Interactions between Carbon Nanomaterials and Biomolecules

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Abstract: Interactions between carbon nanomaterials, including carbon dots, fullerene, carbon nanotube, graphene, and graphene oxide (GO) which is a graphene based material (Fig. 1). Although these nanomaterials mainly consist of only one element carbon, different structures of it show the extraordinary properties. In addition to the architecture itself, the colloids whose solute particle size ranges from \(10^{-9}\) m to \(10^{-8}\) m obtained from the carbon nanomaterial demonstrate excellent functions as well\(^6\). C-Dots mainly act as nonmetal quantum dots, and demonstrate promising potential in the development of bioimaging\(^8\) and other clinic applications\(^7\) owing to its excellent biocompatibility\(^5\). Fullerene is a sphere shaped carbon nanomaterial. The fullerene-biomolecule conjugates are widely investigated for the purpose of medical applications\(^5\). The special structure of the CNT indicates high sensitivity and fast response for the reaction at its interface when used in the fabrication of nano electronic devices\(^10\). A variety of CNT-based nanomachines have been built for biosensing applications. Graphene was recently experimentally isolated but may have been used for centuries, and regarded as the newest carbon nanomaterials. Since its discovery in 2004\(^11\), it has drawn much attention in chemistry and material communities. GO, as one of the graphene based materials, has been investigated a lot in the area of biomedical field as well.

Biomolecule, associated with metabolism, consist of any molecule, including protein, carbohydrate, lipid, DNA, and other big or small molecules, present in the living organism. The biomolecules show an influence on metabolism in different ways. Acute myocardial infarction (AMI) is one clinical form of the cardiovascular diseases. When the person is diagnosed with it, a protein named cardiac troponin I (cTnI) will be released into the blood. Such that cTnI are believed to be the gold standard biomarker for AMI. In addition, carbohydrate growing on the cell surface also plays an important role in metabolism. Many biological processes, such as cell-cell adhesion and protein folding, take place as a result of saccharides on the surface of the cell\(^22\). To overcome the obstacles and make more achievements in solving diagnostic and therapeutic biomedical problems,

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many efforts have been devoted to the research in the field of biomolecules. With its novel features, the family of carbon nanomaterials has been widely investigated with its function in the biomolecule activity, and shows growing biomedical applications. In this paper, different groups of carbon nanomaterials, including zero dimensional, one dimensional, two dimensional nanomaterials, will be discussed based on their interactions with biomolecules.

2 Zero Dimensional Carbon Nanomaterials
Carbon dots (C-Dots), zero dimension nanomaterials, have recently attracted much attention due to its unique properties, such as low cytotoxicity, high photostability, magnificent biocompatibility, and easy conjugation with biomolecules. A myriad of synthesis strategies have been developed to fabricate C-Dots. Oxidative acid treatment is generally used to obtain C-Dots. Liu et al. prepared C-Dots from the combustion soot of candles. Polyacrylamide gel electrophoresis was utilized to purify the C-Dots. The quantum yield (QY) of the C-Dots was relatively low, with the highest value of 1.9%. Laser irradiation method for C-Dots fabrication was reported by Hu et al. This study opened a new way to prepare C-Dots. Graphite powders was irradiated at first. After boiling in perchloric acid, mixing with PEG, centrifugation, and separation, C-Dots were obtained. The QY yields vary from 3% to 8%. Zhai et al. reported a microwave-assisted pyrolysis method. Citric acid, the carbon source, and various amine contained molecules, which are used for surface passivation, were utilized for the C-Dots synthesis. The photoluminescent (PL) QY was 30.2%, and it is comparable to the common fluorescent quantum dots. Electrochemical exfoliation is another way to prepare C-Dots. Li et al. used graphite rods as anode and cathode, with NaOH and EtOH as electrolyte, and the intensity of the current was 10-200 mA cm⁻². Alkaline environment is important for this approach. In 2013, Zhu et al. employed hydrothermal method to form C-Dots. Polymer shaped C-Dots were prepared by condensing citric acid and ethylenediamine. Then it was carbonized to form C-Dots. Different characterizations, including transmission electron microscopy (TEM), atomic force microscopy (AFM), Raman microscopy, NMR spectroscopy, and other techniques, were applied. The QY was as high as 80%, which is suitable for industrial production.

