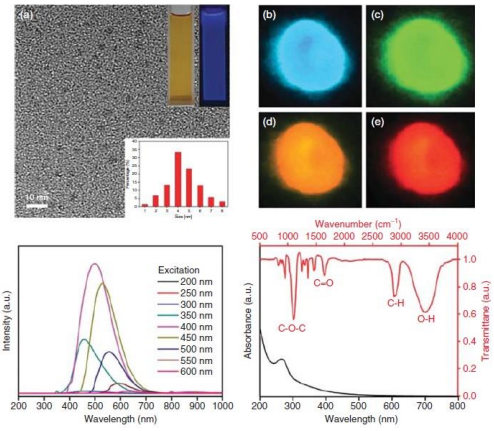

Figure 5. Comparison of HeLa cells stained with fluorescent carbon dots[29]

In addition, the fluorescent carbon dots were able to mark the cell nucleus alone, without staining other parts of the cell, allowing efficient selection of organelles and the precise subcellular location of the fluorescent probes, as noted in Figure 5.

Therefore, it is reasonable to consider fluorescent carbon dots as an ideal target in cellular figures, rather than commercially available dyes.

Despite the fundamental advantages of carbon points for bioimaging applications, including a broad possibility of surface functionalization, strong phospholipid (PL), multicolor emission and excellent biocompatibility, there are limited reports on super-resolution figures with carbon points.

Leménager et al. successfully used depletion microscopy via stimulated emission to observe carbon spots in cells with a resolution of up to 30 nm, achieving an improvement of more than six times the spatial resolution in fixed and living cells compared to confocal microscopy conventional.[30]

In this case, they produced the carbon nanoparticles by laser ablation of a carbon target and passivated them with oligomeric H2NCH2 (CH2CH2O) nCH2CH2CH2NH2 (PEG) finished with diamine (mean n: 35, PEG1500N).

Notably, the successful application of carbon dots for depletion Figures via high-resolution stimulated emission would open up a wide range of applications and provide more possibilities in the life sciences.[30]

Quantum graphene dots could also be used in bioimaging as a new member of the carbon dot group. This possible applicability started to arouse wide interests since Zhu et al. used them successfully for bioimaging for the first time. In this work, they prepared large green photoluminescent graphene quantum dots on a large scale by a one-step solvothermal route with graphene oxide as a carbon source, which could be dissolved in water and most polar organic solvents without other chemical modifications.[31]

Due to their low cytotoxicity, stable photoluminescence, excellent solubility and biocompatibility, the quantum dots of

graphene are particularly suitable for bioimaging. Similar to carbon dots, the quantum dots of graphene exhibit excitation-dependent PL behavior, leading to several visible results: when excited at 488 nm, yellow-green emission is observed and, when excited at 405 nm, a bright blue color is observed.

In addition, Zhu et al. successfully used quantum graphene dots for two-photon luminescence microscopy using the prepared green luminescent graphene quantum dots, graphene quantum m-dots and graphene r-dots with varying degrees of oxidation and surface modification by a solvothermal method and separation in two stages.[27]

In Figure 6, a bright green or blue area can be seen inside the MC3T3 cells under excitation near the infrared (808 nm), which indicates a successful translocation of quantum dots of graphene across the cell membrane. In addition, although there is no more bioconjugation, all three types of quantum dots of graphene could penetrate living cells. Furthermore, these quantum dots of graphene showed high photostability, that is, under continuous excitation for more than 20 minutes, no obvious reduction in PL brightness was observed. Thus, these quantum dots of graphene can act as powerful tools in the conversion bioFigure and with little damage to living cells or tissues.

