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REVIEW ARTICLE

Onion-Like Carbon Nanostructures: An Overview of Bio-Applications

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Abstract: This article presents a brief review of the knowledge concerning onion-like carbons (OLCs). These nanostructures are some of the most fascinating carbon forms due to their unusual structure and physico-chemical properties. Generally, OLCs consist of a hollow-spherical fullerene core surrounded by concentric graphitic layers with increasing diameter. Nevertheless, they can have different size, shape and type of core, which determine their physicochemical properties. In this article, we review the most important literature reports in this area and briefly describe these nanostructures, their physical and chemical properties and their potential uses with a focus on biomedicine.

Keywords: Onion-like nanostructures, multi-layered fullerenes, composites, functionalization, physicochemical properties, bioapplications.

1. INTRODUCTION

It seems that the interest in carbon nanostructures (CNs) has been slowly disappearing. Although the decline in the number of published scientific articles could support this statement, it could not be more misleading. It seems that the scientific research focusing on these nanostructures has reached the next level - we are moving from the basic studies of CNs to their applications. The fascination with the discovery of new allotropic forms of carbon materials (CMs) and the studies of their physicochemical properties are already passing. We are familiar with the advantages and disadvantages of these CNs [1-4], which are mainly made of carbon atoms. We realize that the combination of several hundred or even several thousand carbon atoms in the well-organized macromolecular systems creates interesting structures with unique physicochemical properties. Their uniqueness results not only from the chemical composition and size but also from the fact that the number of carbon atoms and the way they are bonded defines the physicochemical properties of the formed CNs. Among these nanostructures, one may find one-dimensional (1D: carbon nanotubes) [5], two-dimensional (2D: graphene) [6, 7], three-dimensional (3D: diamond and graphite) [8], and those that are considered as idealized zero-dimensional (0D: fullerene and onion-like nanostructure) systems due to their almost perfect spherical structures in the three dimensions [9-12]. Despite almost identical chemical compositions, these nanostructures differ significantly in the physical properties and chemical reactivity. The latter significantly affects their subsequent use. This uniqueness allows searching for special solutions in nanotechnology, medicine or biological sciences.

In this brief discussion, we will try to summarize the achievements for only one group of CNs, commonly known as onion-like carbons (OLCs). In addition to the short characteristics of basic research, including the methods of obtaining these CNs, their structures and physicochemical properties, our considerations will be limited to their potential applications in *biomedicine*. Although this field is not the most widely represented in the literature on these CNs, currently, this field of research seems promising, and the interest in this area is growing rapidly. We will try to prove this statement in our further considerations.

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2. STRUCTURES AND PHYSICAL PROPERTIES OF ONION-LIKE STRUCTURES

OLC structures consist of a hollow-spherical fullerene core surrounded by concentric graphitic layers with increasing diameter (Fig. **1**). The distance between these layers is usually 0.335 nm, and similar as in graphite (0.334 nm) [13]. OLCs consist of quasi-spherical- and polygonal-shaped graphene layers close to one another, due to the van der Waals forces between neighboring shells. That is why they are also referred to as "nano-onions," "carbon nano-onions," "onion-like carbon," or "buckyonions" [14-16]. The number of carbon atoms forming a corresponding layer of spherical OLCs (6-8 graphitic shells and diameter of 5-6 nm) is calculated according to the formula [10]:

$$C_{x(n)} = C_{60} \cdot n^2 \tag{1}$$

where n is the sequential number of the corresponding layer; x (n) defines the number of carbon atoms comprising the layer.

Spherical OLCs have a cage-within-a-cage structure, with smaller fullerenes nested inside larger ones, forming the ideal fullerene series "$C_{60}@C_{240}@C_{540}@\cdot\cdot\cdot@C_{60}n^2$" [10]. In this respect, OLCs are also frequently called "multi-layered fullerenes" [17]. The surface of OLCs is mainly composed of hexagonal rings similar to graphene layers, but they also possess some pentagonal, heptagonal, quadrangular and octagonal rings, and contain some defects and holes [14, 18]. The structural defects present (edges, dangling bonds, vacancies, dislocations) lead to a relatively high chemical reactivity of OLCs. This is one of the main advantages of these CNs.

Although the number of atoms can be calculated using one equation, the number of different CNs classified as OLCs is unlimited. Fig. (**1**) presents images obtained with high-resolution transmission electron microscopy (HRTEM) representing some examples of the diverse types of the OLC nanostructures. The dimensions of the nanostructures fluctuate within the range of 2 and 200 nm. OLCs can be divided according to three parameters: size: "big" or "small"; shape: "spherical" and "polygonal"; type of core: "dense" and "hollow" (empty or filled with different metals) (Fig. **1**). The type of OLCs (size and shape) depends on the type of method and type of starting components used to form these CNs.

Various methods of OLC synthesis have been published. Only a few of them have found widespread use. This is mainly due to the efficiency of the production process and the high purity of the obtained products. The most popular methods include, among others, thermal annealing of nanodiamonds (NDs) [15], arc-discharge process between graphite electrodes submerged in water [19, 20], carbon ion implantation in the presence of metals (Ag, Cu, Co), chemical vapor deposition [21], decomposition of polycyclic aromatic hydrocarbons under laser irradiation [22] and solid-state carbonization of organic compounds [23].

Currently, the most common and economical method used for the production of spherical OLCs is the method proposed by Kuznetsov in 1994 [15], which was further modified and applied by other authors [24-26]. The large-scale production of OLCs (gram quantities, with the onion yield close to 100%) is based on annealing of ND particles (average diameter of 5 nm) at high temperatures in an inert atmosphere under high vacuum. The diameter of the formed OLCs is analogous to the diameter of the used NDs.

Upon high-temperature annealing, the graphitization process of NDs starts on the surface and progressively extends into the bulk of the particle, initially resulting in the formation of graphene shells surrounding an ND core. The formation of perfect graphitic shells occurs only after the complete transformation of the diamond core, at temperatures higher than 1,600°C. This annealing leads to the formation of small particles with 6-8 graphitic shells and diameters of 5-6 nm, and these particles are frequently called "small" OLCs. Thermal annealing of NDs up to 1,700°C leads to the progressive formation of polygonal nanostructures (Fig. **1**).

The types of formed nanostructures, their structures, the numbers of layers and the distances between them, determine their different physical and chemical properties. Because OLCs derived from NDs are the most commonly used nanostructures in many areas, the physicochemical properties of these CNs will be briefly discussed below. We will pay attention mainly to those physical properties that affect the chemical properties of the OLCs, in particular, their chemical reactivity. These properties play major roles in further applications of the CNs. Compared to other CNs, OLCs are not very soluble in many organic and inorganic solvents, which restricts their applications in many fields. Although OLCs have greater surface curvature and strain energy, which leads to higher chemical reactivity [32, 26], their "solubility" is still very low. In the case of CNs, the term "dispersibility" should be used rather than "solubility." Even in the case of modified CNs, it is impossible to obtain real solutions, *i.e.,* completely dissolved CNs, due to the van der Walls forces between carbon nanoparticles.

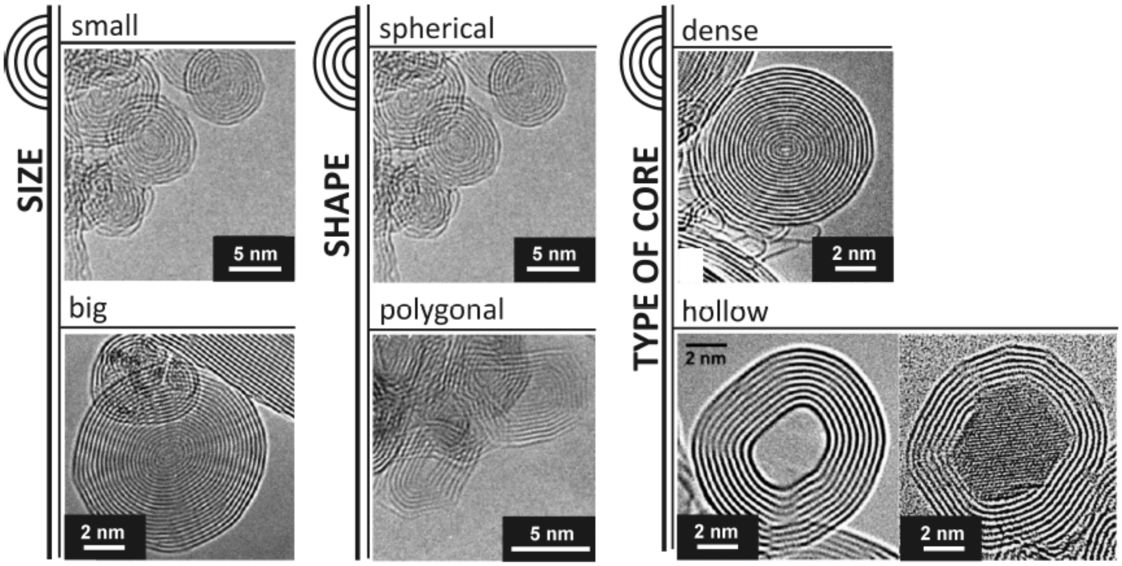

Fig. (1). High-resolution TEM images of OLCs: “big”, “small”, spherical, polygonal, dense and hollow. Reprinted with permission from Ref. [20, 28-31]. Reproduced by permission of AIP Publishing LLC, Elsevier and Wiley & Sons.

As already mentioned, the spherical “small” OLCs are formed during a high-temperature annealing in the progressive graphitization process of NDs, from the external layers into the bulk of the particle. This process was investigated using HRTEM [33], Raman spectroscopy [34, 35], solid state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) [36, 37], porosymetry [38, 39], X-ray emission and X-ray diffraction (XRD) studies [40, 41]. The following conclusions can be drawn: (i) annealing the NDs in the 1,100-1,700°C temperature range leads to the creation of spherical OLCs with a 5-6 nm diameter; (ii) increasing the annealing temperature results in an increasing weight fraction of *sp*^{2}-carbons in the OLCs [42]; (iii) the formation of perfect graphitic shells occurs only after the complete transformation of the diamond core, which occurs at temperatures ca. 1,700°C; and (iv) thermal annealing of NDs up to 1,700°C leads to the progressive formation of polygonal nanostructures.

The textural properties of OLCs, such as the specific surface areas (SSA), densities and average pore sizes, greatly affect the electrochemical and catalytic properties of CNs, which are crucial in the design of electrochemical sensors, including biosensors [43, 44, 45]. These parameters depend on the annealing temperature utilized. OLCs obtained by annealing NDs at 900-1,900°C display large SSA values. The SSAs determined by nitrogen gas adsorption are between ca. 390 and ca. 600 m^2/g. At 1,500°C, NDs are totally transformed into spherical, defect-free OLC structures, which exhibit the maximum SSA values. Additionally, the OLC nanoparticles exhibit only a small fraction (~5 vol.%) of micropores (d < 2 nm), and most of the volume is composed of 0.50-36 nm pores [39, 46]. The OLCs are highly absorptive over a broad microwave and RF frequency range (2−38 GHz) [47] and Terahertz range (12–230 THz) [48], as a result of their small size and high reactivity [47,49]. These unique structural and textural parameters are crucial for the bio-analytical applications, especially when the latter are based on electrochemistry as their fundamental principle.

3. TOWARDS BIO-APPLICATIONS: ADVANTAGES OF FUNCTIONALIZED OLCS

As already mentioned, the low dispersibility and hydrophobic nature of OLCs limit their applications in biosensing, bioimaging and other bioapplications. One of the most popular methods for increasing OLC’s solubility is their functionalization that may be performed in a covalent or non-covalent manner. Considering the application of CNs in biomedicine, their chemical stability and resistance to pH changes are also extremely important factors. Consequently, covalent modifications are more often used in this field.

3.1. Covalent and Non-Covalent Functionalization of OLCs

The hydrophobic nature of OLCs prevents their easy use in biological applications. Therefore, the modification with other compounds or biomolecules is required. The reactivity of fullerene-like structures, including OLCs, decreases with increasing size due to a decrease in the surface curvature [50]. In this respect, the synthesis of OLCs with few layers is highly desired to increase the ability to modify them using chemical reactions. The ability to functionalize OLC surfaces depends on the presence of defects on the carbon surface as well as on the presence of carbon atoms with *sp^2* hybridization [51].

According to theoretical and experimental studies, two types of C–C bonds exist in the OLCs [52-54]: (i) the C-C bond between a hexagon and a pentagon with lengths between 1.455 and 1.460 Å, and (ii) the C-C bond between two hexagons with lengths between 1.400 and 1.391 Å. The lengths of these bonds correspond to the lengths observed for polyenic bonds in polyolefins, which are susceptible to addition reactions [55]. Due to these properties, many covalent reactions were applied such as amidation [56], oxidation [57, 58] 1,3-dipolar cycloaddition [59, 60, 2+1] cycloaddition [61], nucleophilic substitution [62], diazonium addition [63], reduction [62], fluorination [64] and radical addition [65] (Fig. **2**). Attaching of various functional groups on the OLC surface improves their solubility and changes their physicochemical properties (Fig. **2**).

