View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Xiamen University Institutional Repository

学校编码：10384　　分类号＿＿＿密级＿＿＿

学号：20720130153645　　UDC＿＿＿

厦门大学

博　士　学　位　论　文

载羟基喜树碱针形纳米靶向给药系统的构建及性能评价

Preparation and evaluation of HCPT-loaded, needle-shaped, and targeted nanodrug delivery systems

指导教师姓名：　冯祖德教授

侯振清教授

专　业　名　称：　材料学

论文提交日期：　2016 年　　月

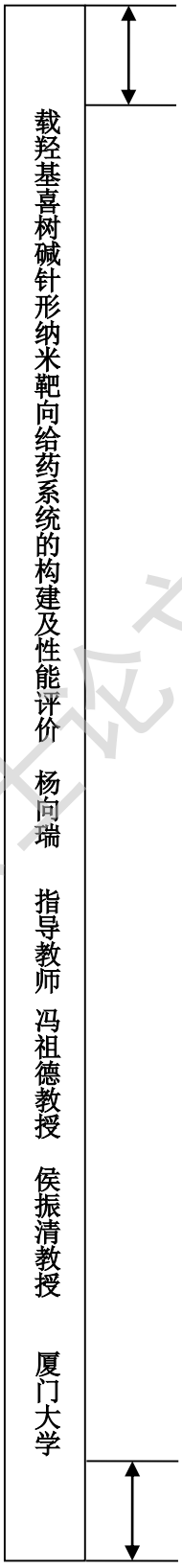
论文答辩日期：　2016 年　　月

学位授予日期：　2016 年　　月

答辩委员会主席：＿＿＿＿＿＿

评　　阅　　人：＿＿＿＿＿＿

厦门大学博硕士论文摘要库



厦门大学博硕士论文摘要库

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为（　　）课题（组）的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学博硕士论文摘要库

厦门大学博硕士论文摘要库

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月

厦门大学博硕士论文摘要库

厦门大学博硕士论文摘要库

摘 要

对于影响纳米粒子细胞摄取因素的研究，人们已经做了非常多的工作，对包括载体材料、粒径、Zeta电位、表面修饰等在内的诸多因素都做了系统的研究。然而在这些研究工作中，所用的绝大多数都是球形纳米粒子，也就是说人们忽略了形状对纳米粒子细胞摄取的影响。近些年来，非球形纳米粒由于其异于球形粒子的优异性质，受到越来越多的关注，研究者也开始对非球形的纳米粒子的性质进行研究，已有大量实验证明，粒子的形状对其细胞摄取有着至关重要的影响，但是它们之间的这种关系还没有完全阐明。

10-羟基喜树碱（10-Hydroxycamptothecin, HCPT）是从我国特有的珙桐科旱莲属植物喜树中分离提纯出来的一种吲哚类的生物碱（即喜树碱）的衍生物，属纯天然广谱抗癌药，研究表明喜树碱及其衍生物可通过作用于 DNA 拓扑异构酶 I 来抑制 DNA 复制、转录和有丝分裂，从而抑制恶性肿瘤的增殖。在自然界中，10-羟基喜树碱是以大尺寸棒状晶体的形式存在，也就是说，其本身就具有成为棒状纳米粒子的潜在可能。

本文以 PEG-*b*-PLGA 为药物载体，以 10-羟基喜树碱为模型药物，采用超声反溶剂法制备出了纵横比较大的针状和棒状的纳米粒，并考察了制备条件对其形貌的影响，然后将两种粒子与本课题组之前制备的球形 10-羟基喜树碱纳米粒子进行对比，考察了它们在体外细胞摄取及细胞毒性的差别，得出结论：针状纳米粒子最容易被细胞摄取。我们又通过改良反溶剂法，制备出了尺寸更小的，同时负载甲氨喋呤和 10-羟基喜树碱的双载药纳米针，并对其体内和体外性质做了研究，结果如下：