Recently, the effect of C-Dots on protein fibrillation was investigated for the first time. Human insulin was selected for the study. Different characterizations, including UV-vis, Fourier transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and transmission electron microscopy (TEM), were performed for the C-Dots prepared from carbon powder. The non-toxic property of C-Dots was demonstrated by incubating it with sea urchin embryos. To monitor the kinetics of human insulin fibrillation, thioflavin T (ThT) fluorophore was used. In addition, circular dichroism (CD) and AFM were applied over different periods of the fibrillation process. According to the fluorescence study, three stages, including lag phase, elongation phase, and saturation phase, were observed. That the interaction between C-Dots and human insulin at the early stage makes the C-Dots inhibit human insulin fibrillation was proposed in the paper. The study displays the inhibiting effect of C-Dots on human insulin fibrillation, and shows the great potential of C-Dots in application of biological systems.

The potential application of C-Dots for bioimaging was studied by Sun et al. for the first time. The C-Dots were produced by laser ablation method with argon as carrier gas. The products did not exhibit photoluminescence until the surface was passivated by attaching organic molecules, and the observed QY ranged from 4 to 10%. The mechanism of C-Dots photoluminescence was also discussed, which is attributed to the surface energy traps as a result of surface passivation. Two-photon fluorescence materials, such as CdSe quantum dots, have promising applications in bioimaging. However, due to the heavy metal contained, the two-photon fluorescence materials have caused serious problems, including hazard on human health and environment. Sun and co-workers moved on to investigate C-Dots for the function of multiphoton bioimaging. The same set up as the previous one was performed to characterize the C-Dots. Human breast cancer MCF-7 cells were cultured to demonstrate the potential of C-Dots in cell imaging by two photon luminescence microscopy. This study shows great capability of C-Dots in bioimaging, and comparable to the best performing semiconductor quantum dots. In 2009, Sun and co-workers discussed the use of C-Dots for in vivo and in vitro applications. In the study, the PEGylated C-Dots were found to show strong fluorescence both in aqueous solution and on the surface. Human breast cancer MCF-7 and human colorectal adenocarcinoma HT-29 cells were utilized for the C-Dots toxicity assessment in vitro, and nontoxic property of C-Dots, comparing to other heavy metal contained quantum dots, were concluded. The toxicity evaluation of C-Dots in vivo were investigated by using CD-1 mice, and these fluorescence nanomaterials demonstrated nontoxic to the selected cell lines. Based on the results, C-Dots showed competitive bioimaging functions to the commercially available heavy metal bearing quantum dots both in vitro and in vivo applications. Tao et al. utilized C-Dots for vivo imaging in the near-infrared (NIR) region for the first time. The C-Dots were obtained from the modified traditional method of using mixed acid to oxidize carbon nanotubes and graphite. AFM, FTIR, UV-Vis, and fluorescence microscopy were em-
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Employed separately for C-Dots characterizations. The in vitro cytotoxicity of C-Dots was tested by incubating C-Dots with the human kidney embryonic 293T cell line. In vivo fluorescence imaging and in vivo biodistribution were demonstrated in mice over time. This work shows the promising application of C-Dots in biomedical imaging.

Fullerene, discovered at Rice University in 1985, was another zero dimension carbon nanomaterial. Since then research regarding to fullerene-biomolecule conjugates, besides the field of solar cell, has drawn much attention. Fullerene is a sphere shaped molecule, and unable to dissolve in water which limits its applications in biotechnology. Due to this reason, water soluble fullerene derivatives are largely needed. The landmark work was the one performed by Friedman et al. in 1993. With the model built via the program DOCK3, that the active site of HIV-1 protease (HIVP) was able to host fullerene derivatives was found. In addition, the inhibition function of the fullerene derivatives to the HIVP was examined experimentally. Followed by this work, numerous efforts were devoted to the field of fullerene biomedical applications, including attaching amino acids and peptides to the fullerene for the ap-

Fig. 1 Zero dimensional carbon nanomaterials, a) carbon dots, and b) fullerene. One dimensional carbon nanomaterial, c) carbon nanotube. Two dimensional carbon nanomaterial, d) graphene, and e) graphene oxide.

Fig. 2 a) Without carbon dots, insulin will fibril within 3.5 h. b) By the function of carbon dots on insulin, the fibrillation is inhibited within 5 days. (Reproduced with permission from reference 20. Copyright 2015 American Chemical Society).