In addition to many reactions typical for organic synthesis of fullerene chemistry, one was conducted that led to the production of unique products with specific physicochemical properties. The first covalent functionalization of “small” OLCs incorporated into self-assembled monolayer with biomolecules on gold surface was reported in 2010 (Fig. **3**) [66]. The oxidized-OLCs (ox-OLCs) were linked directly to the cysteamine layer trough the amine bond. On this modified “layer-by-layer” surface, biotin was attached and interacted with avidin. This system, containing OLCs and biomolecules, is a potential candidate for biosensors and other bio-applications [66].

Fig. (**4**) presents the OLC nanostructures covalently functionalized by fluorophores. The boron dipyrromethene-OLCs (BODIPY-OLCs) (Fig. **4a**) were synthesized in two steps. First, using the Tour reaction, OLCs were functionalized with benzoic acid. Then, employing an ester condensation reaction, OLCs with meso-phenol substituted boron dipyrromethene were synthesized (Fig. **4a**) [68,69]. Additionally, other derivatives of BODIPY were synthesized, such as boron difluoride azadipyrromethene (Fig. **4b**) [70] and π-extended distyryl-substituted boron dipyrromethene (Fig. **4c**). These BODIPY derivatives were used to functionalize “small” OLCs [71]. In addition, the non-covalent functionalization by pyrene-BODIPY conjugates to form π-π-stacking interaction with OLC surfaces was performed by Bartelmess *et al.* [72].

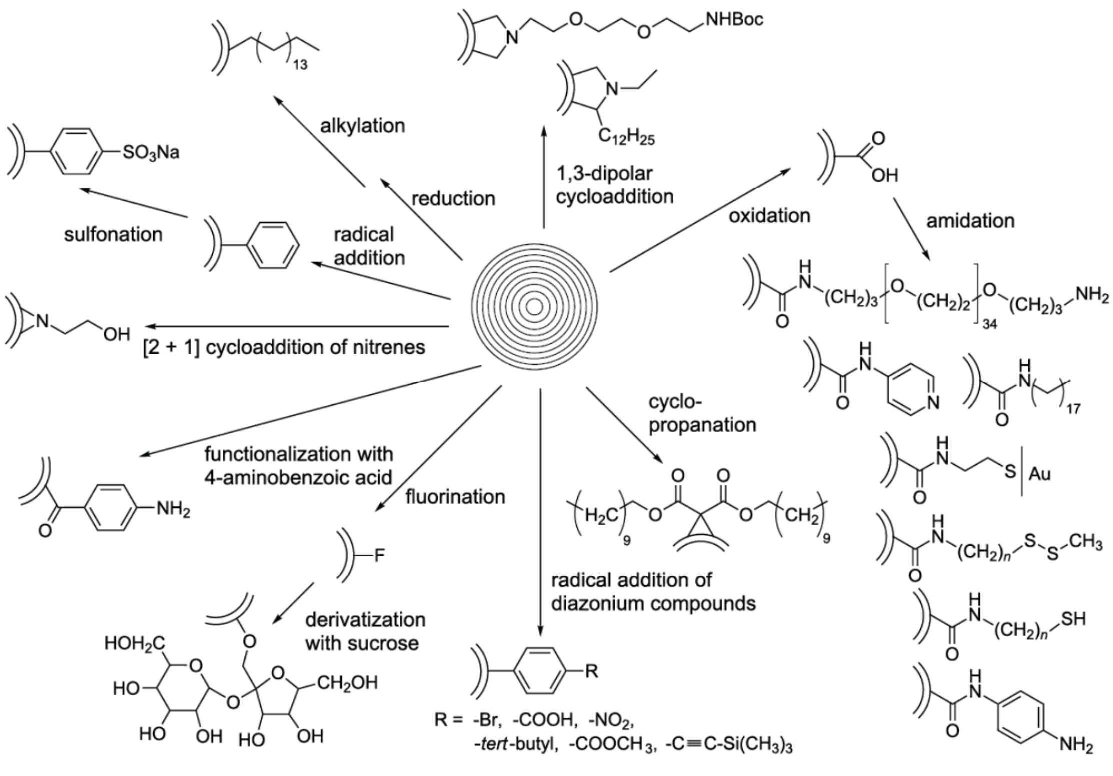

Fig. (2). Covalent functionalization pathways for OLCs. Reprinted with permission from Ref. [67]. Reproduced by permission of Beilstein-Institute.

Another example of fluorescein-functionalized OLC (fluoro-OLC) is shown in Fig. (**4d**). The fluoro-OLC derivative was synthesized in two steps with a by-product, Tour-functionalized OLCs [73]. Frasconi *et al.* synthesized doubly functionalized OLCs with fluorescein and folic acid derivatives linked with a polyethylene glycol chain to OLC surfaces for potential therapeutic and diagnostic applications [74].

3.2. Composites and Doped-OLCs

The formation of OLC composites and doping of carbon nanoparticles with heteroatoms are relatively new and popular methods of their functionalization. Such modification results in the optimization of electrocatalytic properties and the biocompatibility enhances the efficiency and increases biological applicability.

The application of surfactants in combination with OLCs increases the solubility of the latter. This approach allows the formation of water-soluble composites characterized by high hydrophilicity and mechanical stability combined with unique physicochemical properties. The OLCs were successfully modified by

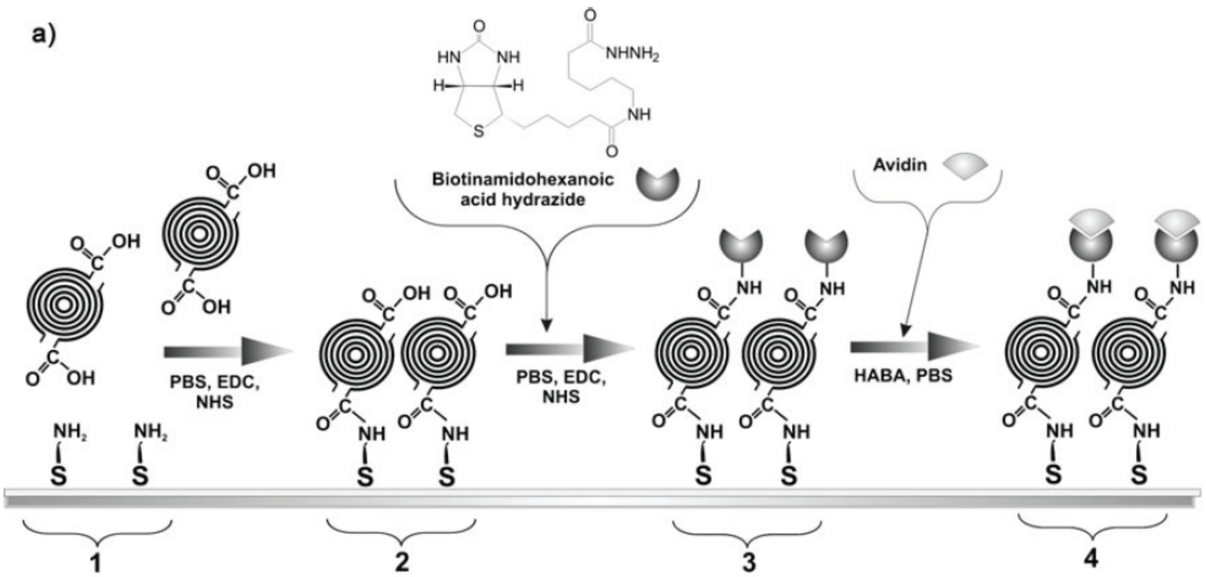

Fig. (3). Scheme of modification "layer-by-layer" gold surface by 1) cysteamine, 2) ox-OLCs, 3) biotin, and 4) avidin. Reprinted with permission from Ref. [66]. Reproduced by permission of Wiley & Sons.

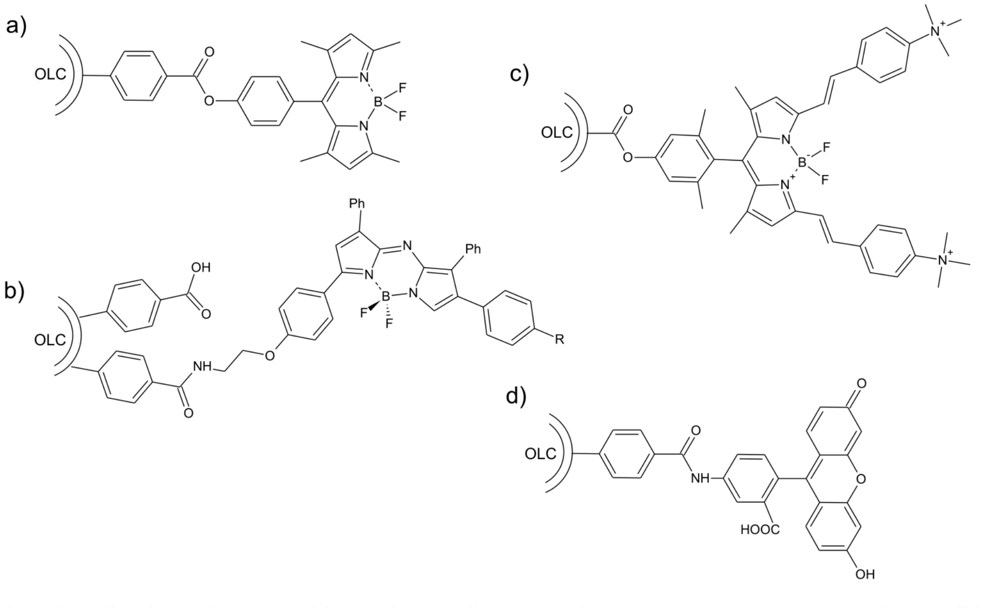

Fig. (4). Covalent functionalization of OLCs with: (**a**) boron dipyrromethene (BODIPY-OLCs), (**b**) boron difluoride azadipyrromethene, (**c**) π-extended distyryl-substituted boron dipyrromethene, (**d**) fluoresceinamine.

non-covalent functionalization with hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), sodium dodecyl benzene sulfonate (SDBS), 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol (Triton X-100) and polyethylene glycol sorbitan monolaurate (Tween 20) [75]. Such modifications provided long-term stability to the composites and high dispersability in aqueous solutions, as confirmed by TEM, dynamic light scattering and zeta potential.

The OLC composites can be used as carriers of different compounds, such as natural polyphenols, which show strong antibacterial properties and are able to protect mammalian cells. The composite containing OLCs and polyethylene glycol (PEG 400) was tested as a carrier of biologically active compounds including 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (β-PGG) [76]. The specific interactions between the OLC/PEG systems, polyphenolic derivatives and a triple-helical collagen-like peptide [(Pro-Hyp-Gly)$_4$-Pro-Hyp-Ala-(Pro-Hyp-Gly)$_5$]$_3$ were described and confirmed by surface plasmon resonance, among other techniques.

The OLC composites can act as platforms for electrostatic immobilization of polyphenolic compounds, such as quercetin, which has anti-inflammatory properties and may be effective in fibromyalgia treatment [77]. For this purpose, the carbon nanoparticles were first modified with poly(4-vinylpyridine-*co*-styrene) or poly(ethylene glycol)/polysorbate 20 in a non-covalent procedure. Next, a modification using 3-mercaptopropionic or 2-mercapto-4-methyl-5-thiazoleacetic acids provided a biocompatible and hydrophilic character to the OLC's surfaces. The OLC systems with incorporated flavonoids, such as quercetin, could be applied in biosensing and drug targeting applications.

The most recent studies showed that nitrogen-rich OLCs are able to reduce oxygen [78]. The strategy described by Chatterjee *et al.* is based on the formation of nitrogen-doped OLCs (N-OLCs) from renewable biological resources, such as collagen. The product contained 7.5% of nitrogen embedded into the carbon molecular skeleton and exhibited an outstanding electrocatalytic activity as a metal-free catalyst alternative to platinum [79]. Similar catalytic properties have been exhibited by nitrogen-doped OLCs prepared by thermal annealing of nanodiamond particles using Kuznetsov's method [44].

One of the most important aspects of bioapplications is finding biocompatible systems with low toxicity and high electrical conductivity. That issue concerns defined systems, which may be applied in biosensors or other biodevices. In this respect, scientists are looking for novel systems, which are capable of ultrafast electric charge transfer. An example of a composite with high conductivity and high permittivity is OLC/polyaniline [80, 81, 82, 83]. The aniline functional groups were introduced through the 4-aminobenzoic acid onto the OLC's surface. The structure of the obtained nanocomposite formed homogeneous, easily dispersible material in protic solvents and showed excellent biocompatibility.

