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多功能纳米复合物的设计、合成及在抗癌治疗与生物分离中的应用

Design and Synthesis of Multifunctional Nanocomposites for Cancer Therapy and Bioseparation

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Design and Synthesis of Multifunctional Nanocomposites for Cancer Therapy and Bioseparation

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摘　要

近年来，纳米材料的生物应用研究受到越来越广泛的关注，纳米材料在疾病检测与诊断、治疗和生物分离等方面都展示出了独特的优越性与发展潜力。针对纳米材料的肿瘤治疗和生物分离应用，本论文重点发展实现相关纳米材料多功能耦合的化学途径，以优化其性能，主要开展的工作包括: (1) 设计、合成钯纳米片/介孔二氧化硅纳米复合载药体系，并应用于肿瘤细胞的近红外光热疗-化学联合治疗；(2) 高分子包裹的磁性纳米颗粒的合成研究及在蛋白质分离纯化中的应用。展开具体的研究内容与研究成果包括以下六个部分：

第一章：从构建纳米材料的结构角度出发，简要总结了多功能纳米复合材料在抗癌联合治疗和蛋白质的分离纯化领域中的应用进展状况，并以此阐述了本论文的研究内容与意义。

第二章：通过层层包裹的方法合成出了介孔二氧化硅包裹的 Pd@Ag 纳米载药体系，研究了该载药体系在体外药物可控释放行为及对癌细胞(HepG2)进行联合治疗的效果。研究表明合成出的 Pd-Ag@MSNs 纳米颗粒尺寸均一、分散性好、比表面积高、介孔孔径大(10 nm 左右)，其药物负载量也高达 49% (w/w)。这些性质特点是一般合成介孔材料的方法很难实现的，并且阿霉素(DOX)负载后的 Pd-Ag@MSNs 纳米颗粒比游离的 DOX 表现出了更好抗癌治疗效果。重要的是，通过配位键负载的 DOX 可以在酸性环境与近红外光照射下实现可控释放，从而使该纳米复合物可以对肝癌细胞同时进行药物化疗和光热治疗，并体现出了优异的协同效应，提高了抗癌治疗效率。

第三章：设计并合成出了钯纳米片包裹的中空介孔二氧化硅新型的纳米载药体系(*hm*-SiO_2-NH_2@Pd)。相对于具有一包一型的核壳结构 Pd-Ag@MSNs 纳米颗粒，每个 *hm*-SiO_2-NH_2@Pd 纳米颗粒外表面吸附的多个钯纳米片可以高效地把近红外光转化成热，从而可以提高纳米载体的光热治疗效果。研究发现用 *hm*-SiO_2-NH_2/DOX@Pd 纳米颗粒进行肿瘤细胞的近红外光热疗-化学联合治疗时，其治疗效果比没有经过光照单独进行化疗和仅用光热治疗的效果之和还要好，体现出了优异的协同效应。

第四章：为了在同一载药体系中同步实现诊断兼治疗的功能，制备了新型的 $Mn_{0.56}Fe_{2.44}O_4@mSiO_2$/Pd 纳米载体。该纳米载体有望用于核磁共振成像(MRI)，而且能对癌症进行化疗和光热疗的联合治疗。

第五章：通过沉淀聚合的方法成功合成出了表面富含 Ni-NTA 分子的 $Fe_3O_4@SiO_2$/P(St-alt-MAA)/Ni-NTA 纳米颗粒。该磁性纳米复合物内核是由超顺磁性的多晶纳米颗粒所组成，从而使整个纳米颗粒具有很好的磁响应性及单分散性；而外壳是由含有很多反应位点的聚(苯乙烯-交替-马来酸酐)高分子所组成，可以用来连接大量的 Ni-NTA 分子。实验表明，所制备的两种磁性纳米对组氨酸标记的蛋白都具有很高的选择性和纯化效率，可以直接在细胞裂解液中分离提取目标蛋白。由于多价效应的存在，$Fe_3O_4@SiO_2$/P(St-alt- MAA)/Ni-NTA 纳米颗粒对组氨酸标记(His-tag)蛋白的纯化能力是 $Fe_3O_4@SiO_2$/ Ni-NTA 纳米颗粒的 4 倍，在低表达量的蛋白分离体系中具有更大的优势。

