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普鲁士蓝纳米材料的生物医学应用研究

The Biomedical Application of Prussian Blue Nanomaterials

张 书 鹏

指导教师姓名: 叶社房 副教授  
周 樨 副教授

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## 摘 要

近年来,肿瘤发病率持续升高,并且日益年轻化,形式十分严峻。由于传统治疗方法具有风险大、毒副作用强等弊端,人们正在积极寻找更安全有效的治疗手段。而肿瘤热疗,作为治疗肿瘤的一种新的有效手段,已成为继手术治疗、放疗、化疗和免疫疗法之后的第五大疗法,如磁流体热疗(MFH)、光热治疗(PTT)等。肿瘤热疗是泛指用加热来治疗肿瘤的一类治疗方法,主要是利用正常组织和肿瘤细胞对温度耐受能力的差异,达到既能使肿瘤细胞凋亡、又不损伤正常组织的治疗目的,具有无创伤、快速、便捷、效果较好、较安全等优势。在现在的研究中,将热疗与其他手段联合,实现诊疗一体已成为研究的热点。但是,目前癌症的早期诊断也同癌症治疗一样,仍然是一项充满难度与挑战性的一项工作。虽然现在的肿瘤诊断方法众多,包括计算机断层扫描技术(CT)、磁共振成像技术(MRI)、超声成像(US)、光声层析成像技术(PAI)等等,各有各的特点,甚至是多种诊断方式的联合,形成一种多模式诊断技术来确定肿瘤的存在与否。现如今,多模式是一种主流,而多模式的诊疗一体化研究更是成为研究热点,其中,制备出一种诊断效果好、治疗效果优良的同时简单、便捷的诊疗试剂仍是不小的一个挑战。

本文设计了一种非常简便的方法,在室温条件下,第一次合成出以普鲁士蓝(PB)和铁酸锰( $\text{MnFe}_2\text{O}_4$ )为基的PB/ $\text{MnFe}_2\text{O}_4$ 磁性普鲁士蓝复合纳米材料,实现了集MRI中的 $T_1/T_2$ ,MFH及PTT等多功能为一体,不仅弥补了单一成像所带来的不足,实现了精准诊疗的目标,同时又可以实现光热治疗与磁热治疗的目的,并且光热治疗与磁热治疗可同时作用,治疗效果更加明显,达到了诊疗一体的目的,且便捷、高效。本文主要的工作包括了如下的几个方面:

1) 实验首先采用水热法制备出粒径较均一(100 nm)、分散性稳定的立方体PB,且表面修饰有-COOH,使粒子带有较强的负电荷。然后再通过共沉淀法制备出因表面带有-NH<sub>2</sub>而带正电荷的20 nm左右磁性 $\text{MnFe}_2\text{O}_4$ 纳米粒子。然后通过正负电荷相互吸引的方法对PB/ $\text{MnFe}_2\text{O}_4$ 这种复合纳米材料的制备进行探索,通过不断的表征和分析,最终选择了质量浓度比为1:2.3(PB: $\text{MnFe}_2\text{O}_4$ =1:2.3)这个比例,从而得到PB/ $\text{MnFe}_2\text{O}_4$ 复合纳米材料,也就是磁性普鲁士蓝复合纳米材料(Magnetic Prussian Blue Nanoparticles, MPB NPs)。

2) 然后通过扫描电镜(SEM)、透射电镜(TEM)、紫外可见光谱(UV-Vis)、振动样品磁强计(VSM)等表征,发现该纳米材料具有很好的水溶性、结构稳定性,以及良好的光热、磁热效果,温度均能达到 42℃ 以上,具有光热治疗方面的应用潜力,并且通过 MRI 检测出其弛豫值  $r_1$  为 5.56 mM<sup>-1</sup>s<sup>-1</sup>,具有较强的  $T_1$  加权造影效果,弛豫值  $r_2$  为 123.46 mM<sup>-1</sup>s<sup>-1</sup>,具有较强的  $T_2$  加权造影效果,表明此材料是一个  $T_1/T_2$  双模态造影剂,可用于  $T_1/T_2$  双模态成像。我们所合成的 MPB NPs 既具有良好的  $T_1/T_2$  双模态造影剂造影功能,又具有良好的热疗效果,是一个优良的诊疗试剂。