The OLC composites were also applied to water purification. The ferromagnetic metal-encapsulated $Fe_{0.64}Ni_{0.36}$OLC material revealed flexibility, stretching ability, capability for oil adsorption and cleansing properties, as proven by removal of methylene blue (MB) from water solution [84]. Similar bioremediation properties for heavy metal ion contaminants, including Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}, were described. The oxidized OLCs possess a 10-fold higher sorption capacity than single-layered fullerenes [85].

4. BIOMEDICAL APPLICATIONS OF OLCS AND THEIR SAFETY CONCERNS

4.1. Toxicological Aspects. Biocompatibility and Biodistribution

The toxicity of CNs utilized in biological and medical applications is a very important issue. The toxicology aspect of OLCs depends on their size, shape and the synthesis procedure. The first toxicity test of OLCs was performed in 2005 [86]. Their biocompatibility and influence on living organism were examined several times by different groups. The studies confirmed non-invasive features of OLCs, such as their low toxicity and weak inflammatory properties (Table **1**).

It was found that "big" OLCs (30 nm) in high doses could seriously affect cellular functions during cell maintenance, growth, and differentiation. These tests were done on human skin fibroblasts [86]. Additionally, the influence of "big" OLCs (30 nm, produced by the arc-discharge method) on human umbilical vein endothelial cells (HUVECs) was examined. OLCs exhibited a dose-dependent inhibitory effect on cell growth. In addition, OLCs could induce apoptosis in HUVECs and DNA damage [87]. It should be emphasized that "big", insoluble OLCs showed less cellular toxicity than multiwalled carbon nanotubes (MWCNTs) [83, 85]. This was confirmed by measuring unpaired electrons of particles and intercellular reactive oxygen species (ROS) in the presence or absence of antioxidant *N*-acetylcysteine (NAC) [88]. In this test, the toxicity of OLCs was negligible.

Table 1. Some examples of biomedical applications of OLCs and their safety concerns.

Functional Group or Structure	Analyses	Target Site	Properties of OLCs	Application	Refs.
PEGylated OLCs	MTS tests SPR spectroscopy analysis	Human fibroblasts Polyphenols	Biocompatible Non-cytotoxic	Drug delivery Drug delivery	[66, 86, 89] [90, 91] [76]
Water-soluble OLCs	Toxicity investigation	*Drosophila melanogaster* *Escherichia coli* *Caenorhabditis elegans*	Non-toxic Biocompatible	Drug delivery *In vivo* fluorescence imaging Biodelivery of drugs	[92, 94]
OLCs, MnO_2/OLCs	Cytotoxicity Hemolysis analysis	4T1 cell line	Negligible toxicity Non-toxic		[95]
Pyrene-BODIPY OLCs	Toxicity investigation Resazurin-based assay	HeLa Kyoto cells	Low toxicity	Imaging agents Target of cancer cells	[70, 72]
Distyryl-substituted B dipyrromethene OLCs	Toxicity investigation Colorimetric assay	MCF-7 and HeLa cells	Cyto-biocompatible	Imaging agents	[71]
BODIPY OLCs	*In vitro* toxicity investigation	MCF-7 cells	Low toxicity	Imaging agents	[69]
BODIPY OLCs, benz-OLCs	Toxicity investigation	Zebrafish	Non-toxic Biocompatible	Imaging agents Intracellular transport	[68]
OLCs/CTAB	Antibacterial activity	*Escherichia coli*	Antibacterial activity		[75]
OLCs/SDS, SDBS, Triton X-100, Tween 20	Antibacterial activity	*Escherichia coli*	Non-active		[75]
Folic acid OLC derivatives	Toxicity investigation	HeLa and KB cells	Low toxicity		[74]
Ox-OLCs	SPR spectroscopy analysis	Biotin-avidin model	Biocompatible	Biosensors	[66]
OLCs/Pt	Reduction of oxygen		Electrocatalytic activity	Biofuel cells	[96]
OLCs/SWCNTs/CS/Au NPs	Electrochemical detection	Carcinoembryonic antigen	High sensitivity Decreasing detection limit	Electrochemical immunosensors	[97]
Diazonium OLC derivatives	Amperometric detection	DNA of human papillomavirus oncogene	Enhancing sensitivity Decreasing detection limit	Amperometric sensors	[98]
*o*AP or Th modified OLCs	Electrochemical detection	Nitrite or ascorbic acids in real samples	Enhancing sensitivity Decreasing detection limit	Biosensors	[99]
N-OLCs	Non-enzymatic electrochemical detection	Hydrogen peroxide Dopamine Epinephrine Serotonin	Electrocatalytic activity High sensitivity and selectivity Decreasing detection limit	Electrochemical sensors	[44, 78, 79] [100]
OLCs/PDDA	Electrochemical detection	Dopamine	Electrocatalytic activity High sensitivity and selectivity	Electrochemical sensors Bio-fueling	[45]
Ox-OLCs	Fluorescence	Al(III) Glucose	Photocatalytic activity High sensitivity and selectivity	Fluorescent sensors	[101, 102]

Abbreviations: PEGylated – OLCs with polyethylene glycol (PEG 400); MTS - ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2-tetrazolium)) salt; SPR – surface plasmon resonance spectroscopy; pyrene-BODIPY - boron dipyrromethene-; CTAB – hexadecyltrimethylammonium bromide; SDS - sodium dodecyl sulfate; SDBS - sodium dodecyl benzene sulfonate; Triton X-100 - 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol; Tween 20 - polyethylene glycol sorbitan monolaurate; Ox-OLCs – oxidized OLCs; SWCNTs – single-walled carbon nanotubes; CS – chitosan; AuNPs – gold nanoparticles; *o*AP – *o*-aminophenol; Th – thionine; N-OLCs – nitrogen-doped OLCs; PDDA - polydiallyldimethylammonium chloride.

The cytotoxicity profiles of "small" OLCs, oxidized OLCs and PEGylated OLCs (OLCs functionalized by polyethylene glycol) were carried out on human fibroblasts [66]. The assay was based on (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2-tetrazolium) salt (MTS), which plays a key role in colorimetric proliferation, cytotoxicity or chemosensitivity tests [89-91]. MTS is bioreduced by NADPH/NADH, which is produced by active mitochondrial dehydrogenase inside living cells. The reduced tetrazolium salt is a colored, water-soluble formazan product which is generated in the presence of a phenazine ethylosulfate (PES). The quantity of formazan that is directly proportional to the number of living cells in the culture is measured by a calorimetric method. The results of MTS assay have shown biocompatibility and non-cytotoxicity of "small" OLCs [66].

In another study, an investigation of the toxicity on fruit flies (*Drosophila melanogaster*) was performed [92]. They were fed nourishment with and without water-soluble OLCs. The morphological appearance was invariable for flies that were fed OLCs and standard food. Moreover, OLCs were easily excreted from *Drosophila melanogaster*. However, it was observed that pupae, male and female adults fed with the OLCs, weigh more than the control flies, but this fact did not affect the normal activity of their life. A recent study showed that OLCs crossed the blood brain barrier (BBB) and entered the brain without influencing the examined organism, in this case – mice [93]. This illustrates an opportunity of OLCs application for drug delivery into brain with a subsequent easy removal from the body. Analogously to the *Drosophila melanogaster* research mentioned above, a gradually decreasing fluorescence intensity of both the excreta and the body was monitored. Within six days, the fluorescence in mice after intravenous tail vein injection decreased to a negligible level, as in untreated mice [93].

Additionally, Revuri *et al.* carried out the cytotoxicity and hemolysis analyses of OLCs and their OLC/MnO_2 composites [95]. OLCs were incubated in a 4T1 cell line for 24 hours and showed negligible toxicity profiles, even at concentrations up to 100 µg/mL. The hemolysis analysis showed no toxicity effects. Only minimal erythrocyte disruptions in the blood were noticed [95].

The near-infrared (NIR) fluorescent OLCs modified by boron difluoride azadipyrromethene (Fig. **4b**) and non-covalently pyrene-BODIPY-functionalized OLCs were tested on HeLa cells [70,72]. The viability of HeLa cells after incubation with five different concentrations of the modified OLCs was determined by a resazurin-based assay. The tests showed a slight reduction in the cell viability. The far-red fluorescent OLCs modified by π-extended distyryl-substituted boron dipyrromethene were tested for cell viability of human breast cancer stem (MCF-7) and HeLa cells. The colorimetric assay (WST1) was conducted with different concentrations of functionalized OLCs for various incubation times. Cell viability values higher than 80% confirmed cyto-biocompatibility of OLCs [71]. The *in vitro* toxicity of BODIPY-OLCs (Fig. **4a**) was also examined. The viability of MCF-7 cells was checked upon their exposure to different concentrations of BODIPY-OLCs and their intermediate products, benz-OLCs. The toxicity measurements after 24, 48 and 72-hour incubation did not show significant toxicity compared with the cell control [69].

In another study, the first-time toxicity evaluation of BODIPY-OLCs on vertebrates was reported [68]. The zebrafish was chosen as a model organism, which exhibits many similarities in major organs, tissues and processes to higher vertebrates [103]. To observe the developmental stages of zebrafish, benz-OLCs and BODIPY-OLCs with different mass concentrations were added to the embryo growth medium. The functionalized OLCs were internalized through both swallowing and skin-absorption. The survival rates after zebrafish treatment with OLCs were higher than 95% compared with untreated zebrafish. In the larvae stages, the differences in such parameters as heartbeat rate and frequency of movements were not observed [68]. Some morphological malformations, such as yolk sac edema, pericardial edema, fin fold abnormalities and tail flexure were observed. However, the total percentages of abnormalities, induced by OLCs, were less than 4% [68]. This study showed the non-toxicity of homogenous well-dispersed functionalized OLCs and their good biocompatibility with the zebrafish body.

In aforementioned research on OLC's composites with surfactants (CTAB, SDS, SDBS, Triton X-100 and Tween 20), their effects on *Escherichia coli* strains were assayed [75]. The OLC/CTAB composite showed only a significantly decreasing cell viability. The non-modified OLCs and their composites containing SDS, Tween 20, Triton X100 or SDBS had no or little effect on bacterial cell viability, when compared to the negative control and pure detergents. Thus, the OLC/CTAB composite influences *E. coli* cultures and has strong antibacterial activity. Moreover, the influence of OLCs and the OLCs functionalized with benzoic acid, pyridinium and pyridine, on morphology and behavior, and the long-term effects on the development and reproductive capability of *Hydra vulgaris* were investigated [104].

The cytotoxicity assay for OLCs multi-functionalized with fluorescence and folic acid derivatives was performed [74]. The cellular viability of HeLa and KB cells after exposure to OLCs at different concentrations and incubation times was evaluated. Cells showed more than 80% viability compared with the control sample [74].

All analyses classified OLCs as biologically safe and non-toxic materials (Table **1**) [104]. Additionally, OLCs have shown weak inflammatory properties and low toxicity and demonstrated an efficient uptake by antigen-presenting cells and transport to lymph nodes [73].

4.2. Multifunctional Nanoparticle in Biomedicine

The multifunctional character of OLCs was described in some reviews [10, 67]. The authors focused on their unique, fascinating properties and wide-range potential applications. The abilities for the functionalization of OLCs depend mainly on the applied synthesis procedure, which determines their further physico-chemical properties, and the presence of different molecules or metals during the OLC formation process [105-107]. The OLCs can be used in different fields, mainly in bioelectronics [108], biocatalysis [44], energy storage [43, 109] and biosensors [66]. The multi-functionality is the key feature of OLCs. From the biomedical point of view, they may be used as tumor markers, and in cell and tissue imaging, and drug delivery or sensing fields [110, 67, 111].

In all cases, the OLCs should have excellent biocompatibility. This parameter is crucial for preserving the activity of biologically important molecules. The OLC materials can perform a support function for different enzymes, for example, alkaline phosphatase, horseradish peroxidase and glucose oxidase [112]. It has been proven that specific immobilization of such biocatalysts on OLC surfaces can preserve the electro-catalytic activity and ensure the long-term stability under variable pH and temperature conditions. The first covalent functionalization of oxidized OLCs with biomolecules was performed using biotin-avidin interactions [66]. These studies showed that protein-like molecules attached to the OLCs retain their biological activity. The non-toxicity of OLCs and their excellent biocompatibility further support using these systems for biosensor construction and for other biological applications.

The abovementioned properties of OLCs are also used in the imaging probe field for cancer cells and other important diseases [113]. For example, an attachment to the OLC's surface of a substituent terminated with bromine allowed a specific fluorophore immobilization, thus leading to the formation of potential imaging agents for biomedical applications. This promising field is described in detail in Chapter 4.3. focusing on optical and biological imaging.

