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类石墨烯二维材料的非共价功能化及生物医学应用研究

Noncovalent functionalization of two-dimensional graphene analogues for biomedical applications

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Noncovalent functionalization of two-dimensional graphene analogues for biomedical applications

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摘 要

类石墨烯二维材料（2D-GAs, 比如过渡金属硫化物纳米片、g-C_3N_4、Bi_2Se_3和黑磷等）由于其独特的成分、结构、理化性质和良好的生物相容性，在生物医学领域展现出前所未有的优势。2D-GAs的制备、表面功能化以及在纳米生物医学领域的应用是目前的研究热点，引起了材料、化学、物理、生物、医学等各个领域科学家的广泛兴趣。

本文对三种2D-GAs（Bi_2Se_3、MoS_2和黑磷）分别进行了表面非共价功能化，探索了这三种2D-GAs在纳米生物医学中的应用。本文主要研究了以下几方面的内容：

一. 探索了2D-硒化铋的表面功能化及其用于阻止$A\beta_{1-42}$聚集。制备了血红素稳定的少层硒化铋（Bi_2Se_3），通过分步离心得到不同尺寸和厚度的少层Bi_2Se_3，并探索了其对$A\beta_{1-42}$纤维化的抑制作用。通过荧光光谱证明越小越薄（3±1 nm）的Bi_2Se_3纳米片展现出更高的$A\beta_{1-42}$纤维化抑制效率，TEM、AFM和DLS结果进一步证实了Bi_2Se_3纳米片能够抑制$A\beta_{1-42}$纤维化。用CD、CV、电化学阻抗和微天平系统地探索了少层Bi_2Se_3与$A\beta_{1-42}$单体之间的相互作用，结果表明少层Bi_2Se_3能抑制$A\beta_{1-42}$纤维化是由于少层Bi_2Se_3对$A\beta_{1-42}$单体强的吸附作用。体外细胞实验证明少层Bi_2Se_3能够将$A\beta_{1-42}$聚集诱导的神经细胞毒性从22%降低到7%，同时还能降低$A\beta_{1-42}$聚集诱导的类过氧化物酶活性。因此，少层Bi_2Se_3对老年痴呆症的治疗具有一定的应用前景；

二. 探索了2D-MoS_2的表面功能化及其用于电化学细胞生物传感器的构建。首先，利用叶酸（FA）作为表面稳定剂在溶液中制备了FA功能化的少层MoS_2（MoS_2/FA）。随后制备了MoS_2/FA修饰的金电极，并且利用MoS_2/FA修饰的金电极识别和固定富含叶酸受体的癌细胞后引起阻抗的变化来检测HeLa细胞。实验结果表明，MoS_2/FA修饰的金电极能够非常灵敏的识别癌细胞，检测线性范围为1 – 10^5 cell mL^{-1}，检测限低至1 cell mL^{-1}，该电极还可以识别其他富含叶酸受体的癌细胞，如MCF-7、MG-60、SMMC-7721等。同时该生物传感器对富含叶酸受体的HeLa细胞具有较好的选择性。还制备了FA稳定的其它2D材料（WS_2/FA、

石墨烯/FA和Bi_2Se_3/FA），发现其识别癌细胞的效果依次是MoS_2/FA、WS_2/FA、石墨烯/FA和Bi_2Se_3/FA，证明MoS_2/FA能高灵敏度识别癌细胞是由于其适中的导电性。MoS_2/FA修饰的金电极也被成功用于临床样品的检测，可选择性识别癌症患者样本；

三.以氧化石墨烯为稳定剂制备了氧化石墨烯和其他2D-GAs的复合材料，并探索了其在类过氧化物酶和葡萄糖传感器领域的应用。石墨烯和其它2D-GAs的复合材料目前主要采用化学气相沉积法制备，产量低，价格昂贵，不适用于溶液体系中的应用。首先，本文以氧化石墨烯（GO）和MoS_2体材料作为原料，利用溶液液相剥离的方法制备了GO稳定的少层MoS_2（MoS_2/GO）。通过控制GO尺寸的大小调节MoS_2/GO的结构，采用UV-vis，DLS，SEM，TEM和AFM对分步离心后所制备的不同尺寸的MoS_2/GO进行了表征。结果表明，GO能够像表面活性剂一样粘附在少层MoS_2的表面形成稳定的MoS_2/GO复合材料。利用这种方法本文也成功制备了GO与其它2D-GAs的复合材料。其次，本文报道了MoS_2/GO复合材料本身具有类过氧化物酶活性，其类过氧化物酶活性比传统辣根过氧化物酶（HRP）的米氏常数低4.35倍，而最大反应速率确是HRP的3.34倍。采用电化学方法探索了高类过氧化物酶活性的机理，证明高的催化活性主要是由于MoS_2/GO表面电子的快速转移以及两者之间的协同作用。再次，探索了可见光和近红外光对MoS_2/GO的类过氧化物酶活性的影响，结果表明，可见光和近红外光都能增强MoS_2/GO的类过氧化物酶。基于MoS_2/GO复合材料的高类过氧化物酶活性，本文构建了过氧化氢传感器。在可见光的作用下，传感器的检测限能够从10 nM降低至2.5 nM。最后，本文还检测了血清中的血糖， MoS_2/GO复合材料在血糖检测中展现出很高的灵敏度和选择性，而且可见光能够将血糖的检测限从0.83 μM降低到65 nM。因此，MoS_2/GO复合材料在血糖检测领域具有广阔的应用前景；