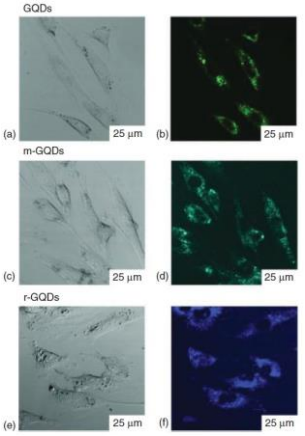

Figure 6. Upward conversion of cellular figure from quantum graphene dots[27]

The following year, Liu et al. synthesized quantum graphene nanodots by a solvothermal method with simple methodology using dimethylformamide (DMF) as a solvent and nitrogen source and systematically investigated the fluorescence induced by two photons using HeLa cells from human cervical carcinoma.[32]

With their tests they were able to significantly extend the fundamental limit of Figure depth with two photons in dispersed tissues. In the incubation with N-GQD (50 µg / mL DMEM of high glucose) at 37 ° C for 2 h, the in vivo HeLa cells were illuminated when excited at 800 nm (with low laser power of 1mW and average power density of 13W / cm2), providing a high-contrast fluorescence Figure of green graphene quantum nanodots distributed around each core. This indicated the ability of quantum graphene nanodots to mark both the cell membrane and the cytoplasm of HeLa cells with insignificant disorders of the nucleus. In addition, using a near-infrared laser as an excitation source, the quantum graphene nanodots could be visualized in high resolution and high signal-to-noise ratio at depths ranging from 0 to 1300 µm in the tissue spectrum, even at 1800 µm depth. Quantum graphene nanodots were also easily identified with an appreciable two-photon fluorescence signal.

On the other hand, the maximum penetration depth of OPFI (a type of organic dye) was only 400 µm, due to the strong dispersion and refraction of the excitation light visible in the hazy tissue ghost, with a high photon absorption cross section of two photons (48000 GM), few photodegradation and photothermal effects under repeated irradiation with infrared laser near femtoseconds, the quantum graphene nanodots prepared by a one-pot solvothermic approach using DMF as a solvent and nitrogen source are powerful tools for bioimaging.[32]

The bioimaging of carbon points becomes more significant when they are useful in bioanalysis also simultaneously, taking advantage of their PL. For example, in 2012, Tian and colleagues used ratiometric sensors to detect Cu2 + in vitro, integrating carbon spots coated with a specific organic molecule N- (2-aminoethyl) -N, N, N'tris

(pyridin-2-methyl) ethane-1,2-diamine (AE-TPEA) and CdSe / ZnS QDs.[33]

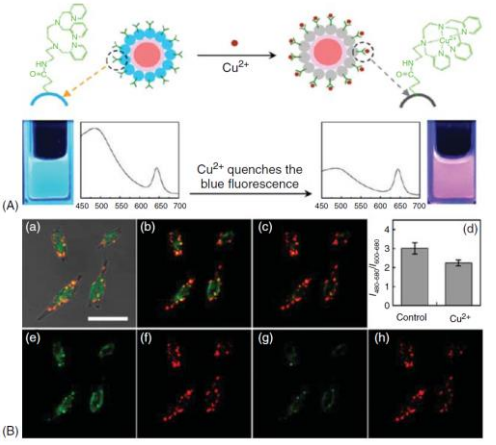

Figure 7. Schematic illustration of Cu^{2+}dual emission fluorescent detection [33]

It is observed in (a) the superposition of bright field Figures and fluorescence of HeLa cells. (b, c) are Figures resulting from the confocal fluorescence of HeLa cells (b) before and (c) after exogenous treatment of the Cu2 + source. (d) Bar graph representing the integrated intensity from 480 to 580 nm over the integrated fluorescence intensity from 600 to 680 nm. It should be noted that the values presented are the average proportion generated from the intensity of three fields selected at random in both channels. (e, g). Confocal fluorescence figures obtained from the 480-580 nm channel before and after the exogenous treatment of the Cu2 + source, while (f, h) are from the 600-680 nm channel.

The fluorescent probe was able to detect Cu2 + in concentrations ranging from 5 to 200 µm in physiological pH. After absorption, as shown in Figure 7, the nanoparticles resided in several intracellular compartments: when treated by an exogenous source of Cu2 +, the color of the probe's fluorescence emission changed from yellow-green to red (only CdSe / ZnS QDs emits) that, using bioimaging, dual-emission hybrid sensors based on carbon points can

offer great promise for the investigation of fundamental biological processes.

