具有高光热转化率和环境pH响应的载药硫化铜纳米粒子

秦玉杰, 韩宇, 靳杭, 高颖, 单晓茜*

上海应用技术大学材料科学与工程学院, 上海 201418

* 联系人, E-mail: shanxiaolian@sit.edu.cn

2019-06-18 收稿, 2019-08-21 修回, 2019-09-29 接受, 2019-10-09 网络版发表

上海市自然科学基金(16ZR1436000)和上海应用技术大学产学研平台(10210Q180024-2016xt024)资助

摘要 采用铜离子与硫离子在溶液中直接反应制备粒径为50~60 nm的硫化铜纳米颗粒(CuS NPs). 在CuS NPs表面修饰了聚多巴胺层, 并利用聚多巴胺(PDA)的非共价键自聚在聚多巴胺层表面负载DOX, 从而得到一种核壳结构纳米粒子——DOX@CuS/PDA(其核为CuS NPs, 壳为担载阿霉素的聚多巴胺). 研究表明, 该硫化铜纳米颗粒具备光热敏感特性, 在功率为1 W/cm²的808 nm近红外光照射下, 光热转化率高达34.2%. 在不同pH下释放的研究结果表明, 在酸性条件下经过72 h体外释放后, DOX@CuS/PDA累积DOX的释放量是其在碱性环境中的6倍, 证明了DOX@CuS/PDA拥有明显的pH刺激响应释放机制. 在体外癌细胞实验结果中, DOX@CuS/PDA可以将光热治疗与化学药物治疗相结合, 癌细胞存活率仅有27%~33%, 有效地提升了肿瘤细胞灭杀效果. 本研究对于光热治疗和化学治疗相结合提供了研究方案, 并且对于吸附载药以及pH响应药物释放研究提供了新认识.

关键词 纳米硫化铜, 聚多巴胺, 近红外光热转化, pH响应

随着肿瘤发病率的不断上升, 对于肿瘤治疗药物的需求愈来愈大^[1-4]. 目前肿瘤药物实际使用效果不够理想的主要原因是肿瘤药物的毒副作用^[5-8]. 例如, 盐酸阿霉素^[9](doxorubicin, DOX)存在着严重心脏毒性, 在杀死肿瘤细胞的同时, 也会对患者的健康组织产生毒害作用, 尤其是心脏. 紫杉醇^[10]被认为是20年以来肿瘤细胞灭杀最好的肿瘤药物, 但是在肿瘤治疗过程中容易引起过敏、心脏毒性、骨髓抑制、血液系统毒性等问题. 功能化的纳米肿瘤药物可以有效地降低肿瘤药物的副作用, 增强肿瘤药物在治疗过程中的效果^[11,12]. 因此, 功能化纳米肿瘤药物的研究已经成为了目前肿瘤药物研究的热点, 在众多肿瘤药物功能化的方法中, 引入纳米粒子实现药物的功能化是目前较为理想的一种方案^[13,14].

聚多巴胺(polydopamine, PDA)是一种内源性含氮

有机化合物, 由于表面存在大量羟基基团, 可以提升药物的亲水性能和生物相容性^[15,16]. 2007年, 美国西北大学Messersmith研究组^[17]通过实验发现: 多巴胺(dopamine, DA)在弱碱性的环境下可以发生自聚行为, 这种自聚产物可以附着在绝大多数固体材料的表面, 反应过程中同时存在着化学反应和非共价键自聚的物理反应. 正是由于PDA优良的吸附性能和高生物活性, 在肿瘤药物应用中有巨大的潜力.

光热治疗(photothermal therapy, PTT)在癌症治疗中的研究已经得到了广泛关注^[18,19]. 其中常作为催化剂的硫化铜纳米粒子由于在近红外光的照射下, 会发生表面等离子共振实现高效的光热转化, 因此在肿瘤的光热治疗中被广泛应用^[20-22]. 2015年, 中国科学院理化技术研究所、美国加州大学洛杉矶分校和意大利理工学院合作研究后发现^[23]: 硫化铜纳米颗粒(copper

引用格式: 秦玉杰, 韩宇, 靳杭, 等. 具有高光热转化率和环境pH响应的载药硫化铜纳米粒子. 科学通报, 2020, 65: 203-212

Qin Y J, Han Y, Jin H, et al. Drug-loaded copper sulfide nanoparticles with high photothermal conversion and pH-stimuli response for drug delivery (in Chinese). Chin Sci Bull, 2020, 65: 203-212, doi: 10.1360/TB-2019-0214

sulfide nanoparticles, CuS NPs)可以实现光热治疗和光动力治疗(photodynamic therapy, PDT). 在808 nm近红外光的照射下, 纳米硫化铜能够发生表面等离子体共振, 产生热量和活性氧簇有效地杀死肿瘤细胞, 显著减小小鼠体内的肿瘤体积^[24-26].