四. 探索了二维材料黑磷烯（BP）的不稳定性在构建高效癌症标记物生物传感器中的应用。本文中，利用负载的纳米金粒子的黑磷烯（BP-Au）的高效还原黄色的4-硝基酚（4-NP）成无色的4-氨基酚（4-AP）的性能可以比色法识别癌症生物标记物。首先将BP和纳米金前体通过简单混合很容易制备BP-Au，BP能够充当还原剂和稳定剂。BP-Au在催化4-NP还原的反应中展现出高效的催化性能（$K = 1120\ s^{-1}\ g^{-1}$）和低的活化能（$Ea= 17.53\ kJ\ mol^{-1}$），主要归因为BP和纳米金粒子

之间的协同作用和半导体材料BP表面丰富的电子。其次，当把癌胚抗原（CEA）抗体吸附在BP-Au表面后，催化4-NP的反应被抑制，加入肿瘤标记物CEA后，CEA与抗体结合，抗原抗体复合物从材料表面脱附下来，催化4-NP的反应得以恢复，利用比色法检测4-NP的浓度改变，从而间接计算血清中CEA的浓度。基于以上原理构建的CEA生物传感器具有较宽的检测线性范围（1 pg mL^{-1}到10 μg mL^{-1}）和较高的灵敏度（0.20 pg mL^{-1}，信噪比为3）。最后，将这种生物传感器用于临床癌症患者血清样品的检测，与酶联免疫吸附实验（ELISA）的结果一致。重要的是我们通过电化学阻抗谱，BCA蛋白试验和微天平的方法证实了提出的检测机理。

关键词：二维石墨烯类似物；黑磷烯；MoS_2；Bi_2Se_3；生物传感器

Abstract

Biocompatible two-dimensional (2D) graphene analogues (e.g., transition metal dichalcogenides nanosheets, g-C_3N_4, Bi_2Se_3, phosphorene, *etc.*), which are referred to as 2D-GAs, have emerged as a new unique family of nanomaterials that show unprecedented advantages and superior performances in biomedicine due to their unique compositional, structural and physicochemical features. Preparation, functionalization and biomedical application of 2D-GAs are hot topics, have attracted extensive attention from scientists of various fields include materials, physics, biology, medical.

The thesis focused on the noncovalent functionalization of three kinds of 2D-GAs: Bi_2Se_3, MoS_2 and phosphorene and their application in nanobiomedical field. The major explored issues of the thesis are outlined as follows:

Firstly, we investigated the surface functionalization of few-layer Bi_2Se_3 and its application in preventing amyloid beta aggregation. Few-layer Bi_2Se_3 was exfoliated in aqueous solution of hemin. Then we separated few-layer Bi_2Se_3 with different sizes and thicknesses by fractional centrifugation, and investigated their inhibition effect on $A\beta_{1\text{-}42}$ aggregation. The fluorescence spectra results show that smaller and thinner few-layer Bi_2Se_3 had higher inhibition efficiency than that of larger and thicker one, which was further confirmed by Transmission electron microscope (TEM), atomic force microscope (AFM) and dynamic light scattering (DLS) mesurements. Analysis of the interaction between few-layer Bi_2Se_3 and $A\beta_{1\text{-}42}$ monomer by cyclic voltammograms, circular dichroism (CD) spectrum and microbalance methods indicates that the significant inhibitory effect was due to the high adsorption capacity of few-layer Bi_2Se_3 for $A\beta_{1-42}$ monomer. In vitro neurotoxicity studies under physiological conditions show that few-layer Bi_2Se_3 could decrease Aβ-mediated peroxidase-like activity and cytotoxicity. Therefore, our work extends the potential applications of two-dimensional few-layer Bi_2Se_3 in AD treatment field.