第六章：针对本论文所进行的研究工作进行了总结及后续的研究做了展望。

关键词：钯纳米片；近红外光；协同效应；磁性分离；组氨酸标记的蛋白

Abstract

Recently, the bioapplications of nanomaterials have attached more and more attention. Especilly in disease diagnosis, disease therapy and bioseparation, nanomaterials exhibit unique superiority and high development potential. Aiming at nanomaterials in the application of the area of anticancer therapy and purification of proteins, this thesis sheds light on developing coupling chemical methods to optimize the properties of multifunctional nanomaterials. The main research include two works：(1) Design and synthesis of Pd nanosheets/mesoporous silica composites used for chemophotothermal treatment of cancer cells, (2) Design and synthesis of polymer coated superparamagnetic nanoparticles used for purification of proteins. The detailed research work and major results are divided into six parts as follows:

Chapter 1. From the point of view of the construction of nanocomposites, we brief review and summarize the research progress on the synthesis of multifunctional nanocomposites for cancer therapy and bioseparation. Also demonstrated that the main content and significance of the dissertation.

Chapter 2. Mesoporous silica-coated Pd@Ag nanoparticles are obtained by using layer-by-layer deposition method, and then we also study the drug-release kinetics from the drug carries and the cell-killing efficacy by the DOX-loaded nanoparticles in vitro. Our experiments revealed that the Pd-Ag@MSNs nanocarries with uniform particle size、large surface arear、big pore diameter(~10 nm)、high drug-loading capacity(49%), which properties are difficult to obtain by other common method for synthesizing of mesoporous silica. Especially, the DOX-loaded core-shell nanoparticles exhibited highter cytotoxicity than free DOX at the same experimental condition. More importantly, DOX molecules are loaded in the mesopores shell through coordination bonds that are reponsive to pH and heat. The release of DOX from our core-shell delivery vehicles into cancer cells can be therefore triggered by the pH drop and also NIR irradiation. It was found out that the combining

chemotherapy and photothermal therapy to the cancer cells demonstrated a synergistic effect, resulting in higher therapeutic efficincy.

Chapter 3. Based on the achievements and shortages of chapter 3, Pd nanosheets coated hollow mesoporous silica nanocarries (*hm*-SiO_2-NH_2@Pd) were successfully designed and prepared. Compared to Pd-Ag@MSNs nanocarries, each *hm*-SiO_2-NH_2@Pd particles' surface absorbed lots of Pd nanosheets which could efficiently convert NIR light into heat, resulting in improving the photothermal therapeutic dfficiency. We found out that the cell-killing efficacy by DOX-loaded *hm*-SiO_2-NH_2@Pd nanoparticles under NIR irriadiation was even higher than the sum of chemotheraphy by DOX-loaded *hm*-SiO_2-NH_2@Pd nanoparticles and photothermal therapy by unloaded *hm*-SiO_2-NH_2@Pd nanoparticles, indicating that the synergistic effect appeared in our drug delivery system. Interestingly, we also found that more Pd nanosheets were taken up by the cancer cells when the Pd nanosheets were coated on the surface of hollow mesoporous nanoparticles. This is a good method of improving nanoparticles internalized into cancer cells, and gives some advice for related research areas.

Chapter 4. In this chater, we have successfully synthesised a novel $Mn_{0.56}Fe_{2.44}O_4$@*m*SiO_2/Pd nanocarriers for cancer diagnosia and treatment. This platform could simultaneously use for MR imaging and the combining chemotherapy and photothermal therapy to the cancer cells. The multifunctional nanocarriers possessing various functions including magnetic tumor-targeting, magnetic resonance imaging and chemo-thermal therapy have potential use in biomedicine.

Chapter 5. Magnetic core-shell Fe_3O_4@SiO_2@poly(styrene-alt-maleic anhydride) spheres enriched with Ni-NTA on their surface have been prepared by precipitation polymerization. The spheres have a core composed of superparamagnetic polycrystalline magnetite, endowing the spheres with excellent magnetic responsivity and dispersity. The shell composition of poly(styrene-alt-maleic anhydride) allows the incorporation of more Ni-NTA affinity sites onto the surface of the magnetic spheres. Our experiments revealed that the two types of magnentic particles could exhibite excellent performance in the direct separation of His-tagged

protein from cells lysates. In addition owing to the multivalency effect, the separation capacity of His-tagged proteins by the as-prepared Fe_3O_4@SiO_2@polymer/Ni-NTA composites was four times as that by Fe_3O_4@SiO_2/Ni-NTA, making them particularly promising for the magnetic separation of low-concentration His-tagged proteins.

Chapter 6. we made some conculusins and gave a prospect on the topics discussed in this dissertation

Keywords: Pd nanosheets; NIR light; Synergistic effect; Magnetic separation; His-tagged proteins.