It was also proved that even small amount of OLC's in polymer matrix can significantly affect the composite electromagnetic (EM) response [114]. In that case the EM characteristic depends on the OLC's structure, size and amount. Macutkevic *et al.* presented the spectral features of OLC-polymethyl methacrylate (PMMA) composite in the Terahertz frequencies [47]. Their EM properties may have potential applications in therapeutic and diagnostic field including selective thermolysis of cancer cells and bioimaging [115].

The outstanding advantage of OLCs is their capability of energy storage [39, 46, 105, 116]. This feature is most often used in supercapacitors, in the electronic or transport industry sectors [117, 118]. A search for supercapacitor devices with excellent electrochemical performance parameters is still required, and this also applies to the biomedical field. For example, the patients with cardiac pacemakers and other implantable electronic medical devices end up requiring re-implantations when the batteries run out. The challenge is now to develop the systems capable of collecting electricity from the body and use it, for example, to power the implants. It is important to develop supercapacitor devices capable of storing the energy inside the body using safe and non-toxic components. Soon, OLCs may be used as ideal candidates to replace the ultra-thin graphene. Presently, OLCs have shown their energy storage capabilities separately [119, 120, 121]; as primary components in various types of composites with: metals [122-124], metal oxides [125-131], metal hydroxides [132], conducting polymers [109, 83, 133, 134] or as doped-carbon nanostructures with heteroatoms [43, 135].

The electrocatalytic properties of OLCs can also be used in biofuel cells, miniaturized devices which use living organisms to produce electricity. Such a way of free electric energy sourcing might be very appreciated in biomedicine to power electric implant devices. In this case, OLCs might support the catalytic effectiveness of electrodes and bioelectrode systems. Yang *et al.*

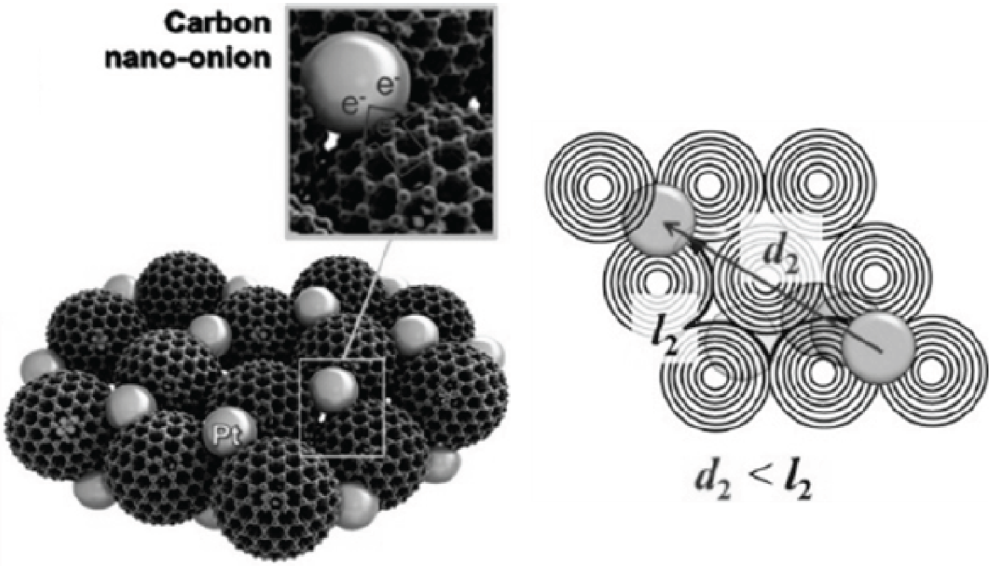

Fig. (5). Islands on a particle for Pt/OLC and Oswald ripening or surface migration. Reprinted with permission from Ref. [96]. Reproduced by permission of American Chemical Society.

used ordered OLCs as a support base for a nanomolecular Pt-catalyst able to reduce oxygen (Fig. **6**) [96].

OLCs can also be used as adsorbents for the removal of bisphenol A (BPA) from aqueous solutions [136]. BPA is a model endocrine-disrupting compound (EDCs), which can affect human beings even at low concentrations. BPA removal is possible with OLCs modified with Fe_2O_3 which provides a magnetic character to this carbon material.

4.3. Imaging (Optical and Biological Imaging)

The optical imaging by oral ingestion of a fluorescent probe is an ideal and the simplest approach to monitor *in vivo* life functions, the growth cycle of living species, biomolecule/chemical tracking or labeling. The main challenge is a selection of a good contrast agent, which should be non-toxic, non-invasive, inert, sufficiently small and water-soluble.

In 2011, Ghosh *et al.* were the first to use water-soluble OLCs (ws-OLCs) as fluorescent reagents to image the *Drosophila melanogaster* life cycle [92]. OLCs were synthesized by pyrolysis of wood wool at 600°C in a muffle furnace under a flow of nitrogen/oxygen mixture (95:5). The water-soluble carbon nanoparticles were added to standard corn syrup-sucrose-agar food. Upon oral ingestion, it enabled observation of *Drosophila melanogaster* full-life cycle over 12 days, from egg to adulthood, including visualization of internal organs [92]. OLCs did not perturb the normal activity of fruit flies. Moreover, they were easily removed from the *Drosophila melanogaster* body, as shown by the loss of fluorescence intensity of excreted materials over 12 days. In 2012, Sonkar and coworkers reported potential application of ws-OLCs (from 10 to 30 nm in diameter) as fluorescent probes for *in vivo* fluorescence imaging [94]. Initially, the primitive prokaryotic cells *(Escherichia coli)* were fed standard Luria-Bertani medium with different OLC concentrations. After simple and non-invasive feeding, the fluorescence experiments were conducted and showed that OLCs may be applied as highly fluorescent bioimaging agents. Moreover, OLC-labeled *E. coli* were fed to more complex eukaryotic (multicellular) *Caenorhabditis elegans*. These studies confirmed that OLCs have a very good biocompability as bioimaging agents, and *E. coli* cells could be a convenient vehicle to transport the ws-OLCs. This easy labeling of unicellular cells and multicellular organisms offers a versatile and ideal platform for targeted bioimaging or biodelivery of drugs with fluorescence monitoring [94].

Dubey *et al.* obtained ws-OLCs by ambient burning of common carbonaceous sources, such as camphor and polystyrene foam under an insufficient oxygen atmosphere [137]. The large fraction of carboxylic groups on the functionalized surface caused a self-passivated fluorescence effect along with photostability. These properties allow their usage as fluorescent contrast agents for biological cell labeling [137]. The OLCs were mixed with a Luria-Bertani medium and incubated with the DH5α strain of *E. coli*. OLC-labeled bacteria had a green fluorescence property [137].

Pyrolysis of natural bile acid (deoxycholic acid (DOCA)) results in the formation of biocompatible, single-phosphor white-light emitting ws-OLCs [95]. White-light-emitting materials have generated interest in recent years due to their high photoluminescence efficiency, color stability and potential applications in biomedicine. These carbon nanoparticles demonstrated multichannel fluorescence imaging and tumor homing properties in the tumor-bearing mice [95].

The structures of fluorescently labeled OLCs as probes for high resolution imagining are shown in Fig. (**4**). The BODIPY-OLCs (Fig. **4a**) were utilized as probes for *in vitro* high-resolution imaging of MCF-7 cells. Functionalized OLCs have shown excellent cellular uptake properties [69]. The confocal microscopic analysis was used to image the intracellular distribution of BODIPY-OLC conjugates, internalized by endocytosis, which were mainly localized in lysosomes [69]. NIR fluorescence imaging is a highly sensitive, high-resolution and non-invasive technique. The OLCs covalently functionalized by boron azadipyrromethene (Fig. **4b**) have been investigated in HeLa Kyoto cells and were imaged using laser-scanning confocal microscopy. OLCs proved NIR fluorescence properties with the on/off reversibility controlled by the pH using phenol/phenolate modulation [70]. Moreover, this ability was demonstrated both in solution and *in vitro* imagining of HeLa Kyoto cells. Furthermore, HeLa cancer cells were also imaged by fluorescent pyrene-BODIPY OLCs (Fig. **4c**) [72]. The "small" OLCs functionalized by π-extended distyryl-substituted boron dipyrromethene were used as fluorescent agents. They exhibited non-toxic behavior and were easily internalized by MCF-7 cells. The high fluorescence intensity may allow using them as therapeutic agents or targeted drug delivery systems due to their uptake by MCF-7 cells. In addition, their localization was identified by a specific lysosome dye (the LysoTracker Green) [71]. Overall, the accumulated results indicate that OLCs multi-functionalized by fluorescence and folic acid derivatives linked to a polyethylene glycol chain possessed an ability to target cancer cells and localize the nanoparticles in the late-endosomal cell compartments [74].

4.4. Biological and Medical Sensing

One of the most important advantages of the onion-like nanostructures is their catalytic character, which may be applied to biosensing (Table **1**). One of the most recent reports concerns the formation of nanocomposite-containing OLCs, single-walled carbon nanotubes (SWCNTs) and chitosan (CS), decorated with gold nanoparticles (AuNPs) [97]. Such nanocomposites were prepared for the development of highly sensitive electrochemical immunosensors for the detection of carcinoembryonic antigen (CEA). The increase in clinical tumor marker CEA may be an early indication of cancerous diseases, such as colon and breast tumors, ovarian carcinoma, colorectal cancer or cystadenocarcinoma [138-143]. The presence of OLCs in this electrochemical immunosensor indirectly affected the 200% increase in the surface area and conductivity effectiveness. Finally, the CEA-immunosensor exhibited a wide linear detection range (100 fg/mL – 400 ng/mL) with a low detection limit (100 fg/mL).

Bartolome *et al.* created a bioelectrode modified with OLCs with the immobilized diazonium salts terminated with carboxylic acid and maleimide groups [98]. Such a promising 'bioplatform' was able to amperometrically detect the model DNA of human papillomavirus oncogene. The presence of OLCs affected the analytical parameters, enhancing the sensitivity and decreasing the detection limit. Similar OLC functionalization by diazonium species was mentioned in the first chapter of *Nanomaterials, Polymers and Devices: Materials Functionalization and Device Fabrication* [144]. It should be noted that the modification by diazonium-based compounds is one of the most efficient and effective methods for the covalent functionalization of carbon nanoparticle surfaces. For the first time, the "click" chemistry using diazonium species attached to the OLCs was described by Flavin *et al.* in 2010 [63].

The larger OLCs with a diameter of approximately 30 nm can also act as supports for glucose oxidase (GOx) in an electrochemical glucose sensor [145]. The enzyme was covalently immobilized on the carbon nanoparticle surface via a carbodiimide treatment procedure, which preserves the bioactivity of GOx. Such GOx/OLCs biosensors achieved relatively high sensitivity (26.5 $\mu A/mM\ cm^2$) with a linear response between 1-10 mM of glucose concentration. The OLC's non-enzymatic alternative glucose biosensor showed a slightly lower sensitivity (21.6 $\mu A/mM\ cm^2$), where the catalytic activity was enhanced by Pt nanoparticles.

The OLC nanoparticles employed as active electrode surfaces were also able to electrochemically detect nitrite and ascorbic acid (AA), which are the most widely used additive stabilizing compounds to different products for human consumption [99]. In that case, the

OLCs were modified with *o*-aminophenol or thionine in the electro grafting and physical adsorption procedures, respectively. The experiments were carried out using real samples (fruits and juice) and indicated great selectivity and specificity.

The incorporation of nitrogen atoms into OLC structures maintains the π-conductive surface of these nanoparticles, which is crucial for applications in bioelectrochemistry and bioelectronics [44]. The main advantage of N-OLCs is their electroactivity in non-enzymatic electrocatalytic reactions. The N-OLCs were used as active materials able to detect hydrogen peroxide (Fig. **6**). Linear increases of reduction currents were observed with increasing H_2O_2 concentrations from 4 to 50 mM (Fig. **6b**), and the reduction of H_2O_2 also occurs at lower over potentials with N-OLCs than with undoped OLCs. H_2O_2 is the most stable of the reactive oxygen species and important for oxidative stress investigations, which are a crucial part of tumors and tissue aging studies [146-149].

The N-OLCs were also studied as ideal materials for the detection of redox-active biomolecules such as dopamine (DA), epinephrine, norepinephrine and serotonin. N-doped OLCs revealed stable electrochemical activity, high sensitivity and selectivity [100]. Recently, Breczko *et al.* for the first time reported that OLCs mixed with polydiallyldimethylammonium chloride (PDDA) showed promising electrochemical properties in DA detection in the presence of interfering chemicals (ascorbic and uric acids). The detection limit for DA using this electrochemical sensor was ~10 μM [45]. It should be noted that OLC/PDDA composites are capable of non-enzymatic oxygen reduction, which makes it a promising material for bio-fueling.