将CuS NPs与PDA相结合, 利用聚多巴胺非共价键吸附药物DOX进行了研究, 制备出能够同时实现近红外光热治疗和pH刺激响应释放为一体的纳米载药粒子. 利用硫离子和铜离子直接合成法, 制得了粒径均一、光热转化率高的球形纳米硫化铜粒子. 相比于传统的纳米硫化铜合成^[27], 反应中不使用有机溶剂, 更绿色环保. 同时, 采用X射线衍射仪(XRD)、透射电子显微镜(TEM)、红外光谱和紫外光谱等对粒子的理化性能进行了表征. 通过近红外光热实验和药物体外释放实验, 验证了合成药物的光热转化性能和pH刺激响应机制. 此外, 发现PDA表面吸附DOX后, 可以在pH刺激下实现药物控制释放.

1 实验

(i) 合成. 实验所用药品(纯度 $\geq 99.9\%$)购自上海阿拉丁生化科技有限公司, 所有药品均直接使用. CuS NPs的合成步骤如下: (1) 将5 g聚乙二醇(平均分子量500)和5 mmol的二水氯化铜溶于50 mL的去离子水中, 充分搅拌至澄清透明. (2) 将50 mmol的九水硫化钠溶于200 mL的去离子水中, 得到硫化钠的水溶液. (3) 在磁力搅拌下缓慢地将硫化钠水溶液加入到氯化铜水溶液中, 持续反应30 min. (4) 将反应液放入45°C的烘箱中温育5 h. (5) 离心和乙醇洗涤后获得CuS NPs, 在50°C烘箱中烘干过夜.

(ii) CuS NPs表面PDA修饰载药. (1) 称取20 mg合成的CuS NPs, 超声分散在10 mL的Tris-HCl缓冲溶液中. (2) 称取10 mg的DA加入到溶液中, 搅拌溶解后, 放入50°C油浴锅中, 磁力搅拌反应4 h. (3) 室温冷却后, 在10000 r/min的转速下离心分离, 将得到的聚多巴胺修饰的纳米粒硫化铜离子(CuS/PDA)重新分散于pH为8.0的磷酸盐缓冲溶液中. (4) 加入20 mg的DOX, 常温下避光搅拌24 h后, 在10000 r/min的转速下离心分离, 磷酸盐缓冲溶液洗涤3次, 得到聚多巴胺修饰的硫化铜载药粒子DOX@CuS/PDA. 上层溶液稀释20倍后, 采用UV-745N紫外-可见分光光度计在490 nm处以磷酸盐缓冲溶液作为参比样测试吸光度, 计算载药量.

(iii) 红外光谱测试. 样品和干燥的溴化钾放入研

钵中研磨粉碎后, 压片制样, 使用IRAFFINITY-1傅里叶变换红外光谱仪测试. 通过红外特征峰的对比验证实验方案的可行性和正确性.

(iv) 粉末X射线衍射(XRD). 实验制得的CuS NPs经过研磨后用于粉末XRD测试, 衍射仪为D/max 2200PC X射线衍射仪, 实验温度为298 K, 扫描范围为0°~70°, 测角仪精度 2θ 为0.002°.

(v) 纳米粒子的TEM测试. 实验制得的反应产物超声分散在去离子水中, 配成浓度适当的纳米粒子溶液, 滴在铜网上, 并在射灯下烘干. TEM为美国FEI公司生产, 型号为Tecnai G2 20 TWIN. 加速电压为200 kV, 点分辨率为0.27 nm, 线分辨率为0.144 nm. TEM结果可以清楚地看到CuS NPs和DOX@CuS/PDA的形貌结构.