3) 我们再在动物水平上去验证材料的性能,选择 Balb/C 小鼠进行 MRI 及热疗实验。从小鼠的磁共振成像效果可以看出,材料具有较好的  $T_1$  及  $T_2$  效果,并且显示在 1 h 时效果较好。接着进行小鼠的热疗实验,结果发现材料+磁热、材料+光热、材料+磁热+光热组的小鼠的肿瘤区域,在实验过程中,5 min 内温度均显著上升,均能超过 42 °C,具有较好的热疗效果,并且在后续的治疗中发现,肿瘤逐渐减小,直至消失,而其他组肿瘤均显著增大,对比说明材料具有优良的热疗效果,并且材料+磁热+光热组效果更明显,升温更快,治疗效果更好,肿瘤消失更快。根据上述结果,这种 MPB NPs 具有较好的生物相容性、 $T_1/T_2$  造影效果以及热疗效果,显示出这种材料在诊疗领域具有极大的应用潜力。

**关键词:** 纳米材料; 复合材料; 磁性普鲁士蓝; 磁共振造影; 热疗

## Abstract

In recent years, the tumor incidence increasing rapidly and the patient become more and more younger. The situation is very serious. Since the traditional treatment have some disadvantages need to be solved, such as high risk and side effects, people are looking for a more safe and effective treatment actively. Tumor hyperthermia, as a new and effective means for the treatment of tumor, has been the fifth largest therapy, after the surgical treatment, radiotherapy, chemotherapy and immunotherapy. It contains many kinds of treatment means, such as Magnetic Fluid Hyperthermia (MFH), and Photothermal Therapy(PTT), etc. Tumor hyperthermia, which increases the temperature of tumor area by heating, and because of the temperature tolerance of the normal tissue and tumor cells, it can make tumor cell apoptosis, and not damage to normal tissue, it's advantage is much, such as no trauma, quickly, convenient, efficient and safer, etc. Up to now, it has been a hot research connect with the diagnosis. Compared to the cancer treatment, the early diagnosis of cancer, is still full of difficult and challenge. Although there are many kinds of tumor diagnosis methods, including the Computed tomography (CT), Magnetic Resonance Imaging (MRI) and Ultrasound imaging (US), Photoacoustic imaging (PAI), etc, each have their own characteristic, and if need, even can form a multimodal diagnostic system to detect whether the tumor exists or not. Now, the multi-model is mainstream patter, especially the research of diagnosis and treatment, but, the materials with good effect, excellent treatment effect, simple and fast synthesis is still quite a challenge.

In this paper, we designed a very simple method to synthesis the Magnetic Prussian Blue nanoparticles (MPB NPs) for the first time at room temperature, which composite by Prussian blue (PB) and Manganese ferrite ( $\text{MnFe}_2\text{O}_4$ ), implements the MRI  $T_1/T_2$ , PTT and MFH in a multi-functional platform, not only makes up for the shortage result from the single imaging, achieve the goal of accurate diagnosis, but also achieve the purpose of PTT and MFH, especially the PTT and MFH can work together, more effectively. It can realize diagnose and treatment, in the meantime, it is more convenient and efficient. In this paper, the main work includes the following

several aspects:

1) At first, we synthesized the uniform and stable particle of PB by the hydrothermal method, the size is about 100 nm, and the group of -COOH is in the particles surface, which makes the particles with negative charge. Then prepared the MnFe<sub>2</sub>O<sub>4</sub> magnetic nanoparticles with the surface with -NH<sub>2</sub> positively charged by coprecipitation method, and the particle size is about 20 nm. And then through the method of positive and negative charges attract each other, the PB and MnFe<sub>2</sub>O<sub>4</sub> composite together and form the MPB NPs. And through continuous characterization and analysis, finally choose the mass concentration ratio of 1:2.3 (PB: MnFe<sub>2</sub>O<sub>4</sub> = 1:2.3), the shape of particle is better in this proportion.