The ws-OLCs modified with numerous carboxyl functionalities showed significantly enhanced photocatalytic activities and were able to detect Al(III) ion in a highly selective fluorescent way. Aluminum is the third most-abundant element in the Earth's crust and it is often present in water as a result of acid rains [150, 151]. The amount of aluminum is constantly growing due to its high demand in industry. An increasing human exposure to aluminum can cause Alzheimer's and Parkinson's disease [152, 153], Down syndrome and even breast cancer [154]. The stable green emission of ws-OLCs can be used as a fluorescent sensor for the sensitive detection of Al(III) ions [101]. The fluorescence of such materials is highly specific and allows an achievement of 0.77 μM detection limit even in the presence of other metal ions and anions.

Due to their fluorescent abilities, the ws-OLCs were also tested as glucose-sensitive materials [102]. The sensing process was based upon a simple fluorescence "turn off/on" technique after methylene blue immobilization. The selectivity for glucose was explored in reference to the potential interfering compounds, such as DA, UA and amino acids. The detection limit for ws-OLC is 1.3 x 10^{-2} M, and the detection process is simple and very fast.

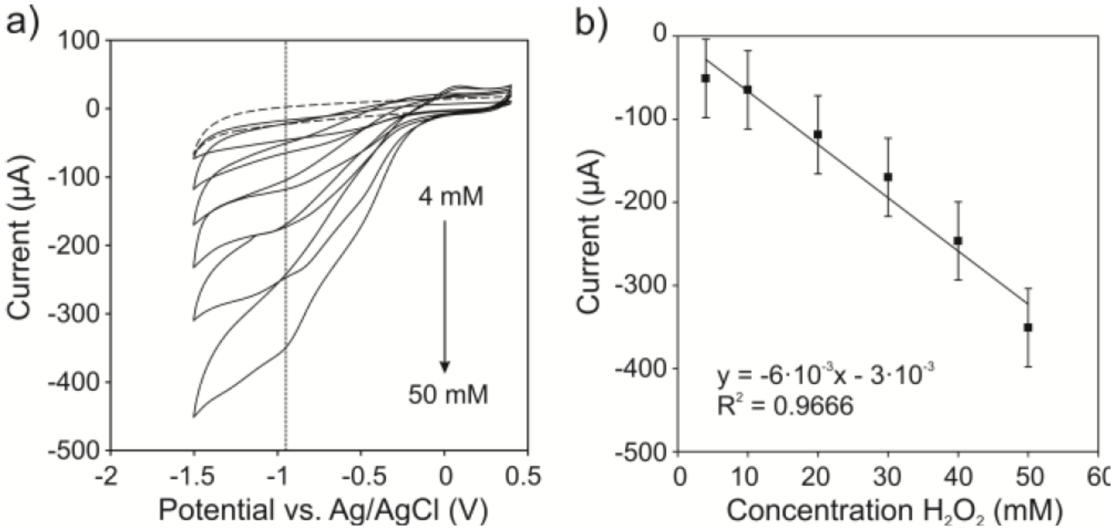

Fig. (6). (a) CV curves of a GCE covered with N-OLCs in 0.01 M PBS (pH=7.4) in the absence (dashed black curves) and the presence (solid black curves) of H_2O_2 at different concentrations (4, 10, 20, 30, 40, 50 mM), at a scan rate of 10 mV/s. **(b)** The dependence of reduction peak current on the H_2O_2 concentration. Reprinted with permission from Ref. [44]. Reproduced by permission of Wiley & Sons.

CONCLUSION

The OLC structures are unique carbon nanostructures due to their size, shape, physicochemical properties, homogeneity and purity. The greatest interest among scientists is caused by spherical, small OLCs, whose surface may be easily modified, significantly changing their dispersibility in polar solvents and physicochemical properties. Easy covalent and non-covalent modifications of the OLC's surface and easy doping with other atoms or metals lead to multifunctionalization of these nanoparticles. Multifunctionality is the key feature of OLCs, which may be used as tumor markers, in cell and tissue imaging, and drug delivery or sensing fields. OLCs have been employed in biosensing, bioimaging, bioelectrochemistry, electrochemical immunosensors, detection of active biomolecules, electrocatalysis, sorption and intracellular transport.

Nanomaterials used in biological areas should be characterized by their biocompatibility and non-toxicity. Still, given their unique properties, they could be very promising nanoparticles for biomedical applications.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Gu, W.; Yushin, G. Review of Nanostructured Carbon Materials for Electrochemical Capacitor Applications: Advantages and Limitations of Activated Carbon, Carbide-Derived Carbon, Zeolite-Templated Carbon, Carbon Aerogels, Carbon Nanotubes, Onion-like Carbon, and Graphene: Nanostructured Carbon Materials for Electrochemical Capacitor Applications. *Wiley Interdiscip. Rev. Energy Environ.*, **2014**, *3*, 424–473.

[2] Al-Jumaili, A.; Alancherry, S.; Bazaka, K.; Jacob, M. Review on the Antimicrobial Properties of Carbon Nanostructures. *Materials*, **2017**, *10*, 1066.

[3] Froudakis, G.E. Hydrogen Storage in Nanotubes & Nanostructures. *Mater. Today*, **2011**, *14*, 324–328.

[4] Jun Han, Z.; Rider, A.E.; Ishaq, M.; Kumar, S.; Kondyurin, A.; Bilek, M.M.M.; Levchenko, I.; Ostrikov, K. (Ken). Carbon Nanostructures for Hard Tissue Engineering. *RSC Adv.*, **2013**, *3*, 11058.

[5] Kumar, S.; Rani, R.; Dilbaghi, N.; Tankeshwar, K.; Kim, K.-H. Carbon Nanotubes: A Novel Material for Multifaceted Applications in Human Healthcare. *Chem. Soc. Rev.*, **2017**, *46*, 158–196.

[6] Torres, T. Graphene Chemistry. *Chem. Soc. Rev.*, **2017**, *46*, 4385–4386.

[7] Solís-Fernández, P.; Bissett, M.; Ago, H. Synthesis, Structure and Applications of Graphene-Based 2D Heterostructures. *Chem. Soc. Rev.*, **2017**, *46*, 4572–4613.

[8] Falcao, E.H.; Wudl, F. Carbon Allotropes: Beyond Graphite and Diamond. *J. Chem. Technol. Biotechnol.*, **2007**, *82*, 524–531.

[9] *Fullerenes: Chemistry, Physics, and Technology*; Kadish, K.M.; Ruoff, R.S., Eds.; Wiley-Interscience: New York, **2000**.

[10] Mykhailiv, O.; Zubyk, H.; Plonska-Brzezinska, M.E. Carbon Nano-Onions: Unique Carbon Nanostructures with Fascinating Properties and Their Potential Applications. *Inorganica Chim. Acta*, **2017**, *468*, 49–66.

[11] *CRC Concise Encyclopedia of Nanotechnology*; Kharisov, B.I.; Kharissova, O.V.; Ortiz Méndez, U., Eds.; CRC Press: Boca Raton, **2016**.

[12] *Carbon Nanomaterials Sourcebook*; Sattler, K.D., Ed.; CRC Press, Taylor & Francis Group, CRC Press is an imprint of the Taylor & Francis Group, an informa business: Boca Raton, **2016**.

[13] Bacon, R. Growth, Structure, and Properties of Graphite Whiskers. *J. Appl. Phys.*, **1960**, *31*, 283.

[14] Bates, K.R.; Scuseria, G.E. Why Are Buckyonions Round? *Theor. Chem. Acc. Theory Comput. Model. Theor. Chim. Acta*, **1998**, *99*, 29–33.

[15] Kuznetsov, V.L.; Chuvilin, A.L.; Butenko, Y.V.; Mal'kov, I.Y.; Titov, V.M. Onion-like Carbon from Ultra-Disperse Diamond. *Chem. Phys. Lett.*, **1994**, *222*, 343–348.

[16] Santiago, D.; Rodríguez-Calero, G.G.; Palkar, A.; Barraza-Jimenez, D.; Galvan, D.H.; Casillas, G.; Mayoral, A.; Jose-Yacamán, M.; Echegoyen, L.; Cabrera, C.R. Platinum Electrodeposition on Unsupported Carbon Nano-Onions. *Langmuir*, **2012**, *28*, 17202–17210.

[17] Wang, B.-C.; Wang, H.-W.; Chang, J.-C.; Tso, H.-C.; Chou, Y.-M. More Spherical Large Fullerenes and Multi-Layer Fullerene Cages. *J. Mol. Struct. THEOCHEM*, **2001**, *540*, 171–176.

[18] Saxby, J.D.; Chatfield, S.P.; Palmisano, A.J.; Vassallo, A.M.; Wilson, M.A.; Pang, L.S.K. Thermogravimetric Analysis of Buckminsterfullerene and Related Materials in Air. *J. Phys. Chem.*, **1992**, *96*, 17–18.

[19] Sano, N.; Wang, H.; Chhowalla, M.; Alexandrou, I.; Amaratunga, G.A.J. Synthesis of Carbon "Onions" in Water. *Nature*, **2001**, *414*, 506–507.

[20] Sano, N.; Wang, H.; Alexandrou, I.; Chhowalla, M.; Teo, K.B.K.; Amaratunga, G.A.J.; Iimura, K. Properties of Carbon Onions Produced by an Arc Discharge in Water. *J. Appl. Phys.*, **2002**, *92*, 2783–2788.

[21] Chen, X..; Deng, F..; Wang, J..; Yang, H..; Wu, G..; Zhang, X..; Peng, J..; Li, W.. New Method of Carbon Onion Growth by Radio-Frequency Plasma-Enhanced Chemical Vapor Deposition. *Chem. Phys. Lett.*, **2001**, *336*, 201–204.

[22] Gao, Y.; Zhou, Y.S.; Park, J.B.; Wang, H.; He, X.N.; Luo, H.F.; Jiang, L.; Lu, Y.F. Resonant Excitation of Precursor Molecules in Improving the Particle Crystallinity, Growth Rate and Optical Limiting Performance of Carbon Nano-Onions. *Nanotechnology*, **2011**, *22*, 165604.

[23] Zhao, M.; Song, H.; Chen, X.; Lian, W. Large-Scale Synthesis of Onion-like Carbon Nanoparticles by Carbonization of Phenolic Resin. *Acta Mater.*, **2007**, *55*, 6144–6150.

[24] Palkar, A.; Melin, F.; Cardona, C.M.; Elliott, B.; Naskar, A.K.; Edie, D.D.; Kumbhar, A.; Echegoyen, L. Reactivity Differences between Carbon Nano Onions (CNOs) Prepared by Different Methods. *Chem. – Asian J.*, **2007**, *2*, 625–633.

[25] Andersson, O.E.; Prasad, B.L.V.; Sato, H.; Enoki, T.; Hishiyama, Y.; Kaburagi, Y.; Yoshikawa, M.; Bandow, S. Structure and Electronic Properties of Graphite Nanoparticles. *Phys. Rev. B*, **1998**, *58*, 16387–16395.

[26] Rettenbacher, A.S.; Elliott, B.; Hudson, J.S.; Amirkhanian, A.; Echegoyen, L. Preparation and Functionalization of Multilayer Fullerenes (Carbon Nano-Onions). *Chem. - Eur. J.*, **2006**, *12*, 376–387.

[27] Zeiger, M.; Jäckel, N.; Mochalin, V.N.; Presser, V. Review: Carbon Onions for Electrochemical Energy Storage. *J Mater Chem A*, **2016**, *4*, 3172–3196.

[28] Tomita, S.; Sakurai, T.; Ohta, H.; Fujii, M.; Hayashi, S. Structure and Electronic Properties of Carbon Onions. *J. Chem. Phys.*, **2001**, *114*, 7477.

[29] Roy, D.; Chhowalla, M.; Wang, H.; Sano, N.; Alexandrou, I.; Clyne, T..; Amaratunga, G.A.. Characterisation of Carbon Nano-Onions Using Raman Spectroscopy. *Chem. Phys. Lett.*, **2003**, *373*, 52–56.

[30] Gan, Y.; Banhart, F. The Mobility of Carbon Atoms in Graphitic Nanoparticles Studied by the Relaxation of Strain in Carbon Onions. *Adv. Mater.*, **2008**, *20*, 4751–4754.

[31] Banhart, F.; Redlich, P.; Ajayan, P.M. The Migration of Metal Atoms through Carbon Onions. *Chem. Phys. Lett.*, **1998**, *292*, 554–560.

[32] Wajs, E.; Molina-Ontoria, A.; Nielsen, T.T.; Echegoyen, L.; Fragoso, A. Supramolecular Solubilization of Cyclodextrin-Modified Carbon Nano-Onions by Host–Guest Interactions. *Langmuir*, **2015**, *31*, 535–541.

[33] Bogdanov, K.; Fedorov, A.; Osipov, V.; Enoki, T.; Takai, K.; Hayashi, T.; Ermakov, V.; Moshkalev, S.; Baranov, A. Annealing-Induced Structural Changes of Carbon Onions: High-Resolution Transmission Electron Microscopy and Raman Studies. *Carbon*, **2014**, *73*, 78–86.