Fig. 3 a) Differential pulse voltammetry (DPV) was suppressed after the phosphorylation of Tyrosine (Tyr) in the peptide residue. b) The oxidation current signal was recorded in the presence of kinase inhibitor which is able to prevent the phosphorylation of Tyr in the peptide residue.

Fig. 4 i) Fluorophore attached DNA was absorbed onto graphene oxide, and its fluorescence was quenched because of Förster resonance energy transfer (FRET). ii) Specific sequence ssDNA was added to the surface, which leads to the fluorescence restoration.

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applications in the neural diseases, binding DNA to the fullerene for the use of breaking DNA, and immobilizing carbohydrate onto the fullerene for photodynamic therapy. Nowadays research with regard to the field of fullerene-biomolecule is still active, and more applications of fullerene also needs to develop.

3 One Dimensional Carbon Nanomaterials

Carbon nanotubes (CNTs) are important one dimensional nanomaterials which have a cylindrical nanostructure. The CNTs length to diameter ratio could be up to 10^5 (ref. 3). In 1991, Iijima reported a similar method to producing fullerene to prepare CNTs. The CNTs were obtained on the negative carbon electrode via arc-discharge method. In addition, different synthesis strategies have been developed to construct the CNTs, such as laser ablation, and chemical vapor deposition. The needle shaped carbon nanomaterial shows excellent properties like fullerene, and have been investigated by numerous researchers for the valuable applications in the field of nanobiotechnology.

In 1974, individual molecules were introduced into electronic devices for the first time. Since then molecular electronics attracted much attention, due to the high sensitivity, fast response, and low detection limits. Carbon nanotubes are capable of decreasing over-potential and improving sensitivity when incorporated into electronics. Variable CNT-based devices have been fabricated to detect biomolecules, such as proteins, DNA, and others. In CNT-based field effect transistor (FET), the CNT which can provide faster electron transfer kinetics plays a role of channel, and the charge of biomolecules like protein bind to the device followed by serving the gate for the current channel, and the charge of biomolecules like protein bind to the device followed by serving the gate for the current channel. It was concluded that phosphorylation could prevent phosphorylated Tyr oxidizing at approximately 0.65 V. The research is important for the pharmaceutical development, since PTKs is related to cancer. Another CNT-based FET was built by Wang and Musameh. The results confirmed the CNT could accelerate the electron transfer kinetics. The CNT modified electrode showed high sensitivity and stability to insulin sensing by amperometric measurements. More interestingly, CNTs could be internalized by the cell via tip recognition and rotation according to the report by Shi et al. With the aid of computer based model and experiments, they found that the receptor on the cell membrane could bind to the tip of CNT. In such a way, the CNT is capable of entering the cell, and providing a new approach to send the cargo attached to the CNT into the cell for therapeutic applications. CNT can also be used in energy storage. Lee and Lee studied the potential of CNTs in hydrogen storage through density-functional calculations. Two different sites for H2 chemisorption were found inside the empty space of CNT. The storage capacity of over 14 wt% (160 kg H2/m3) were predicted in the paper. However, the experimental reports with regard to the CNT-hydrogen storage can not achieve such a high level. Chen et al. utilized the aligned CNT for hydrogen adsorption study at room temperature under 10 atm pressure. The capacity of storage could only get 5-7 wt%, which is much smaller than the computational study, but the pretreatment by heating samples to 300°C could increase storage capacity up to 13 wt%.

4 Two Dimensional Carbon Nanomaterials

Graphene is an atomically thin and two dimensional carbon nanomaterials. Since its first experimental discovery in 2004, graphene has gained lots of popularity in biological systems and applications of therapeutic properties due to its unique structure, such as extremely large surface area on both sides and one atomic thickness. To improve the sensitivity of biosensing system, graphene has been widely used to fabricate more efficient nanodevices. As a novel semiconductor, graphene shows extraordinary properties comparing to conventional industrial materials, such as silicon. For example, there exists many electron collisions when current flows through silicon, which limits its wider applications. Not only its fantastic electronic properties, graphene is an outstanding conductor of heat, and also the world’s thinnest and strongest material. However, unlike other semiconductor materials, there is no band gap between conduction and valence band in graphene, bringing up problems for fabricating nano electronic devices. For graphene based transistors, once the device is turned on, it is hard to be switched off. To overcome such obstacles, many groups are trying to modify the graphene to open up the band gap in graphene, thereby improving the sensitivity of the nano bioelectronics. Covalently and noncovalently bonded strategies are generally adopted to solve the problem. Recently, Mann et al. developed a method to modify the graphene via the aromatic moieties by noncovalent strategy. The approach studied in this paper can provide promising applications in the field of sensing and biosensing. Graphene oxide (GO) is a derivative carbon material of graphene. It contains carboxyl groups at the edges, hydroxyl and epoxide groups mainly at the basal plane, and some carbon sp² domains. Such different oxygen containing groups provide more active reacting sites to link
the molecule to the surface via covalent or noncovalent bound strategies\(^{50}\). In 2009, Lu et al.\(^{51}\) immobilized dye labeled ssDNA onto the GO via the ionic interaction (Fig. 4). Since the small distance between the probe DNA and GO, the conjugated organic dye was quenched after deposition. The restoration of dye fluorescence proved the detection of target DNA, which is due to its interaction with the released DNA from the GO.