(1) 采用超声反溶剂法成功制备出了羟基喜树碱纳米针。进行了制备条件的优化实验。优化条件为采用丙酮作为溶剂，投料比为 1：1，超声功率为 200 W，超声时间为 3 s，间隙 3 s，冰浴条件下反复 150 次。根据优化实验结果，对优化条件下制备的羟基喜树碱纳米针的粒径、Zeta 电位、载药量、包封率、形貌、HCPT 的存在状态进行分析。结果表明：羟基喜树碱纳米针为两端尖，中部略宽，长 5 μm，宽 400 nm 的针状结构，粒径分布较为均一，分散性较好，动态光散射分析

厦门大学博硕士论文摘要库

其平均粒径为 356.7 nm，多分散系数为 0.063，zeta 电位为-10.9 mV。羟基喜树碱纳米针的载药量为 55.84%，包封率为 90.32%，且缓释效果良好，在 pH=7.4 的缓冲溶液中，能够于 400 h 内缓慢的释放药物。

(2) 体外细胞摄取及细胞毒性实验。体外细胞摄取实验表明，不管是三种癌细胞（HeLa 细胞，MG-63 细胞和 MCF-7 细胞）还是正常细胞（MC3T3-E1 细胞），对针状纳米粒子的细胞摄取的速率最快，数量也最多，而对相同尺寸，相同纵横比的棒状纳米粒子则要小一些，而对尺寸只有 150 nm 的球状纳米粒子，其细胞摄取的速率和数量都是远远小于针状纳米粒的。对此可能的原因我们也做了探讨，这应该与细胞膜在细胞摄取过程中的运动有关，我们定义了一个角度Φ，代表细胞摄取过程中，包裹纳米粒子的细胞膜运动方向的角度，角度越小，越容易被摄取。体外细胞毒性实验表明，三种纳米粒子的细胞毒性也与其细胞摄取情况一样，都是针状纳米粒子 > 棒状纳米粒子 > 球状纳米粒子。

(3) PEG-*b*-PLGA-MTX 复合物的制备。对载体材料 PEG-*b*-PLGA 进行了甲氨喋呤的修饰，通过酯化反应，将甲氨喋呤连接到 PEG-*b*-PLGA 上，对其进行了红外光谱的表征，证明了该聚合物-药物轭合物的成功合成。然后通过紫外分光光度法，计算出其甲氨喋呤在轭合物中的百分含量。

(4) 双载药纳米针的制备。利用丙酮-乙醇混合溶液为溶剂，在小剂量条件下，将上述制备的聚合物-药物轭合物和 10-羟基喜树碱制备成尺寸更小的双载药纳米针。并对该纳米针进行了性质的表征，结果表明：双载药纳米针同样为两端尖，中部略宽的针状结构，但其长度约 1 μm，宽约 100 nm，粒径分布较为均一，分散性较好，动态光散射分析其平均粒径为 102.6 nm，多分散系数为 0.049，zeta 电位为-19.3 mV，其羟基喜树碱的载药量为 62.56%，包封率为 92.43%，甲氨喋呤的载药量为 2.03%。该双载药纳米针缓释效果良好，在 pH=7.4 的缓冲溶液中，能够于 400 h 内缓慢的释放两种药物。

(5) 双载药纳米针的体外及体内性质。结果如下：体外细胞摄取实验表明，HeLa 细胞对双载药纳米针的细胞摄取的速率和数量都要远远大于相同尺寸相同形貌的无甲氨喋呤修饰的羟基喜树碱纳米针，同时做了叶酸竞争性抑制试验，表明在叶酸存在条件下，双载药纳米针的细胞摄取明显下降，说明双载药纳米针是通过与叶酸受体的特异性结合介导的特异性吞噬进入细胞的。体外细胞毒性实验

厦门大学博硕士论文摘要库

也得出类似结论，即双载药纳米针的细胞毒性更大。活体荧光成像结果表明双载药纳米针在肿瘤部位的药物浓度要高于单载药纳米针，小鼠抗肿瘤实验说明，双载药纳米针的体内抗癌效果要远远好于相同药物剂量下的市售制剂以及单载药纳米针制剂。