2) and then characterized the MPB NPs by Scanning Electron Microscope(SEM), Transmission Electron Microscope(TEM), Ultraviolet and Visible spectrophotometer (UV-Vis), Vibrating Sample Magnetometer(VSM), found that the nanometer material has good water solubility, structural stability, strong Near infrared absorption capacity and good magnetic effect, and the temperature of materials can reach more than 42 °C in both MFH and PTT experiment, with the perfect potential applications of hyperthermia treatment. And through MRI detected, the relaxation values of  $r_1$  is 5.56 mM<sup>-1</sup>s<sup>-1</sup>, have stronger  $T_1$ -weighted imaging effect, and the relaxation value of  $r_2$  is 123.46 mM<sup>-1</sup>s<sup>-1</sup>, with strong  $T_2$  weighted imaging effect, showed that the material is a  $T_1/T_2$  dual modal contrast agent, can be used for  $T_1/T_2$  dual modal imaging. Our synthesized MPB NPs has both good  $T_1/T_2$  dual modal contrast imaging (MRI) features, and good effect of heat treatment, is a good diagnostic reagents.

3) Then verified the performance of the material in the animal level, the Balb/C mice was choosed and used it for MRI and hyperthermia treatment. Big change from the effect of MRI between before injection and after could been found in mice, especially in the 1h, demonstrating that the material has good effect of  $T_1$  and  $T_2$ . And then in the hyperthermia treatment, the result showed that found the group of materials + magnetic, materials + Laser, and materials+ magnetic+ Laser had good effect, the temperature of tumor area has exceeded 42 °C in the process of experiment, especially the group of materials+ magnetic+ Laser, the temperature was significantly

increased within 5 min, the effect of hyperthermia experiment was very well, and in the follow-up treatment, the tumor size decrease, till to disappear (especially the group of materials+ magnetic+ Laser), but the other group was not. Based on the above results, the MPB NPs has good biocompatibility,  $T_1/T_2$  imaging effect and excellent heat effect, showed that the material has great potential applications in the field of diagnosis and treatment.

**Key words:** Nanoparticle; Composite materials; MPB NPs; MRI; Thermal therapy

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## 第一章 绪论

### 1 引言

#### 1.1 纳米材料

纳米材料是指在三维空间中至少有一维处于纳米范围(1-100 nm)的材料<sup>[1]</sup>, 纳米粒子处于原子簇和宏观物体之间的过渡区, 它们既不是典型的微观系统也不是典型的宏观系统, 它的特点在于其比表面积很大<sup>[2]</sup>, 表面原子是既无长程有序又无短程有序的非晶层, 而粒子内部的原子又呈现有序的排列。与传统的体块材料相比, 其在电磁学、热学、光学、化学等方面都显示出许多奇特的用途, 呈现出许多特殊的物理与化学性质, 诸如小尺寸效应<sup>[3]</sup>、表面效应<sup>[4]</sup>、量子尺寸效应<sup>[5]</sup>和宏观量子隧道效应<sup>[6]</sup>等。

##### 1.1.1 小尺寸效应

一般来讲, 随着颗粒尺寸的量变, 在一定条件下会引起颗粒性质的质变。对纳米颗粒而言, 尺寸变小, 同时其比表面积亦显著增加, 从而磁性、内压、光吸收、热阻、化学活性、催化性及熔点等都较普通粒子发生了很大的变化, 产生一系列新奇的性质, 这称为小尺寸效应 (Small Size Effect), 简单的来说就是随着材料尺寸变小, 材料的性能发生了改变。比如金属块状物体对光吸收不明显, 而金属纳米颗粒对光吸收显著增加, 并产生吸收峰的等离子共振频移<sup>[7]</sup>; 小尺寸的纳米颗粒磁性与大块材料有明显的区别, 并且与大尺寸固态物质相比, 纳米颗粒的熔点会显著下降, 例如2 nm的金颗粒熔点为600 K, 随着粒径增加熔点迅速上升, 块状金为1337 K。

##### 1.1.2 表面效应

由于颗粒的表面积与直径的平方成正比 ( $S=4\pi R^2$ ), 其体积与直径的立方成正比 ( $V=4\pi R^3/3$ ), 故其比表面积 (表面积/体积) 与直径成反比, 因此随着粒径减小, 比表面积显著增加, 颗粒表面原子数增多, 表面原子所占的百分数将会



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