(vi) 近红外光热测试. 实验制得的CuS NPs和DOX@CuS/PDA通过超声分散制得浓度为200 $\mu\text{g/mL}$ 的水溶液. 取200 μL 滴在96孔板中, 使用型号为MDL-N-808的光分析仪用808 nm近红外光照射溶液, 并使用FLIR AX5 SERIES红外相机记录整个升温过程. 通过软件FLIR Tools+导出数据, 经过Origin 2018软件拟合计算光热转化率.

(vii) DOX标准曲线建立. 用电子天平(GL124-1SCN)称取适量DOX(精确至0.0001 g), 配置成一定浓度的磷酸盐缓冲溶液, 并按照体积进行稀释, 得到不同浓度的DOX溶液(浓度分别为: 0.03925, 0.01963, 0.003925, 0.001963和0.0003925 mg/mL). 用型号为UV-745N紫外-可见分光光度计在490 nm处以磷酸盐缓冲溶液作为参比样测试吸光度, 使用Origin 2018软件进行拟合, 得到DOX浓度和吸光度之间的关系.

(viii) 体外释放实验. 首先配置磷酸盐缓冲溶液: 0.24 g磷酸二氢钾、1.42 g磷酸氢二钠、8.0 g氯化钠和0.2 g氯化钾溶解在去离子水中, 并用1 L的容量瓶定容. 用0.1 mol/L的氢氧化钠和0.1 mol/L的盐酸溶液分别调节磷酸盐缓冲溶液的pH, 得到4种缓冲溶液, pH分别为5.0, 6.0, 7.4和8.0. 取4份5 mg的DOX@CuS/PDA粒子分别超声分散于5 mL四种不同pH磷酸盐缓冲溶液中, 装入透析袋密封后浸入对应的80 mL磷酸盐缓冲溶液中. 固定在型号为THZ-100的恒温摇床上, 在转速100 r/min, 温度37°C下进行药物体外释放实验. 在设定时间点取10 mL样品, 用紫外-可见分光光度计在490 nm处以相同pH磷酸盐缓冲液为参比样测试样品吸光度. 每次取完样后, 将烧杯中溶液全部倒出, 并补充等量新鲜的磷酸盐缓冲溶液.

(ix) 抗癌细胞实验. 将HaLa细胞接种到96孔板中, 并在5% CO₂, 37°C细胞培养箱中孵育24 h. 将细胞分成2份, 每份分成7组, 分别是Control, NIR, CuS NPs, DOX, CuS NPs+NIR, DOX@CuS/PDA和DOX@CuS/PDA+NIR. 每组设置5个重复, 共同孵育5 h后, 使用808 nm的近红外光以1 W/cm²的功率照射NIR组4 min, 随后继续培养19 h. 将未进入细胞内的材料用磷酸盐缓冲溶液洗涤3次, 收集细胞并分散在200 μ L的磷酸盐缓冲溶液中. 活细胞的数量采用MTT比色法检测^[28], 以Control组作为对照组.

2 结果与讨论

2.1 XRD

采用XRD对所制备的样品成分进行了分析. 如图1所示, 图谱中出现了8个不同的衍射峰, 位于 10.81°, 27.68°, 29.28°, 31.78°, 32.85°, 47.94°, 52.71°和59.35°, 分别对应(002), (101), (102), (103), (006), (110), (108)和(116)晶面, 根据标准卡片(JCPDS No. 06-0464)可知样品是硫化铜. XRD图谱中样品的峰较为尖锐, 表明得到的CuS NPs结晶度较高. 同时, XRD图谱没有发现杂质峰, 表明所制备的CuS NPs纯度较高.

2.2 红外测试结果

通过傅里叶变换红外光谱, 分析所合成的DOX@-CuS/PDA. 图2中, DOX@CuS/PDA在1600 cm⁻¹处有芳环振动峰. 在700~800 cm⁻¹之间, DOX@CuS/PDA比DA的振动强度有所降低, 这主要是由于DA聚合为PDA过程中, 芳环被取代、芳烃数量减少所引起的. 另外, 在DOX@CuS/PDA红外图谱中, 1250 cm⁻¹处出现了DOX中特有的-C-O-C特征峰, 可以证明DOX被吸附在CuS/PDA表面.