关键词：羟基喜树碱 纳米针 甲氨喋呤 靶向 细胞摄取

厦门大学博硕士论文摘要库

Abstract

The cellular uptake of the particles is a paramount factor to the therapeutic effect of drug delivery systems. To facilitate the cellular uptake of the nanoparticles, the concentration of the research has been focused on controlling the size of the particles. Although the effect of size on the cellular uptake into nonphagocytic cells is still far from being fully understood, it is always accepted that the size of nanoparticles might play the most important role in cellular uptake. It was once believed that the upper limit of the size of the nanoparticles that could be internalized into nonphagocytic cells via nonspecific endocytosis was 150 nm. And the uptake of nanoparticles will increase with the particle size of less than 50 nm, and decrease for larger nanoparticles. We notice that all these studies were performed based on spherical nanoparticles, and neglected the influence of the particle shape. This was most likely due to the lack of easy-to-use methods available to control the particle shape. Since the last decade, the non-spherical particle shape have been attracting more and more attentions for their potential use in drug delivery system, due to the great progress made in attaining diversely shaped particles. Although the effect of particle shape on drug delivery has not been thoroughly examined, there have been already evidences showing that many properties of the particles are particle shape dependent.

10-hydroxycamptothecin (HCPT) is a promising broad-spectrum antitumor agent which targets nuclear enzyme topoisomerase I and has achieved remarkable success in the early clinical trials. Nevertheless, the clinical application of HCPT was now largely hampered because of the suboptimal pharmaceutical properties, such as poor solubility, poor stability, short half-life and so on. This would lead to a quite low therapeutic efficiency and a number of side effects to normal tissues such as thrombocytopenia, neutropenia, and diarrhea. Therefore, finding a novel formulation suitable for the delivery of HCPT is of great significance in fully unleashing the potential of HCPT. What's more, the natural HCPT is in the form of rod-shaped crystals, which means that HCPT has the potential of forming nanorods.

厦门大学博硕士论文摘要库

Hence, in this study, we will fabricate a new kind of high-aspect-ratio, pointed-end and HCPT-loaded nanoneedles with sustained drug release. To implement this idea, the anti-solvent co-precipitation of PEG-*b*-PLGA and HCPT will be employed under sonication and ice-bath to control the nucleation of the nanoneedles with nanocrystalline HCPT. In this fabrication, the core of HCPT will be wrapped with PEG-*b*-PLGA as steric stabilizers. *In vitro s*tudies will be then systematically carried out to examine the effect of the HCPT-loaded nanoneedles against cancer cells and normal cells compared with the rod-shaped particles and the nanospheres.

The contents are summarized as follows:

(1) Preparation and characterization of HCPT-loaded nanoneedles. In this work, our aim was to obtain the needle-shaped nanoparticles. For this purpose, influences of different initial conditions, including the ratio of HCPT to PEG-*b*-PLGA, and the power of sonication, were investigated to obtain the optimal formulation condition. The results were as follows: the organic solvents was acetone; the ratio of HCPT to PEG-*b*-PLGA was 1:1; the power of the sonication was 200 W; the sonication time was sonication for 3 s, interval for 3 s and repeated for 150 times in ice-bath. The obtained nanoneedles were characterized by scanning electron microscopy (SEM)/transmission electron microscopy (TEM), dynamic light scattering (DLS), and X-ray diffraction (XRD). Based on these experimental results, it was proposed that HCPT-loaded nanoneedles exhibited well dispersed, fairly uniform size and a pointed-end, needle-like shape with a length of about 5 μm and a width of about 400 nm. The DLS analysis illustrated that the size of the nanoneedles was 356.7 nm, the zeta potential was -10.9 mV and the PDI was 0.063. The drug-loading and entrapment efficiency respectively of nanoneedles were 55.84% and 90.32%. Their release behavior in vitro showed that the nanoneedles could slowly release the drug in 400 h in PBS (pH=7.4).