To understand the roles of GO in interacting with biomolecules, Li et al.\(^{52}\) studied the effect of GO as a quencher on the fluorescent assay of amino acids, peptides, and proteins. The work started with the fluorescence quenching of L-Tyrosin (Tyr) and L-tryptophan (Trp) by GO. That the hydrophobic interaction and electrostatic interaction between amino acids and GO could contribute to the quenching effect was concluded in the study. Also the quenching mechanism was discussed, and it was attributed mainly to the static quenching. To test the fluorescence quenching of peptides and proteins by GO, amyloid β-protein 40 (Aβ 40), human islet amyloid polypeptide (hIAPP) and bovine serum albumin (BSA), human serum albumin (HSA) were selected separately for the investigation. The work deeply addressed the interaction between GO and biomolecules, and is important before applying GO to actual applications. GO can also be used for the selectivity and sensitivity enhancement. Followed by this study, the influence of GO on the determination of Trp was studied\(^{53}\). Different parameters including pH, temperature, incubation time, and chemical reagents were screened to optimize the conditions. By oxidizing the products from reaction of formaldehyde and Trp with the hydrogen peroxide, the selectivity and sensitivity were enhanced in the presence of GO.

One important issue when applying GO to biosensing system is that the selective detection of a specific protein from a biological fluid which contains various proteins. To examine the interaction between lysozyme, which exist abundantly in body fluid, and GO for biosensing, Li et al.\(^{54}\) demonstrated the strong and selective adsorption of lysozyme on GO. The strong quenching effect of GO on lysozyme confirmed the lysozyme and GO interaction, and this interaction was further characterized by zeta potential, dynamic light scattering (DLS), and AFM. The nature of the interaction was determined to be electrostatic interaction. After lysozyme adsorbed onto GO, that adding pH 11.5 NaOH and precipitating it with CaCl\(_2\) could release lysozyme from the GO surface. What’s more, their strong interactions made lysozyme from binary and ternary protein mixtures adsorpt onto GO as well. This study well addressed the fundamental problem, selective detection of abundant proteins, for the further development of GO based diagnostics approach.

Besides peptides and proteins, the interaction between GO different lipid models were also investigated by Li et al.\(^{55}\). To understand how the GO acts in the interaction with lipids, the Langmuir monolayer was used, and five different lipids with the same alkyl chain but different head groups were selected. The results showed that it is electrostatic interaction that governed the lipids and GO interaction. An “edge-in” orientation of GO was proposed for inserting GO into lipids.

5 Conclusion

The interactions between biomolecules and carbon nanomaterials offer an excellent chance for the chemists and material scientists to dig into the field of nanobiotechnology. A myriad of new discoveries in terms of carbon nanomaterials with biomolecules have been made in the past decades. From the views of different forms of carbon nanomaterial, recent developments and practical biomedicine applications were briefly summarized. Not only fibrillation inhibition function of carbon quantum dots, also bioimaging ability of these nano sized carbon dots shows great potential in practical applications. Fullerenes, another style of zero dimensional carbon nanomaterial, conjugated with other biomolecules also attracts much attention due to the special properties. The one dimensional nanomaterial carbon nanotube (CNT) is usually employed for the biosensing system. The high sensitivity from CNT based nanobiocircuits gains its highly popularity. Graphene was recently discovered, but graphene itself and its derivate such as graphene oxide have been the hot topic in the area of biotechnology. Research regarding to the interactions between these graphene based materials and biomolecules harvested much in the past decades. Although the carbon in its nano scale shows unique properties, challenges including biocompatibility and cost efficiency, coexist with opportunities. More and more new sightings still need to be discovered.

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