2.3 微观结构

采用TEM对CuS NPs和DOX@CuS/PDA的微观形貌进行了观察, TEM结果如图3所示. 图3(a)表明, CuS NPs分布均匀没有发生团聚, 以单层蜂窝状排列, 粒子形状为均匀球形, 其粒径约50~60 nm, 其中CuS NPs表面少量的有机物为制备过程中的聚乙二醇. 从图3(b)可知, DOX@CuS/PDA的粒径约80~100 nm, 相比于图3(a), CuS NPs表面明显有一层有机物, 可以推断这层有机物是PDA和DOX的复合物. TEM测试结果证明, 在聚

图1 CuS NPs的XRD图谱
Figure 1 XRD spectra of CuS NPs

图2 (网络版彩色)DOX(a), DA(b)和DOX@CuS/PDA(c)的红外光谱图
Figure 2 (Color online) Infrared spectra of DOX (a), DA (b) and DOX@CuS/PDA (c)

图3 两种纳米粒子的微观形貌. (a) CuS NPs; (b) DOX@CuS/PDA
Figure 3 The TEM images of nanoparticles. (a) CuS NPs; (b) DOX@CuS/PDA

乙二醇中通过硫离子与铜离子直接合成硫化铜的方法可以得到形貌均一、分散均匀的球状纳米硫化铜粒子. 在DOX@CuS/PAD表面的半透明有机层结合红外分析

证明了PDA成功地包裹在CuS NPs表面。

2.4 近红外光测试结果

图4(a)为100 $\mu\text{g/mL}$ DOX@CuS/PDA的紫外-可见吸收光谱, 在800~900和950~1000 nm波长内拥有较高的吸收率。本实验选择808 nm的近红外光作为光源, 主要考虑了以下原因: (1) 在800~900和950~1000 nm两段波长中, 800~900 nm的波长比更短, 穿透力更强, 能量密度更高; (2) 近红外光已经在理疗中得到使用, 未见有致癌、致畸现象, 更安全可靠; (3) 808 nm近红外光源在其他行业已经得到充分发展, 光源设备的可靠性高。光热实验装置如图4(b)所示, 激光从正上方照射96孔板中的溶液, 通过正下方的红外摄像机拍摄整个升温过程, 随后用软件导出时间和温度数据, 最终得到时间和温度曲线。图4(c)~(e)分别是空白对照样(去离子水), 纳米硫化铜溶液和DOX@CuS/PDA溶液升温过程的热成像仪截图, 所测定的样品升温曲线如图5所示。

从图5(a)中的温度变化曲线可见, 200 μL 的CuS NPs水溶液和DOX@CuS/PDA水溶液在波长为808 nm, 功率为1 W/cm^2 的近红外照射下迅速升温。CuS NPs水溶液在120 s照射后, 温度达到54°C, 升高了27°C。DOX@CuS/PDA水溶液照射120 s后, 从27°C升高至41.4°C; PDA水溶液在120 s照射后从25.9°C升高至28.4°C, 升高了2.5°C; 而去离子水在同样条件下, 120 s内温度几乎没有发生变化。通过式(3)和(4)以及图5(b)的拟合曲线可知, 系统向环境的传热系数 $\tau_s=241.559 \text{ s}$, 将 τ_s 带入式(2), 计算得到系统向环境中传导的能量。样品质量为0.2 g, 808 nm近红外激光功率为1 W/cm^2 , 96

孔板单孔的面积为0.32 cm^2 , 因此有效功率 $I=0.32 \text{ W}$ 。最后, 根据式(1), 计算得出CuS NPs的光热转化率为34.2%, DOX@CuS/PDA的光热转化效率为19.3%, PDA的光热转化率为3.3%。光热转化效率计算公式^[29]如下所示:

$$\eta = \frac{hs(T_{\max} - T_{\text{surr}}) - Q}{I(1 - 10^{-A_{808}})}, \quad (1)$$

$$hs = \frac{m_D c_D}{\tau_s}, \quad (2)$$

$$\theta = \frac{T - T_{\text{surr}}}{T_{\max} - T_{\text{surr}}}, \quad (3)$$

$$t = -\tau_s \ln \theta, \quad (4)$$

其中, h 和 s 是传热系数和容器表面积, m_D 是样品的质量, C_D 是水的比热容, $T_{\max}$ 是升温过程的最高温度, T_{surr} 是环境温度, Q 是水吸收的热量, I 是有效激光功率, η 是转化效率。