Secondly, functionalization of few-layer MoS_2 and its application for electrochemical cell biosensor are explored. First, we use folic acid (FA) to exfoliate few-layer MoS_2 in aqueous solution to produce few-layer MoS_2 stabilized by FA (MoS_2/FA) by a one-pot method. Secondly, we fabricated MoS_2/FA-modified gold electrode (AuE/MoS_2/FA) and use the impedance change resulting from the interaction of AuE/MoS_2/FA and cancer cells for cancer cell testing. The results indicate AuE/MoS_2/FA can sensitively detect HeLa cells. The linear range for HeLa cell detection by the optimized AuE/MoS_2/FA is from 1 to 10^5 cell mL^{-1} with a detection limit of 1 cell mL^{-1}. Experimental results have shown that AuE/MoS_2/FA can also recognize other folate receptor-rich cancer cells, such as MCF-7, MG-63 and SMMC-7721 cells. Furthermore, the biosensor also shows good selectivity for folate receptor-rich cancer cells. We also prepared FA-stablized other 2D-GAs (WS_2/FA, graphene/FA and Bi_2Se_3/FA). The cancer cell detection efficiency of the 2D-GAs is in the following order: MoS_2/FA, WS_2/FA, grapheme/FA and Bi_2Se_3/FA, indicating the high sensitivity of AuE/MoS_2/FA is attributing to the intermediate conductivity. Finally, the proposed biosensor is applied to detecte clinic samples. The result indicates AuE/MoS_2/FA could selectively recognize samples of cancer patients.

Thirdly, hybrids 2D-GAs and graphene were prepared by using GO as a stabilizer, and the application of hybrids in intrinsic peroxidase-like activity and gluocose biosensor were investigated. Hybrids of 2D-GAs and graphene are mainly prepared by chemical vapor deposition method, suffer from low yield, critical preparation conditions and could not be used in aqueous solution. Firstly, a hybrid of MoS_2 and graphene oxide (GO) (MoS_2/GO) is prepared by a one-pot liquid-phase-exfoliation process by using GO and bulk MoS_2 as raw materials. The exfoliated MoS_2 by ultrasound waves is stabilized by amphiphilic GO in aqueous solution. The size of GO plays an important role in tuning the structure of MoS_2/GO. Ultraviolet–visible absorption spectrum, DLS, scanning electron microscope, TEM and AFM are used to characterize the structure of MoS_2/GO produced by fractional centrifugation after exfoliation of MoS_2 in GO suspension. The results indicate that GO is adhered to the surface of MoS_2 to stabilize MoS_2/GO in solution. Furthermore,

this preparation method can also be used to prepare the hybrids of GO and other two-dimensional materials. Secondly, an intrinsic peroxidase-like activity of MoS_2 and graphene oxide (MoS_2/GO) hybrid is demonstrated and the hybrid is also used to detect glucose with high sensitivity. The results show that the hydrid has highest catalytic activity and the Michaelis constant of this hybrid is 4.35 times lower and the maximal reaction velocity is 3.34 times higher than those of horseradish peroxidase, respectively. Electrochemical technologies are used to investigate the enhancing mechanism. The results show that the excellently catalytic performance could be attributed to the fast electron transfer on the surface of MoS_2/GO and the synergistic interaction of two components. Thirdly, effect of visible light and near-infrared light on the peroxidase-like activity of hybrid is also investigated. The results show that visible light and near-infrared light could both enhance the peroxidase-like activity of MoS_2/GO. The limit of detection for H_2O_2 can be reduced from 10 nM to 2.5 nM with visible light. Finally, the hybrid is further used to detect glucose in serum with and without light. The results show that the hybrid has high selectivity and sensitivity for glucose detection in serum and the limit of detection for glucose is reduced from 0.83 μM to 65 nM with visible light. Therefore, the hybrid may have a potential application in glucose detection in serum with high sensitivity and selectivity.

Finally, the instability of black phosphorus (BP) was investigated and demonstrated to have a potential application in biosensor. Here, high efficiently reducing yellow 4-nitrophenol to colorless 4-aminophenol by gold nanoparticles/BP (BP-Au) hybrid was used to detect cancer biomarker with colorimetry. Firstly, BP-Au hybrid was facilely synthesized by simply mixing Au precursor with BP, serving as reducing and stabilizing agent. BP-Au shows outstanding catalytic activity (K= 1120 s^{-1} g^{-1}) and low activation energy (17.53 kJ mol^{-1}) for reducing 4-nitrophenol, which is attributed to the synergistic interaction of gold nanoparticles and BP, and rich electrons of BP. Secondly, the catalytic activity of BP–Au could be reversibly switched from “inactive” to “active” upon treatment with antibody and antigen in solution, thus providing a powerful and versatile basis for label–free colorimetric detection of biomarkers. The sensor shows a wide detection range (1 pg mL^{-1} to 10 μg

mL^{-1}), high sensitivity (0.20 pg mL^{-1}, signal-to-noise ratio is 3) and selectivity for the carcinoembryonic antigen (CEA). Finally, the biosensor was used to detect CEA in colon and breast cancer serum samples with satisfactory results. Notably, the suggested detection mechanism was supported by electrochemical impedance spectroscopy, BCA protein assay and microbalance.

Key words: two-dimensional graphene analogues; black phosphorene; MoS_2; Bi_2Se_3; biosensor

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