[34] Ferrari, A.C. Raman Spectroscopy of Graphene and Graphite: Disorder, Electron–phonon Coupling, Doping and Nonadiabatic Effects. *Solid State Commun.*, **2007**, *143*, 47–57.

[35] Mykhaylyk, O.O.; Solonin, Y.M.; Batchelder, D.N.; Brydson, R. Transformation of Nanodiamond into Carbon Onions: A Comparative Study by High-Resolution Transmission Electron Microscopy, Electron Energy-Loss Spectroscopy, X-Ray Diffraction, Small-Angle X-Ray Scattering, and Ultraviolet Raman Spectroscopy. *J. Appl. Phys.*, **2005**, *97*, 074302.

[36] Crestani, M.G.; Puente-Lee, I.; Rendón-Vazquez, L.; Santiago, P.; del Rio, F.; Morales-Morales, D.; García, J.J. The Catalytic Reduction of Carbon Dioxide to Carbon Onion Particles by Platinum Catalysts. *Carbon*, **2005**, *43*, 2621–2624.

[37] Panich, A.M.; Shames, A.I.; Sergeev, N.A.; Olszewski, M.; McDonough, J.K.; Mochalin, V.N.; Gogotsi, Y. Nanodiamond Graphitization: A Magnetic Resonance Study. *J. Phys. Condens. Matter*, **2013**, *25*, 245303.

[38] Butenko, Y.V.; Kuznetsov, V.L.; Chuvilin, A.L.; Kolomiichuk, V.N.; Stankus, S.V.; Khairulin, R.A.; Segall, B. Kinetics of the Graphitization of Dispersed Diamonds at "low" Temperatures. *J. Appl. Phys.*, **2000**, *88*, 4380.

[39] Portet, C.; Yushin, G.; Gogotsi, Y. Electrochemical Performance of Carbon Onions, Nanodiamonds, Carbon Black and Multiwalled Nanotubes in Electrical Double Layer Capacitors. *Carbon*, **2007**, *45*, 2511–2518.

[40] Tomita, S.; Burian, A.; Dore, J.C.; LeBolloch, D.; Fujii, M.; Hayashi, S. Diamond Nanoparticles to Carbon Onions Transformation: X-Ray Diffraction Studies. *Carbon*, **2002**, *40*, 1469–1474.

[41] Okotrub, A.V.; Bulusheva, L.G.; Kuznetsov, V.L.; Butenko, Y.V.; Chuvilin, A.L.; Heggie, M.I. X-Ray Emission Studies of the Valence Band of Nanodiamonds Annealed at Different Temperatures. *J. Phys. Chem. A*, **2001**, *105*, 9781–9787.

[42] Kuznetsov, V.L.; Moseenkov, S.I.; Elumeeva, K.V.; Larina, T.V.; Anufrienko, V.F.; Romanenko, A.I.; Anikeeva, O.B.; Tkachev, E.N. Comparative Study of Reflectance Properties of Nanodiamonds, Onion-like Carbon and Multiwalled Carbon Nanotubes. *Phys. Status Solidi B*, **2011**, *248*, 2572–2576.

[43] Mykhailiv, O.; Brzezinski, K.; Sulikowski, B.; Olejniczak, Z.; Gras, M.; Lota, G.; Molina-Ontoria, A.; Jakubczyk, M.; Echegoyen, L.; Plonska-Brzezinska, M.E. Boron-Doped Polygonal Carbon Nano-Onions: Synthesis and Applications in Electrochemical Energy Storage. *Chem. – Eur. J.*, **2017**, *23*, 7132–7141.

[44] Mykhailiv, O.; Zubyk, H.; Brzezinski, K.; Gras, M.; Lota, G.; Gniadek, M.; Romero, E.; Echegoyen, L.; Plonska-Brzezinska, M.E. Improvement of the Structural and Chemical Properties of Carbon Nano-Onions for Electrocatalysis. *ChemNanoMat*, **2017**, *3*, 583–590.

[45] Breczko, J.; Plonska-Brzezinska, M.E.; Echegoyen, L. Electrochemical Oxidation and Determination of Dopamine in the Presence of Uric and Ascorbic Acids Using a Carbon Nano-Onion and Poly(diallyldimethylammonium Chloride) Composite. *Electrochimica Acta*, **2012**, *72*, 61–67.

[46] Pech, D.; Brunet, M.; Durou, H.; Huang, P.; Mochalin, V.; Gogotsi, Y.; Taberna, P.-L.; Simon, P. Ultrahigh-Power Micrometre-Sized Supercapacitors Based on Onion-like Carbon. *Nat. Nanotechnol.*, **2010**, *5*, 651–654.

[47] Macutkevic, J.; Adomavicius, R.; Krotkus, A.; Seliuta, D.; Valusis, G.; Maksimenko, S.; Kuzhir, P.; Batrakov, K.; Kuznetsov, V.; Moseenkov, S.; Shenderova, O.; Okotrub, A.V.; Langlet, R.; Lambin, P. Terahertz Probing of Onion-like Carbon-PMMA Composite Films. *Diam. Relat. Mater.*, **2008**, *17*, 1608–1612.

[48] Shenderova, O.; Grishko, V.; Cunningham, G.; Moseenkov, S.; McGuire, G.; Kuznetsov, V. Onion-like Carbon for Terahertz Electromagnetic Shielding. *Diam. Relat. Mater.*, **2008**, *17*, 462–466.

[49] Su, X.; Zhang, J.; Jia, Y.; Liu, Y.; Xu, J.; Wang, J. Preparation and Microwave Absorption Property of Nano Onion-like Carbon in the Frequency Range of 8.2–12.4 GHz. *J. Alloys Compd.*, **2017**, *695*, 1420–1425.

[50] Andreas Hirsch. Principles of Fullerene Reactivity. In *Fullerenes and Related Structures*; **1999**; Vol. Volume 199 of the series Topics in Current Chemistry pp 1-65, pp. 1–65.

[51] Hamon, M.A.; Hu, H.; Bhowmik, P.; Niyogi, S.; Zhao, B.; Itkis, M.E.; Haddon, R.C. End-Group and Defect Analysis of Soluble Single-Walled Carbon Nanotubes. *Chem. Phys. Lett.*, **2001**, *347*, 8–12.

[52] David, W.I.F.; Ibberson, R.M.; Matthewman, J.C.; Prassides, K.; Dennis, T.J.S.; Hare, J.P.; Kroto, H.W.; Taylor, R.; Walton, D.R.M. Crystal Structure and Bonding of Ordered C60. *Nature*, **1991**, *353*, 147–149.

[53] Klein, D.J.; Seitz, W.A.; Schmalz, T.G. Icosahedral Symmetry Carbon Cage Molecules. *Nature*, **1986**, *323*, 703–706.

[54] Thilgen, C.; Herrmann, A.; Diederich, F. The Covalent Chemistry of Higher Fullerenes: C70 and Beyond. *Angew. Chem. Int. Ed. Engl.*, **1997**, *36*, 2268–2280.

[55] Cataldo, F. Polymeric Fullerene Oxide (Fullerene Ozopolymers) Produced by Prolonged Ozonation of C60 and C70 Fullerenes. *Carbon*, **2002**, *40*, 1457–1467.

[56] Palkar, A.; Kumbhar, A.; Athans, A.J.; Echegoyen, L. Pyridyl-Functionalized and Water-Soluble Carbon Nano Onions: First Supramolecular Complexes of Carbon Nano Onions. *Chem. Mater.*, **2008**, *20*, 1685–1687.

[57] Plonska-Brzezinska, M.E.; Dubis, A.T.; Lapinski, A.; Villalta-Cerdas, A.; Echegoyen, L. Electrochemical Properties of Oxidized Carbon Nano-Onions: DRIFTS-FTIR and Raman Spectroscopic Analyses. *ChemPhysChem*, **2011**, *12*, 2659–2668.

[58] Plonska-Brzezinska, M.E.; Lapinski, A.; Wilczewska, A.Z.; Dubis, A.T.; Villalta-Cerdas, A.; Winkler, K.; Echegoyen, L. The Synthesis and Characterization of Carbon Nano-Onions Produced by Solution Ozonolysis. *Carbon*, **2011**, *49*, 5079–5089.

[59] Georgakilas, V.; Guldi, D.M.; Signorini, R.; Bozio, R.; Prato, M. Organic Functionalization and Optical Properties of Carbon Onions. *J. Am. Chem. Soc.*, **2003**, *125*, 14268–14269.

[60] Cioffi, C.T.; Palkar, A.; Melin, F.; Kumbhar, A.; Echegoyen, L.; Melle-Franco, M.; Zerbetto, F.; Rahman, G.M.A.; Ehli, C.; Sgobba, V.; Guldi, D.M.; Prato, M. A Carbon Nano-Onion-Ferrocene Donor-Acceptor System: Synthesis, Characterization and Properties. *Chem. - Eur. J.*, **2009**, *15*, 4419–4427.

[61] Zhou, L.; Gao, C.; Zhu, D.; Xu, W.; Chen, F.F.; Palkar, A.; Echegoyen, L.; Kong, E.S.-W. Facile Functionalization of Multilayer Fullerenes (Carbon Nano-Onions) by Nitrene Chemistry and “Grafting From” Strategy. *Chem. - Eur. J.*, **2009**, *15*, 1389–1396.

[62] Molina-Ontoria, A.; Chaur, M.N.; Plonska-Brzezinska, M.E.; Echegoyen, L. Preparation and Characterization of Soluble Carbon Nano-Onions by Covalent Functionalization, Employing a Na–K Alloy. *Chem. Commun.*, **2013**, *49*, 2406.

[63] Flavin, K.; Chaur, M.N.; Echegoyen, L.; Giordani, S. Functionalization of Multilayer Fullerenes (Carbon Nano-Onions) Using Diazonium Compounds and “Click” Chemistry. *Org. Lett.*, **2010**, *12*, 840–843.

[64] Liu, Y.; Vander Wal, R.L.; Khabashesku, V.N. Functionalization of Carbon Nano-Onions by Direct Fluorination. *Chem. Mater.*, **2007**, *19*, 778–786.

[65] Rettenbacher, A.S.; Perpall, M.W.; Echegoyen, L.; Hudson, J.; Smith, D.W. Radical Addition of a Conjugated Polymer to Multilayer Fullerenes (Carbon Nano-Onions). *Chem. Mater.*, **2007**, *19*, 1411–1417.

[66] Luszczyn, J.; Plonska-Brzezinska, M.E.; Palkar, A.; Dubis, A.T.; Simionescu, A.; Simionescu, D.T.; Kalska-Szostko, B.; Winkler, K.; Echegoyen, L. Small Noncytotoxic Carbon Nano-Onions: First Covalent Functionalization with Biomolecules. *Chem. - Eur. J.*, **2010**, *16*, 4870–4880.

[67] Bartelmess, J.; Giordani, S. Carbon Nano-Onions (Multi-Layer Fullerenes): Chemistry and Applications. *Beilstein J. Nanotechnol.*, **2014**, *5*, 1980–1998.

[68] d’ Amora, M.; Rodio, M.; Bartelmess, J.; Sancataldo, G.; Brescia, R.; Cella Zanacchi, F.; Diaspro, A.; Giordani, S. Biocompatibility and Biodistribution of Functionalized Carbon Nano-Onions (F-CNOs) in a Vertebrate Model. *Sci. Rep.*, **2016**, *6*.

[69] Bartelmess, J.; De Luca, E.; Signorelli, A.; Baldrighi, M.; Becce, M.; Brescia, R.; Nardone, V.; Parisini, E.; Echegoyen, L.; Pompa, P.P.; Giordani, S. Boron Dipyrromethene (BODIPY) Functionalized Carbon Nano-Onions for High Resolution Cellular Imaging. *Nanoscale*, **2014**, *6*, 13761–13769.

[70] Giordani, S.; Bartelmess, J.; Frasconi, M.; Biondi, I.; Cheung, S.; Grossi, M.; Wu, D.; Echegoyen, L.; O’Shea, D.F. NIR Fluorescence Labelled Carbon Nano-Onions: Synthesis, Analysis and Cellular Imaging. *J Mater Chem B*, **2014**, *2*, 7459–7463.

[71] Lettieri, S.; Camisasca, A.; d’Amora, M.; Diaspro, A.; Uchida, T.; Nakajima, Y.; Yanagisawa, K.; Maekawa, T.; Giordani, S. Far-Red Fluorescent Carbon Nano-Onions as a Biocompatible Platform for Cellular Imaging. *RSC Adv.*, **2017**, *7*, 45676–45681.

[72] Bartelmess, J.; Frasconi, M.; Balakrishnan, P.B.; Signorelli, A.; Echegoyen, L.; Pellegrino, T.; Giordani, S. Non-Covalent Functionalization of Carbon Nano-Onions with pyrene–BODIPY Dyads for Biological Imaging. *RSC Adv.*, **2015**, *5*, 50253–50258.