图5(c)的5次重复升温-降温曲线结果表明, CuS NPs的每个升温-降温的循环表现几乎相同, 且没有明显的性能劣化, 证明了CuS NPs具有稳定的近红外光热性质。从以上得到的数据结果可以确定CuS NPs拥有非常优秀的光热转化能力。

2.5 标准曲线、载药量和体外释放结果

为验证DOX@CuS/PDA的pH响应释放能力, 本研究进行了体外药物释放实验。如图6(a)所示, 将吸光度和DOX浓度进行线性拟合后, R^2 值高达0.99992, 拟合得到DOX浓度和吸光度之间的函数关系为 $Y=0.05108X-0.0003$ 。为了计算CuS/PDA粒子的载药量, 载药离心后

图4 近红外光热测试。(a) DOX@CuS/PDA的紫外-可见吸收光谱。(b) 近红外测试装置图。去离子水(c), CuS NPs(d)和DOX@CuS/PDA(e)的红外热成像图

Figure 4 Near-infrared photothermal test. (a) UV-visible absorption spectrum of DOX@CuS/PDA. (b) Near-infrared test device diagram. The infrared thermal imaging of deionized water for Control (c), CuS NPs (d), and DOX@CuS/PDA (e)

图5 (网络版彩色)近红外光热转化结果。(a) 近红外照射下的样品升温曲线;(b) 通过冷却区间拟合得到传热系数 $\tau_s=241.559$ s; (c) 200 µg/mL 硫化铜纳米粒子溶液5次升温-降温曲线

Figure 5 (Color online) Near-infrared photothermal conversion. (a) Temperature curve under near infrared illumination. (b) Heat transfer constant from the system is determined to be $\tau_s=241.559$ s from cooling period. (c) Temperature evolution of CuS NPs during 5 heating-cooling cycles

图6 (网络版彩色)药物释放曲线。(a) DOX的标准曲线;(b) DOX@CuS/PDA不同pH下的累积释放曲线

Figure 6 (Color online) Drug release curve. (a) Standard curve of DOX. (b) Release cumulative of DOX@CuS/PDA at different pH

的上层溶液被稀释20倍后,在490 nm波长下进行了紫外吸光度测量,吸光度为0.703。根据标准曲线计算出浓度后,得到载药量为19.1%。图6(b)的累积释放曲线表明,在4种pH下,72 h药物释放量有着明显差异。其中,在pH 5.0的磷酸盐缓冲溶液中,72 h的累积释药量高达90%;pH 6.0的磷酸盐缓冲溶液中72 h累积释放量为45%;而在pH 7.4的弱碱性环境中,72 h累积释放量仅

有15%。累积释放结果证明了DOX@CuS/PDA能够在弱酸条件刺激下迅速地释放DOX。PDA表面的非共价键吸附载药的pH响应释放,主要是因为聚多巴胺形成过程中存在非共价键自聚过程。

如图7所示,通过分析聚多巴胺的合成反应过程,研究了其pH响应的原理。在聚多巴胺合成过程中,dopamine和leukodopamine-chrome通过非共价键自聚成

图 7 (网络版彩色)聚多巴胺合成化学方程式^[30]和DOX吸附原理
Figure 7 (Color online) Reaction schematic of PDA^[30] and adsorption principle of DOX

(dopamine)₂/DHI physical trimer, 其可以与dopamine DHI-DHI trimeric conjugate发生非共价键聚合得到PDA^[31,32]. 在弱碱性溶液中, (dopamine)₂/DHI physical trimer可以通过非共价键吸附dopamine DHI-DHI trimeric conjugate, 形成PDA分子. 通过对比DOX结构式和PDA反应过程中的分子结构式可以发现, 两者之间存在很多相似点: 例如类似的苯环平面结构, 存在大量的羟基, 含有氨基基团. 因此, 在弱碱性环境中, PDA可以以非共价键自聚的方式将DOX吸附在表面上, 从而达到载药目的. 而在弱酸性条件则发生逆反应过程, DOX从PDA的表面逐渐地脱落, 因此在弱酸性条件下DOX@CuS/PDA药物释放速度会更快, 药物释放量也会更大.