Figures in vivo

In addition to relatively abundant reports related to carbon dot-based bio-probes for in vitro bioimaging, there have been several pioneering studies on obtaining in vivo bioFigures in recent years.

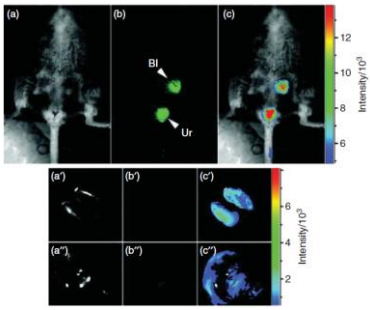

Figure 8. Intravenous injection of carbon dot solution in mice [23]

In 2009 Yang and other researchers, hot-pressed a mixture of graphite powder and cement, carbon dots synthesized via laser ablation (using a Q-switched Nd: YAG laser emitting at 1064 nm, 10Hz) with passivation of the surface of the PEG diamine. For the circulation of the entire body, a solution of carbon spots (440 µg in 200 µL) was injected intravenously into mice, after the abdomen was scraped to detect fluorescence of the points trapped in the organs during circulation. As shown in Figure 8, only emissions from the bladder area were observed and the bright fluorescence in the urine became visible in the Figure after 3 h, the photoactivated location microscopy allowed the display of the carbon spots injected intravenously.

Tao et al. prepared carbon nanodots from carbon nanotubes and graphite by a treatment with mixed acid, with which the in vivo fluorescence bioimage was performed (Figure 8).[26]

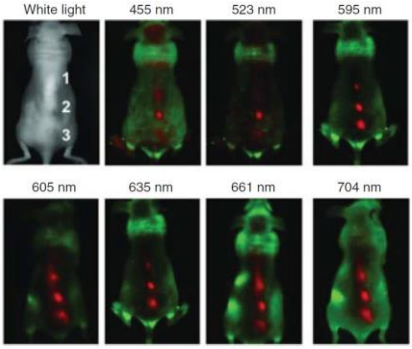

Figure 8. In vivo fluorescence figures of a mouse injected with carbon nanodots[26]

The researchers used several excitation wavelengths (for example, 455, 523, 595, 605, 635, 661 and 704 nm) and differentiated the background autofluorescence (green) by spectral demystification, the points injected subcutaneously (red) displayed Figures bright fluorescence (figure 9), among which those taken under longer wavelength excitation (595 nm and beyond) showed a much better separation between signal and background, as the tissue autofluorescence background decreased at longer wavelengths long. In addition, no perceptible toxicity of the carbon dots was found in the treated animals, thus showing that the carbon dots have an exceptional potential in the application of biomedical figures such as optical nanosounds.[26]

Carbon dots prepared from green carbon sources, such as milk, hold great promise for in vivo bioimaging due to their favorable photoluminescent properties. As a proof of concept in bioimaging applications, Wang et al.[34] synthesized and used carbon points from milk by microwave assisted methods for in vitro and in vivo bioimaging. In their work, HeLa cells were stained with carbon dots with distinct blue, green and red colors under excitation of 405, 488 and 543 nm, respectively, showing that most of the carbon points were distributed in the cell membrane and cytoplasmic areas , as evidenced by the strong existing fluorescence, which contrasts sharply with the

weak fluorescent signals from the carbon spots located in the cell nucleus, as can be seen in Figure 9.

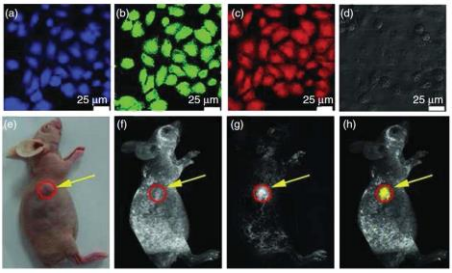

Figure 9. Laser scanning confocal microscopy figures of HeLa cells marked with a carbon dot[34]

Figure (a) shows the result of the excitation at 405 nm; passage of the emission band: 420-480 nm; in b) excitation at 488 nm; passage of the emission band: 500-580 nm; in c) excitation at 543 nm; passage of the emission band: 580–660 nm and (d) bright field (scale bar at 25 µm). In the figures (e - h) are fluorescence Figures of a mouse with a U87 MG tumor after intratumoral injection of carbon dots, in (f) the autofluorescence of the mouse, (g) fluorescence of the carbon dots (with excitation at 455 nm, emission at 500-650 nm and exposure time 200 ms) and (h) merged Figures.