[73] Yang, M.; Flavin, K.; Kopf, I.; Radics, G.; Hearnden, C.H.A.; McManus, G.J.; Moran, B.; Villalta-Cerdas, A.; Echegoyen, L.A.; Giordani, S.; Lavelle, E.C. Functionalization of Carbon Nanoparticles Modulates Inflammatory Cell Recruitment and NLRP3 Inflammasome Activation. *Small*, **2013**, *9*, 4194–4206.

[74] Frasconi, M.; Marotta, R.; Markey, L.; Flavin, K.; Spampinato, V.; Ceccone, G.; Echegoyen, L.; Scanlan, E.M.; Giordani, S. Multi-Functionalized Carbon Nano-Onions as Imaging Probes for Cancer Cells. *Chem. – Eur. J.*, **2015**, *21*, 19071–19080.

[75] Bobrowska, D.M.; Czyrko, J.; Brzezinski, K.; Echegoyen, L.; Plonska-Brzezinska, M.E. Carbon Nano-Onion Composites: Physicochemical Characteristics and Biological Activity. *Fuller. Nanotub. Carbon Nanostructures*, **2017**, *25*, 185–192.

[76] Bobrowska, D.M.; Brzezinski, K.; Plonska-Brzezinska, M.E. PEGylated Carbon Nano-Onions Composite as a Carrier of Polyphenolic Compounds: A Promising System for Medical Applications and Biological Sensors. *Colloid Interface Sci. Commun.*, **2017**, *21*, 6–9.

[77] Plonska-Brzezinska, M.E.; Brus, D.M.; Breczko, J.; Echegoyen, L. Carbon Nano-Onions and Biocompatible Polymers for Flavonoid Incorporation. *Chem. – Eur. J.*, **2013**, *19*, 5019–5024.

[78] Zhang, Y.; Reed, A.; Kim, D.Y. Nitrogen Doped Carbon Nano-Onions as Efficient and Robust Electrocatalysts for Oxygen Reduction Reactions. *Curr. Appl. Phys.*, **2018**, *18*, 417–423.

[79] Chatterjee, K.; Ashokkumar, M.; Gullapalli, H.; Gong, Y.; Vajtai, R.; Thanikaivelan, P.; Ajayan, P.M. Nitrogen-Rich Carbon Nano-Onions for Oxygen Reduction Reaction. *Carbon*, **2018**, *130*, 645–651.

[80] Papathanassiou, A.N.; Plonska-Brzezinska, M.E.; Mykhailiv, O.; Echegoyen, L.; Sakellis, I. Combined High Permittivity and High Electrical Conduc-tivity of Carbon Nano-Onion/Polyaniline Composites. *Synth. Met.*, **2015**, *209*, 583–587.

[81] Plonska-Brzezinska Marta E.; Breczko Joanna; Palys Barbara; Echegoyen Luis. The Electrochemical Properties of Nanocomposite Films Obtained by Chemical In Situ Polymerization of Aniline and Carbon Nanostructures. *ChemPhysChem*, **2012**, *14*, 116–124.

[82] Plonska-Brzezinska Marta E.; Mazurczyk Julita; Palys Barbara; Breczko Joanna; Lapinski Andrzej; Dubis Alina T.; Echegoyen Luis. Preparation and Characterization of Com-

posites That Contain Small Carbon Nano-Onions and Conducting Polyaniline. *Chem. – Eur. J.*, **2012**, *18*, 2600–2608.

[83] Papathanassiou, A.N.; Mykhailiv, O.; Echegoyen, L.; Sakellis, I.; Plonska-Brzezinska, M.E. Electric Properties of Carbon Nano-Onion/Polyaniline Composites: A Combined Electric Modulus and Ac Conductivity Study. *J. Phys. Appl. Phys.*, **2016**, *49*, 285305.

[84] Zhang, C.; Li, J.; Shi, C.; He, C.; Liu, E.; Zhao, N. Self-Anchored Catalysts for Substrate-Free Synthesis of Metal-Encapsulated Carbon Nano-Onions and Study of Their Magnetic Properties. *Nano Res.*, **2016**, *9*, 1159–1172.

[85] Seymour, M.B.; Su, C.; Gao, Y.; Lu, Y.; Li, Y. Characterization of Carbon Nano-Onions for Heavy Metal Ion Remediation. *J. Nanoparticle Res.*, **2012**, *14*, 1087.

[86] Ding, L.; Stilwell, J.; Zhang, T.; Elboudwarej, O.; Jiang, H.; Selegue, J.P.; Cooke, P.A.; Gray, J.W.; Chen, F.F. Molecular Characterization of the Cytotoxic Mechanism of Multiwall Carbon Nanotubes and Nano-Onions on Human Skin Fibroblast. *Nano Lett.*, **2005**, *5*, 2448–2464.

[87] Xu, Y.; Wang, S.-Y.; Yang, J.; Gu, X.; Zhang, J.; Zheng, Y.-F.; Yang, J.; Xu, L.; Zhu, X.-Q. Multiwall Carbon Nano-Onions Induce DNA Damage and Apoptosis in Human Umbilical Vein Endothelial Cells: Biological Effects of MWCNOs on HUVECs. *Environ. Toxicol.*, **2013**, *28*, 442–450.

[88] Kang, S.; Kim, J.-E.; Kim, D.; Woo, C.G.; Pikhitsa, P.V.; Cho, M.-H.; Choi, M. Comparison of Cellular Toxicity between Multi-Walled Carbon Nanotubes and Onion-like Shell-Shaped Carbon Nanoparticles. *J. Nanoparticle Res.*, **2015**, *17*, 378.

[89] Malich, G.; Markovic, B.; Winder, C. The Sensitivity and Specificity of the MTS Tetrazolium Assay for Detecting the *in Vitro* Cytotoxicity of 20 Chemicals Using Human Cell Lines. *Toxicology*, **1997**, *124*, 179–192.

[90] Berridge, M.V.; Tan, A.S. Characterization of the Cellular Reduction of 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction. *Arch. Biochem. Biophys.*, **1993**, *303*, 474–482.

[91] Debnam, P.M.; Shearer, G. Colorimetric Assay for Substrates of NADP+-Dependent Dehydrogenases Based on Reduction of a Tetrazolium Dye to Its Soluble Formazan. *Anal. Biochem.*, **1997**, *250*, 253–255.

[92] Ghosh, M.; Sonkar, S.K.; Saxena, M.; Sarkar, S. Carbon Nano-Onions for Imaging the Life Cycle of Drosophila Melanogaster. *Small*, **2011**, *7*, 3170–3177.

[93] Pakhira, B.; Ghosh, M.; Allam, A.; Sarkar, S. Carbon Nano Onions Cross the Blood Brain Barrier. *RSC Adv.*, **2016**, *6*, 29779–29782.

[94] Sonkar, S.K.; Ghosh, M.; Roy, M.; Begum, A.; Sarkar, S. Carbon Nano-Onions as Nontoxic and High-Fluorescence Bioimaging Agent in Food Chain—An *In Vivo* Study from Unicellular E. Coli to Multicellular C. Elegans. *Mater. Express*, **2012**, *2*, 105–114.

[95] Revuri, V.; Cherukula, K.; Nafiujjaman, M.; Cho, K.J.; Park, I.-K.; Lee, Y. White-Light-Emitting Carbon Nano-Onions: A Tunable Multi-Channel Fluorescent Nanoprobe for Glutathione-Responsive Bioimaging. *ACS Appl. Nano Mater.*, **2017**.

[96] Yang, J.; Kim, S.H.; Kwak, S.K.; Song, H.-K. Curvature-Induced Metal–Support Interaction of an Islands-by-Islands Composite of Platinum Catalyst and Carbon Nano-Onion for Durable Oxygen Reduction. *ACS Appl. Mater. Interfaces*, **2017**, *9*, 23302–23308.

[97] Rizwan, M.; Elma, S.; Lim, S.A.; Ahmed, M.U. AuNPs/CNOs/SWCNTs/Chitosan-Nanocomposite Modified Electrochemical Sensor for the Label-Free Detection of Carcinoembryonic Antigen. *Biosens. Bioelectron.*, **2018**, *107*, 211–217.

[98] Bartolome, J.P.; Echegoyen, L.; Fragoso, A. Reactive Carbon Nano-Onion Modified Glassy Carbon Surfaces as DNA Sensors for Human Papillomavirus Oncogene Detection with Enhanced Sensitivity. *Anal. Chem.*, **2015**, *87*, 6744–6751.

[99] Bartolome, J.P.; Fragoso, A. Electrochemical Detection of Nitrite and Ascorbic Acid at Glassy Carbon Electrodes Modified with Carbon Nano-Onions Bearing Electroactive Moieties. *Inorganica Chim. Acta*, **2017**, *468*, 223–231.

[100] Yang, J.; Zhang, Y.; Kim, D.Y. Electrochemical Sensing Performance of Nanodiamond-Derived Carbon Nano-Onions: Comparison with Multiwalled Carbon Nanotubes, Graphite Nanoflakes, and Glassy Carbon. *Carbon*, **2016**, *98*, 74–82.

[101] Tripathi, K.M.; Tran, T.S.; Kim, Y.J.; Kim, T. Green Fluorescent Onion-Like Carbon Nanoparticles from Flaxseed Oil for Visible Light Induced Photocatalytic Applications and Label-Free Detection of Al(III) Ions. *ACS Sustain. Chem. Eng.*, **2017**, *5*, 3982–3992.

[102] Tripathi, K.M.; Bhati, A.; Singh, A.; Gupta, N.R.; Verma, S.; Sarkar, S.; Sonkar, S.K. From the Traditional Way of Pyrolysis to Tunable Photoluminescent Water Soluble Carbon Nano-Onions for Cell Imaging and Selective Sensing of Glucose. *RSC Adv.*, **2016**, *6*, 37319–37329.

[103] Sprague, J.; Bayraktaroglu, L.; Clements, D.; Conlin, T.; Fashena, D.; Frazer, K.; Haendel, M.; Howe, D.G.; Mani, P.; Ramachandran, S.; Schaper, K.; Segerdell, E.; Song, P.; Sprunger, B.; Taylor, S.; Slyke, V.; E, C.; Westerfield, M. The Zebrafish Information Network: The Zebrafish Model Organism Database. *Nucleic Acids Res.*, **2006**, *34*, D581–D585.

[104] Marchesano, V.; Ambrosone, A.; Bartelmess, J.; Strisciante, F.; Tino, A.; Echegoyen, L.; Tortiglione, C.; Giordani, S. Impact of Carbon Nano-Onions on Hydra Vulgaris as a Model Organism for Nanoecotoxicology. *Nanomaterials*, **2015**, *5*, 1331–1350.

[105] McDonough, J.K.; Frolov, A.I.; Presser, V.; Niu, J.; Miller, C.H.; Ubieto, T.; Fedorov, M.V.; Gogotsi, Y. Influence of the Structure of Carbon Onions on Their Electrochemical Performance in Supercapacitor Electrodes. *Carbon*, **2012**, *50*, 3298–3309.

[106] Mykhailiv, O.; Lapinski, A.; Molina-Ontoria, A.; Regulska, E.; Echegoyen, L.; Dubis, A.T.; Plonska-Brzezinska, M.E. Influence of the Synthetic Conditions on the Structural and Electrochemical Properties of Carbon Nano-Onions. *ChemPhysChem*, **2015**, *16*, 2182–2191.

[107] Plonska-Brzezinska, M.E.; Molina-Ontoria, A.; Echegoyen, L. Post-Modification by Low-Temperature Annealing of Carbon Nano-Onions in the Presence of Carbohydrates. *Carbon*, **2014**, *67*, 304–317.

[108] Sek, S.; Breczko, J.; Plonska-Brzezinska, M.E.; Wilczewska, A.Z.; Echegoyen, L. STM-Based Molecular Junction of Carbon Nano-Onion. *ChemPhysChem*, **2013**, *14*, 96–100.

[109] Mykhailiv, O.; Imierska, M.; Petelczyc, M.; Echegoyen, L.; Plonska-Brzezinska, M.E. Chemical versus Electrochemical Synthesis of Carbon Nano-Onion/Polypyrrole Composites for Supercapacitor Electrodes. *Chem. – Eur. J.*, **2015**, *21*, 5783–5793.

[110] Mykhailiv, O.; Zubyk, H.; Plonska-Brzezinska, M.E. Carbon Nano-Onions: Unique Carbon Nanostructures with Fascinating Properties and Their Potential Applications. *Inorganica Chim. Acta*, **2017**, *468*, 49–66.

[111] Kausar, A. Carbon Nano Onion as Versatile Contender in Polymer Compositing and Advance Application. *Fuller. Nanotub. Carbon Nanostructures*, **2017**, *25*, 109–123.