2.6 体外癌细胞实验

通过肿瘤细胞实验测试了样品对肿瘤细胞的灭杀效率. 图8中DOX组与CuS NPs+NIR组的实际浓度按照DOX@CuS/PDA中含有的DOX, CuS NPs进行了等量换算. 由图8可知, CuS NPs组与NIR组的癌细胞数量并没有出现明显的细胞死亡或增殖, 证明了CuS NPs和单独近红外照射对细胞并没有抑制效果. 通过CuS NPs组与CuS NPs+NIR组HaLa细胞存活率的对比可知, CuS NPs+NIR组的细胞存活率比CuS NPs组降低了约20%, 表明CuS NPs结合近红外光照有一定的瘤细胞抑制作用. DOX组在50 µg/mL浓度下的癌细胞存活率为80%, 在100 µg/mL浓度下的癌细胞存活率为75%; 相同浓度下, CuS NPs+NIR组癌细胞存活率分别为76%和66%, DOX@CuS/PDA+NIR组癌细胞存活率分别为34%和27%, 癌细胞存活率远小于DOX组与CuS NPs+NIR组, 证明了光热治疗与化学治疗相结合明显提升了对癌细胞的抑制效果. 为了系统地验证光热治疗和化学治疗之间是否存在协同作用, 进行了显著性差异分析和协同系数的计算:

$$\frac{f_a}{f_b} = \left(\frac{D}{IC_{50}} \right)^m, \quad (5)$$

$$\log\left(\frac{f_a}{f_b}\right) = m\log(D) + m\log(IC_{50}), \quad (6)$$

$$CI = \frac{D_{A+B}}{D_A} + \frac{D_{A+B}}{D_B}, \quad (7)$$

其中, f_a 是一种药物与细胞作用后的抑制率, f_b 是生存率, IC_{50} 为半致死量, D 为药物浓度, CI 为协同系数,

图8 癌细胞存活率图. *, $P<0.1$; **, $P<0.01$; ***, $P<0.001$

Figure 8 Survival rate of cancer cell. *, $P<0.1$; **, $P<0.01$; ***, $P<0.001$

D_{A+B} 为组合药物浓度^[33,34]. 通过设立标准线性方程和实验结果的拟合, 分别求出DOX组、CuS NPs+NIR组和DOX@CuS/PDA+NIR组对应的 m 值. 设定抑制率为50%时, 求出DOX组、CuS NPs+NIR组和DOX@CuS/PDA+NIR组对应的抑制浓度 D . 通过式(5)计算得到协同系数 CI . $CI<0.1$ 时, 说明2种治疗方法具有超强协同作用; $0.1<CI<0.3$ 时, 2种治疗方法具有强协同作用; $0.3<CI<0.7$ 时, 2种治疗方法具有协同作用; $0.7<CI<0.9$ 时, 则具有轻微协同作用; $CI>1$ 时为拮抗作用. 通过计算得到, 本研究体系 CI 为0.06175, 具有超强协同作用, 说明了光热治疗和化学治疗组合使用时具有良好的协同作用. 综上所述, DOX@CuS/PDA是一种潜在的将光热治疗/化学治疗相结合的纳米载药粒子, 在肿瘤治疗中有着广阔应用前景.

3 总结

本工作主要研究了一种可以实现光热转化和pH响应药物释放相结合的载药纳米粒子DOX@CuS/PDA. 样品中的CuS NPs在808 nm近红外光照射下, 将光能转化成热能, 利用热效应杀死肿瘤细胞. 同时, 研究发现DOX@CuS/PDA在弱酸环境下, 具有较高的药物释放速度和累积释放量. 进一步研究了载体表面非共价键吸附和pH响应控制释放机理, 为纳米载药系统提供了新思路. 在体外癌细胞实验中, DOX@CuS/PDA+NIR可以将光热治疗与化学治疗相互结合, 显著地抑制了癌细胞的生长. 与单一光热治疗或单一化学治疗相比

(癌细胞存活率为60%~70%), 光热治疗与化学治疗相结合的癌细胞存活率只有27%~34%, 有效地提升了药物肿瘤灭杀效果. DOX@CuS/PDA为光热治疗与化学治疗相结合的研究和应用提供了一种研究途径.

致谢 感谢上海交通大学附属第九人民医院临床研究中心提供实验器材和相关测试的帮助.