As for in vivo bioimaging, nude female mice with U87 MG human glioblastoma tumors were injected intratumorally with carbon dots and then photographed by the Maestro in vivo imaging system. Significantly, the tumor tissues distributed with the carbon dots exhibited strong and spectrally resolved signals, suggesting the potential application of in vivo Figures using the prepared carbon dots, as seen in Figure 9 (e-h).

Likewise, the carbon dots synthesized from tire soot that can be found everywhere - are also very promising for in vivo bioimaging. Ko et al. used fluorescent carbon spots derived from tire soot by mixing the materials collected from the combustion of various parts of tires with nitric acid and then demonstrated the near-carbon infrared fluorescence using C6 (a lineage of glioma cells cells) cells for in vitro and in vivo bioimaging.[35]

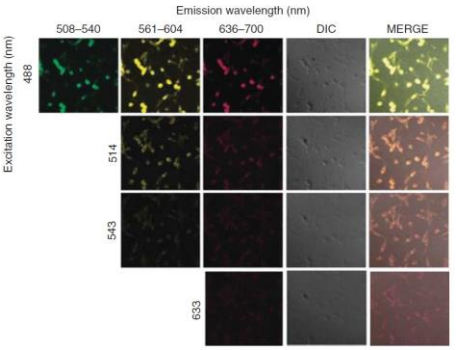

Figure 10. Microscopic confocal analysis of carbon spots derived from tire soot [35]

As shown in Figure 10, microscopic confocal analysis proved that the carbon dots were able to easily enter C6 cells, even in the absence of any additional functionality on the surface of the carbon dots. C6 cells with and without carbon capture were then implanted subcutaneously in the right thigh and in the left thigh of nude mice, respectively.

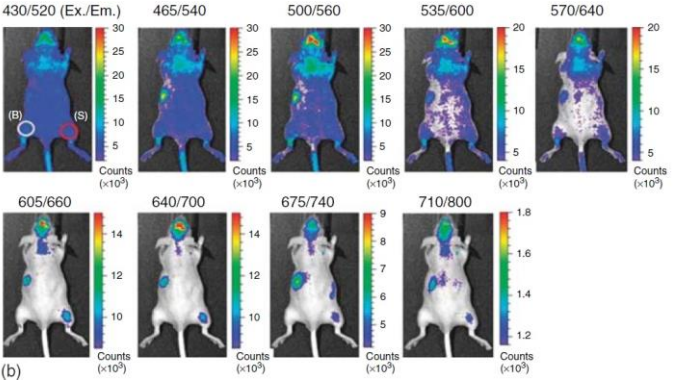

Figure 11. In vivo figures obtained from the injection of carbon spots derived from tire soot in mice[35]

As shown in Figure 11, in vivo Figures of C6 cells injected subcutaneously containing carbon dots were acquired with a series of excitation wavelengths (430, 465, 500, 535, 570, 605, 640, 675 and 710). Obviously, the fluorescence intensity decreased at longer excitation wavelengths, but a relatively strong fluorescence signal

that can be obtained differentiated from the background at emission wavelengths of 660, 700, 740 and 800 nm. Due to their near infrared properties, carbon dots were particularly advantageous for obtaining high resolution in vivo Figures.

CONCLUSION

Recent representative achievements were presented from the use of carbon points for bioimaging applications. Although the application of carbon dots as a type of nanomaterial for bioimaging has only been proven recently, it has attracted worldwide attention due to its easy preparation, fascinating photophysical properties, excellent stability, low toxicity and versatile surface chemistry.