(2) In vitro cellular uptake and cytotoxicity assay of HCPT-loaded nanoneedles. The cellular uptake test was carried out by using three types of cancer cells (HeLa cells, MG-63 cells, and MCF-7 cells) and one type of normal cells (MC3T3-E1 cells). The rod-shaped nanoparticle with the similar aspect ratio and the

厦门大学博硕士论文摘要库

nanospheres with a much smaller size were used as control. Interestingly, the results of these tests were all the same: the cellular uptake of the nanoneedles was a little stronger than that of the nanorods. And the cellular uptake of the nanospheres was the least in the three types of nanoparticles. We discussed the possible reasons for the phenomenon. We define the angle Φ to roughly illustrate the role of shape in nonphagocytosis uptake. Two vectors ($\bar{M}$ and $\bar{N}$) were used to show the flow directions of the cell membranes. Φ is the angle between $\bar{M}$ and $\bar{N}$. The cell membranes flow easly, when the Φ is small. And the cytotoxicity test indicated that the order of the killing ability was NDs > NRs > NSs, which was in according with the results the cellular uptake test.

(3) The synthesis of the PEG-b-PLGA-MTX conjugate. We conjugate MTX to PEG-b-PLGA via an ester bond. The FTIR spectrum illustrated that the ester bond was exist and the conjugate PEG-b-PLGA-MTX was successfully synthesized. We calculated the percentage of the MTX in the conjugation via ultraviolet spectroscopy.

(4) Preparation and characterization of dual drug loaded nanoneedles. We improved the sonication assisted anti-solvent recrystallization method by using the solvent of acetone-ethanol mixtures and smaller dosages. Both MTX and HCPT loaded nanoneedles with a smaller size were successfully synthesized via the optimized method. The SEM images illustrated that the dual drug loaded nanoneedles exhibited well dispersed, fairly uniform size and a pointed-end, needle-like shape with a length of about 1 μm and a width of about 100 nm. The DLS analysis illustrated that the size of the nanoneedles was 102.6 nm, the zeta potential was -19.3 mV and the PDI was 0.049. The drug-loading and entrapment efficiency of HCPT in the dual drug loaded nanoneedles were 62.56% and 92.43%, respectively. And the drug-loading of MTX was 2.03%. Their release behavior in vitro showed that the dual drug loaded nanoneedles could slowly release both of the drug in 400 h in PBS (pH=7.4).

(5) The in vitro and in vivo studies. The in vitro and in vivo studies were then systematically carried out to examine the effect of the dual drug loaded nanoneedles against cancer cells. The cellular uptake test was carried out with HeLa cells and used HCPT-loaded nanoneedles with the same size as control. The cellular uptake of the dual drug loaded nanoneedles was much faster and higher than that of the HCPT-loaded

厦门大学博硕士论文摘要库

nanoneedles. And this enhancement was disappeared, when the folate receptors on the cell membranes were blocked with folate. This stated that the enhanced cellular uptake of the dual drug loaded nanoneedles was caused by the affinity between MTX and the folate receptors in target cells, which would lead to the receptor-mediated endocytosis. The cytotoxicity test indicated that the killing ability of the dual drug loaded nanoneedles was stronger than that of the individual drugs, the mixture of individual drugs, or the mixture of MTX and the HCPT-loaded nanoneedles. This illustrated the good synergistic effect and targeting property of the dual drug loaded nanoneedles. The in-vivo fluorescence test also stated the good targeting property of the dual drug loaded nanoneedles. The in-vivo antitumor experiment showed the excellent anticancer property and the slight side effect.

Keywords： HCPT ·MTX ·Nanoneedles ·Targeting ·Cellular uptake

厦门大学博硕士论文摘要库

目录

厦门大学博硕士论文摘要库

厦门大学博硕士论文摘要库

厦门大学博硕士论文摘要库

Degree papers are in the “Xiamen University Electronic Theses and Dissertations Database”.

Fulltexts are available in the following ways:

1. If your library is a CALIS member libraries, please log on http://etd.calis.edu.cn/ and submit requests online, or consult the interlibrary loan department in your library.

2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.

厦门大学博硕士论文摘要库