[112] Sok, V.; Fragoso, A. Preparation and Characterization of Alkaline Phosphatase, Horseradish Peroxidase, and Glucose Oxidase Conjugates with Carboxylated Carbon Nano-Onions. *Prep. Biochem. Biotechnol.*, **2017**, *0*, 1–8.

[113] Bartelmess, J.; Baldrighi, M.; Nardone, V.; Parisini, E.; Buck, D.; Echegoyen, L.; Giordani, S. Synthesis and Characterization of Far-Red/NIR-Fluorescent BODIPY Dyes, Solid-State Fluorescence, and Application as Fluorescent Tags Attached to Carbon Nano-Onions. *Chem. – Eur. J.*, **2015**, *21*, 9727–9732.

[114] Kuzhir, P.P.; Paddubskaya, A.G.; Maksimenko, S.A.; Kuznetsov, V.L.; Moseenkov, S.; Romanenko, A.I.; Shenderova, O.A.; Macutkevic, J.; Valušis, G.; Lambin, P. Carbon Onion Composites for EMC Applications. *IEEE Trans. Electromagn. Compat.*, **2012**, *54*, 6–16.

[115] Shuba, M.V.; Slepyan, G.Y.; Maksimenko, S.A.; Hanson, G.W. Radiofrequency Field Absorption by Carbon Nanotubes Embedded in a Conductive Host. *J. Appl. Phys.*, **2010**, *108*, 114302.

[116] Han, F.-D.; Yao, B.; Bai, Y.-J. Preparation of Carbon Nano-Onions and Their Application as Anode Materials for Rechargeable Lithium-Ion Batteries. *J. Phys. Chem. C*, **2011**, *115*, 8923–8927.

[117] Koudoumas, E.; Kokkinaki, O.; Konstantaki, M.; Couris, S.; Korovin, S.; Detkov, P.; Kuznetsov, V.; Pimenov, S.; Pustovoi, V. Onion-like Carbon and Diamond Nanoparticles for Optical Limiting. *Chem. Phys. Lett.*, **2002**, *357*, 336–340.

[118] Tomita, S.; Fujii, M.; Hayashi, S. Optical Extinction Properties of Carbon Onions Prepared from Diamond Nanoparticles. *Phys. Rev. B*, **2002**, *66*.

[119] Mohapatra, D.; Badrayyana, S.; Parida, S. A Facile Method for High Yield Synthesis of Carbon Nano Onions for Designing Binder-Free Flexible Supercapacitor. In; **2017**; p. 050069.

[120] Plonska-Brzezinska, M.E.; Echegoyen, L. Carbon Nano-Onions for Supercapacitor Electrodes: Recent Developments and Applications. *J. Mater. Chem. A*, **2013**, *1*, 13703–13714.

[121] Gao, Y.; Zhou, Y.S.; Qian, M.; He, X.N.; Redepenning, J.; Goodman, P.; Li, H.M.; Jiang, L.; Lu, Y.F. Chemical Activation of Carbon Nano-Onions for High-Rate Supercapacitor Electrodes. *Carbon*, **2013**, *51*, 52–58.

[122] Suryawanshi, S.R.; Kaware, V.; Chakravarty, D.; Walke, P.S.; More, M.A.; Joshi, K.; Rout, C.S.; Late, D.J. Pt-Nanoparticle Functionalized Carbon Nano-Onions for Ultra-High Energy Supercapacitors and Enhanced Field Emission Behaviour. *RSC Adv.*, **2015**, *5*, 80990–80997.

[123] Zhang, C.; Li, J.; Shi, C.; He, C.; Liu, E.; Zhao, N. Effect of Ni, Fe and Fe-Ni Alloy Catalysts on the Synthesis of Metal Contained Carbon Nano-Onions and Studies of Their Electrochemical Hydrogen Storage Properties. *J. Energy Chem.*, **2014**, *23*, 324–330.

[124] Grądzka, E.; Winkler, K.; Borowska, M.; Plonska-Brzezinska, M.E.; Echegoyen, L. Comparison of the Electrochemical Properties of Thin Films of MWCNTs/C60-Pd, SWCNTs/C60-Pd and Ox-CNOs/C60-Pd. *Electrochimica Acta*, **2013**, *96*, 274–284.

[125] Mohapatra, D.; Parida, S.; Singh, B.K.; Sutar, D.S. Importance of Microstructure and Interface in Designing Metal Oxide Nanocomposites for Supercapacitor Electrodes. *J. Electroanal. Chem.*, **2017**, *803*, 30–39.

[126] Mohapatra, D.; Parida, S.; Badrayyana, S.; Singh, B.K. High Performance Flexible Asymmetric CNO-ZnO//ZnO Supercapacitor with an Operating Voltage of 1.8V in Aqueous Medium. *Appl. Mater. Today*, **2017**, *7*, 212–221.

[127] Bobrowska, D.M.; Brzezinski, K.; Echegoyen, L.; Plonska-Brzezinska, M.E. A New Perspective on Carbon Nano-Onion/Nickel Hydroxide/Oxide Composites: Physicochemical Properties and Application in Hybrid Electrochemical Systems. *Fuller. Nanotub. Carbon Nanostructures*, **2017**, *25*, 193–203.

[128] Muniraj, V.K.A.; Kamaja, C.K.; Shelke, M.V. RuO2·nH2O Nanoparticles Anchored on Carbon Nano-Onions: An Efficient Electrode for Solid State Flexible Electrochemical Supercapacitor. *ACS Sustain. Chem. Eng.*, **2016**, *4*, 2528–2534.

[129] Borgohain, R.; Selegue, J.P.; Cheng, Y.-T. Ternary Composites of Delaminated-MnO2/PDDA/Functionalized-CNOs for High-Capacity Supercapacitor Electrodes. *J. Mater. Chem. A*, **2014**, *2*, 20367–20373.

[130] Azhagan, M.V.K.; Vaishampayan, M.V.; Shelke, M.V. Synthesis and Electrochemistry of Pseudocapacitive Multilayer Fullerenes and MnO2 Nanocomposites. *J. Mater. Chem. A*, **2014**, *2*, 2152–2159.

[131] Borgohain, R.; Li, J.; Selegue, J.P.; Cheng, Y.-T. Electrochemical Study of Functionalized Carbon Nano-Onions for High-Performance Supercapacitor Electrodes. *J. Phys. Chem. C*, **2012**, *116*, 15068–15075.

[132] Plonska-Brzezinska, M.E.; Brus, D.M.; Molina-Ontoria, A.; Echegoyen, L. Synthesis of Carbon Nano-Onion and Nickel Hydroxide/Oxide Composites as Supercapacitor Electrodes. *RSC Adv.*, **2013**, *3*, 25891–25901.

[133] Plonska-Brzezinska Marta E.; Lewandowski Mikołaj; Błaszyk Małgorzata; Molina-Ontoria Agustin; Luciński Tadeusz; Echegoyen Luis. Preparation and Characterization of Carbon Nano-Onion/PEDOT:PSS Composites. *ChemPhysChem*, **2012**, *13*, 4134–4141.

[134] Breczko, J.; Winkler, K.; Plonska-Brzezinska, M.E.; Villalta-Cerdas, A.; Echegoyen, L. Electrochemical Properties of Composites Containing Small Carbon Nano-Onions and Solid Polyelectrolytes. *J. Mater. Chem.*, **2010**, *20*, 7761–7768.

[135] Liu, Y.; Kim, D.Y. Enhancement of Capacitance by Electrochemical Oxidation of Nanodiamond Derived Carbon Nano-Onions. *Electrochimica Acta*, **2014**, *139*, 82–87.

[136] Zhou, M.; Li, Q.; Zhong, S.; Chen, J.; Lin, H.; Wu, X.-L. Facile Large Scale Fabrication of Magnetic Carbon Nano-Onions for Efficient Removal of Bisphenol A. *Mater. Chem. Phys.*, **2017**, *198*, 186–192.

[137] Dubey, P.; Tripathi, K.M.; Sonkar, S.K. Gram Scale Synthesis of Green Fluorescent Water-Soluble Onion-like Carbon Nanoparticles from Camphor and Polystyrene Foam. *RSC Adv.*, **2014**, *4*, 5838.

[138] Gu, X.; She, Z.; Ma, T.; Tian, S.; Kraatz, H.-B. Electrochemical Detection of Carcinoembryonic Antigen. *Biosens. Bioelectron.*, **2018**, *102*, 610–616.

[139] Yu, Y.; Zhang, Q.; Buscaglia, J.; Chang, C.-C.; Liu, Y.; Yang, Z.; Guo, Y.; Wang, Y.; Levon, K.; Rafailovich, M. Quantitative Real-Time Detection of Carcinoembryonic Antigen (CEA) from Pancreatic Cyst Fluid Using 3-D Surface Molecular Imprinting. *The Analyst*, **2016**, *141*, 4424–4431.

[140] Peng, J.; Lai, Y.; Chen, Y.; Xu, J.; Sun, L.; Weng, J. Sensitive Detection of Carcinoembryonic Antigen Using Stability-Limited Few-Layer Black Phosphorus as an Electron Donor and a Reservoir. *Small*, **2017**, *13*, 1603589.

[141] Su, B.-B. Role of Serum Carcinoembryonic Antigen in the Detection of Colorectal Cancer before and after Surgical Resection. *World J. Gastroenterol.*, **2012**, *18*, 2121.

[142] Kabel, A.M. Tumor Markers of Breast Cancer: New Prospectives. *J. Oncol. Sci.*, **2017**, *3*, 5–11.

[143] Hampton, R.; Walker, M.; Marshall, J.; Juhl, H. Differential Expression of Carcinoembryonic Antigen (CEA) Splice Variants in Whole Blood of Colon Cancer Patients and Healthy Volunteers: Implication for the Detection of Circu-

lating Colon Cancer Cells. *Oncogene*, **2002**, *21*, 7817–7823.

[144] Gopalsamy, K.; Xu, Z.; Gao, C.; Kong, E.S.W. The Functionalization of Carbon Nanotubes and Nano-Onions. In *Nanomaterials, Polymers, and Devices*; Kong, E.S.W., Ed.; John Wiley & Sons, Inc, **2015**; pp. 1–18.

[145] Mohapatra, J.; Ananthoju, B.; Nair, V.; Mitra, A.; Bahadur, D.; Medhekar, N.V.; Aslam, M. Enzymatic and Non-Enzymatic Electrochemical Glucose Sensor Based on Carbon Nano-Onions. *Appl. Surf. Sci.*, **2018**.

[146] Nita, M.; Grzybowski, A. The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults. *Oxid. Med. Cell. Longev.*, **2016**, *2016*, 1–23.

[147] Prasad, T.K. Evidence for Chilling-Induced Oxidative Stress in Maize Seedlings and a Regulatory Role for Hydrogen Peroxide. *PLANT CELL ONLINE*, **1994**, *6*, 65–74.

[148] de Haan, J.B.; Bladier, C.; Griffiths, P.; Kelner, M.; O'Shea, R.D.; Cheung, N.S.; Bronson, R.T.; Silvestro, M.J.; Wild, S.; Zheng, S.S.; Beart, P.M.; Hertzog, P.J.; Kola, I. Mice with a Homozygous Null Mutation for the Most Abundant Glutathione Peroxidase, Gpx1, Show Increased Susceptibility to the Oxidative Stress-Inducing Agents Paraquat and Hydrogen Peroxide. *J. Biol. Chem.*, **1998**, *273*, 22528–22536.

[149] Sies, H. Hydrogen Peroxide as a Central Redox Signaling Molecule in Physiological Oxidative Stress: Oxidative Eustress. *Redox Biol.*, **2017**, *11*, 613–619.

[150] Smith, S.B.; Shute, J.M. Separation and Detection of the Aluminum Ion in Qualitative Analysis. *J. Chem. Educ.*, **1955**, *32*, 380.

[151] Vallejos, S.; Muñoz, A.; Ibeas, S.; Serna, F.; García, F.C.; García, J.M. Selective and Sensitive Detection of Aluminium Ions in Water via Fluorescence "turn-On" with Both Solid and Water Soluble Sensory Polymer Substrates. *J. Hazard. Mater.*, **2014**, *276*, 52–57.

[152] Kawahara, M.; Kato-Negishi, M. Link between Aluminum and the Pathogenesis of Alzheimer's Disease: The Integration of the Aluminum and Amyloid Cascade Hypotheses. *Int. J. Alzheimers Dis.*, **2011**, *2011*, 1–17.

[153] Di Lorenzo, F.; Di Lorenzo, B. Iron and Aluminum in Alzheimer's Disease. *Neuro Endocrinol. Lett.*, **2013**, *34*, 504–507.

[154] Mannello, F.; Ligi, D.; Canale, M. Aluminium, Carbonyls and Cytokines in Human Nipple Aspirate Fluids: Possible Relationship between Inflammation, Oxidative Stress and Breast Cancer Microenvironment. *J. Inorg. Biochem.*, **2013**, *128*, 250–256.