Therefore, these types of carbon-based biosondes are highly suitable for long-term and real-time in vitro and in vivo imaging applications, featuring bright and stable fluorescent signals for direct, long-term visualization of biological labeling.

The versatility of carbon points offers enormous possibilities in a wide range of in vitro and in vivo imaging applications, including real-time cell tracking, high resolution multiplexed vascular imaging, intraoperative imaging guidance and so on.

In addition, recent improvements in the design and manufacture of carbon dot probes, together with the potential virtue of this technology, have shifted the focus from preparing single-component probes to the manufacture of hybrid nanostructures composed of various targeting, imaging and therapeutic modules. For example, when integrated with drugs or nucleic acid therapy, carbon dots can act as traceable delivery vehicles. In order to expand the functionality of the carbon points, it is very important to design multifunctional nano devices based on carbon points that can integrate the Figure, the drug load and even the detection capabilities in a single nanoparticle. However, the existing carbon dot probes are still not ideal enough, leaving much room for the advancement of new engineering for the functional surface of the carbon dots. The carbon

points have great potential for the application and Development of Nanomedicine Strategies for Advanced Theranostics.

REFERENCES

1. Mathur, R.B.; Singh, B.P.; Pande, S.; *Carbon nanomaterials: synthesis, structure, properties and applications*. CRC Press: New York, 2017.
2. Gonçalves, G.; Marques. P.; Vila, M.; *Graphene-based Materials in Health and Environment*. Springer: Genebra, 2016.
3. Jelinek, R.; *Carbon Quantum Dots: Synthesis, Properties and Applications*. Springer: Genebra, 2017.
4. Chen, C.; Wang, H.; *Biomedical Applications and Toxicology of Carbon Nanomaterials*. Wiley: Weinheim, 2016.
5. Sharon, M.; Mewada, A.; *Carbon Dots as Theranostic Agents*. Wiley: Hoboken, 2018.
6. Ray, S.C.; Jana, R.N.; *Carbon Nanomaterials for Biological and Medical Applications*. Elsevier: Amsterdam, 2017.
7. Rocha-Filho, R. C.; *Química Nova na Escola*, **1996**, 4, 7.
8. Hui, Y.Y.; Chang, H.C.; Dong, H.; Zhang, X.; *Carbon Nanomaterials for Bioimaging, Bioanalysis, and Therapy*. Wiley: Chennai, 2019.
9. Klingeler, R.; Sim, R.B.; *Carbon Nanotubes for Biomedical Applications*. Springer: Heidelberg, 2011.
10. Sattler, K.D.; *Carbon nanomaterials sourcebook: Graphene, Fullerenes, Nanotubes, and Nanodiamonds*. CRC Press: Boca Raton, 2016.
11. Zhang, M.; Naik, R.R.; Dai, L.; *Carbon Nanomaterials for Biomedical Applications*. Springer: New York, 2016.
12. Loss, M.R.; *Nanociência e nanotecnologia: compósitos termofixos reforçados com nanotubos de carbono*. Interciência: Rio de Janeiro, 2014.
13. Arnault, J.C.; *Nanodiamonds: Advanced Material Analysis, Properties and Applications*. Elsevier: New York, 2017.
14. Atchudan, A.; Edison, T.N.J.I.; Perumal, S.; Selvam, N.C.S.; Lee, Y.R.; *Journal of Photochemistry & Photobiology A*: *Chemistry*. **2019**, 372, 99.
15. Cheng, C.; Xing, M.; Wu, Q.; *Optical Materials*. **2019**, 88, 353.
16. Das, B.; Dadhich, P.; Pal, P.; Thakur, S.; Neogi, S.; Dhara, S.; *Acta Part A: Molecular and Biomolecular Spectroscopy*. **2020**, 227, 117669.
17. Roy, E.; Patra, S.; Madhuri, R.; Sharma, P.K.; *Chemical Engineering Journal*. **2019**, 356, 1070.
18. Qin, K.; Zhang, D.; Ding, Y.; Zheng, X.; Xiang, Y.; Hua, J.; Zhang, Q.; Ji, X.; Lid, B.; Wei, Y.; *Analyst*. **2020**, 145, 177.
19. Qu, Z.; Liu, L.; Sun, T.; Hou, J.; Sun, Y.; Yu, M.; Diao, Y.; Lu, S.; Zhao, W.; Wang, L.; *Nanotechnology*. **2020,** 31, 175102.
20. Yang, Y.Z.; Xiao, N.; Liu, S.G.; Han, L.; Li, N.B.; Luo, H.Q.; *Materials Science & Engineering C*. **2020**, 108, 110401.

21. Zhang, S.; Ji, X.; Liu, J.; Wang, Q.; Jin, L.; *Acta Part A: Molecular and Biomolecular Spectroscopy*. **2020**, 227, 117677.
22. Giannelis, E.P.; Bourlinos, A.B.; Stassinopoulos, A.; Anglos, D.; Zboril, R.; Karakassides, M.; *Small*. **2008**, 4, 455.
23. Yang, X.R.; Zhu, H.; Wang, X.L.; Li, Y.L.; Wang, Z.J.; Yang, F.; *Chem. Commun*. **2009**, 45, 5118.
24. Li, Q.; Liu, R.L.; Wu, D.Q.; Liu, S.H.; Koynov, K.; Knoll, W.; *Angew. Chem. Int. Ed*. **2009**, 48, 4598.
25. Sun, Y.P.; Yang, S.T.; Wang, X.; Wang, H.F.; Lu, F.S.; Luo, P.G.; Cao, L.; Meziani, M.J.; Liu, J.H.; Liu, Y.F.; Chen, M.; Huang, Y.P.; *J. Phys. Chem. C*, **2009**, 113, 18110.
26. Tao, H.Q.; Yang, K.; Ma, Z.; Wan, J.M.; Zhang, Y.J.;, Kang, Z.H.; Liu, Z.; *Small*. **2011**, 8, 281.
27. Zhu, S.J.; Zhang, J.H.; Tang, S.J.; Qiao, C.; Wang, L.; Wang, H.; Liu, X.; Li, B.; Li, Y.; Yu, W.; Wang, X.; Sun, H.; Yang, B.; *Adv. Funct. Mater*. **2012**, 22, 4732.
28. Sun, Y.P.; Yang, S.T.; Cao, L.; Luo, P.G.; Lu, F.S.; Wang, X.; Wang, H.F.; Meziani, M.J.; Liu, Y.F.; Qi, G.; *J. Am. Chem. Soc*. **2009**, 131, 11308.
29. Kong, W.Q.; Liu, R.H.; Li, H.; Liu, J.; Huang, H.; Liu, Y.; Kang, Z.H.; *J. Mater. Chem. B*. **2014,** 2, 5077.
30. Leménager, G.; Luca, E.D.; Sun, Y.P.; Pomp, P.P.; *Nanoscale*. **2014**, 6, 8617.
31. Zhu, S.J.; Zhang, J.H.; Qiao, C.; Tang, S.J.; Li, Y.; Yuan, W.; Li, B.; Tian, L.; Liu, F.; Hu, R.; Gao, H.; Wei, H.; Zhang, H.; Sun, H.; Yang, B.; *Chem. Commun*. **2011**, 47, 6858.
32. Liu, Q.; Guo, B.D.; Rao, Z.Y.; Zhang, B.H.; Gong, J.R.; *Nano Lett*. **2013**, 13, 2436.
33. Tian, Y.; Zhu, A.; Qu, Q.; Shao, X.; Kong, B.; *AnChem. Int. Ed*. **2012**, 51, 7185.
34. Wang, J.; Peng, F.; Lu, Y.M.; Zhong, Y.L.; Wang, S.Y.; Ji, X.Y.; Zhou, Y.F.; Yang, L.; Xu, M.F.; Su, Y.Y.; Liao, L.S.; He, Y.; *Adv. Opt. Mater*. **2019**, 3, 103.
35. Ko, H.Y.; Chang, Y.W.; Paramasivam, G.; Jeong, M.S.; Cho, S.J.; Kim, S.; *Chem. Commun*. **2019**, 49, 10290.