参考文献

- Chen W, Sun K, Zheng R, et al. Cancer incidence and mortality in China, 2014. *Chin J Cancer Res*, 2018, 30: 1–12
- Bagherabad B M, Afzaljavan F, ShahidSales S, et al. Targeted therapies in pancreatic cancer: Promises and failures. *J Cell Biochem*, 2019, 120: 2726–2741
- Bahreyni A, Ghorbani E, Fuji H, et al. Therapeutic potency of oncolytic virotherapy-induced cancer stem cells targeting in brain tumors, current status, and perspectives. *J Cell Biochem*, 2019, 120: 2766–2773
- Ebrahimi S, Ghorbani E, Shafiee M, et al. Therapeutic potency of oncolytic virotherapy in breast cancer targeting, current status and perspective. *J Cell Biochem*, 2019, 120: 2801–2809
- Bruix J, Qin S, Merle P, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (resorce): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*, 2017, 389: 56–66
- Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: Atlas, a randomised trial. *Lancet*, 2013, 381: 805–816
- Grothey A, Cutsem E V, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (correct): An international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*, 2013, 381: 303–312
- Mehanna H, Robinson M, Hartley A, et al. Radiotherapy plus cisplatin or cetuximab in low-risk human papillomavirus-positive oropharyngeal cancer (De-ESCALaTe HPV): An open-label randomised controlled phase 3 trial. *Lancet*, 2019, 393: 51–60
- Tap W D, Jones R L, Van Tine B A, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: An open-label phase 1b and randomised phase 2 trial. *Lancet*, 2016, 388: 488–497
- Sikov W M, Berry D A, Perou C M, et al. Olaratumab and doxorubicin versus doxorubicin alone for treatment of soft-tissue sarcoma: An open-label phase 1b and randomised phase 2 trial. *J Clin Oncol*, 2015, 33: 13–21
- Jafari S, Soleimani M, Salehi R. Nanotechnology-based combinational drug delivery systems for breast cancer treatment. *Int J Polym Mater Polym Biomater*, 2018, 68: 859–869
- Szuplewska A, Rekorajaska J A, Poczanska E, et al. Magnetic field-assisted selective delivery of doxorubicin to cancer cells using magnetoliposomes as drug nanocarriers. *Nanotechnology*, 2019, doi: 10.1088/1361-6528/ab1019d1083
- Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: Drug nanocarriers, the future of chemotherapy. *Eur J Pharm Biopharm*, 2015, 93: 52–79
- Chang L S, Barroso-Sousa R, Tolaney S M, et al. Endocrine toxicity of cancer immunotherapy targeting immune checkpoints. *Endocr Rev*, 2019, 40: 17–65
- Cheng W, Liang C, Xu L, et al. Tpgs-functionalized polydopamine-modified mesoporous silica as drug nanocarriers for enhanced lung cancer chemotherapy against multidrug resistance. *Small*, 2017, 13: 1700623
- Yang H C, Luo J, Lv Y, et al. Surface engineering of polymer membranes via mussel-inspired chemistry. *J Membr Sci*, 2015, 483: 42–59
- Lee H, Dellatore S M, Miller W M, et al. Mussel-inspired surface chemistry for multifunctional coatings. *Science*, 2007, 318: 426–430
- Tong C, Zhang X, Fan J, et al. PEGylated mBPEI-rGO nanocomposites facilitate hepatocarcinoma treatment combining photothermal therapy and chemotherapy. *Sci Bull*, 2018, 63: 935–946
- Deng L, Xu Y, Sun C, et al. Functionalization of small black phosphorus nanoparticles for targeted imaging and photothermal therapy of cancer. *Sci Bull*, 2018, 63: 917–924
- Li B, Wang Q, Zou R, et al. Cu₇S₄ nanocrystals: A novel photothermal agent with a 56.7% photothermal conversion efficiency for photothermal therapy of cancer cells. *Nanoscale*, 2014, 6: 3274–3282
- Zha Z, Zhang S, Deng Z, et al. Enzyme-responsive copper sulphide nanoparticles for combined photoacoustic imaging, tumor-selective chemotherapy and photothermal therapy. *Chem Commun*, 2013, 49: 3455–3457
- Zhang S, Zha Z, Yue X, et al. Gadolinium-chelate functionalized copper sulphide as a nanotheranostic agent for MR imaging and photothermal destruction of cancer cells. *Chem Commun*, 2013, 49: 6776–6778
- Wang S, Riedinger A, Li H, et al. Plasmonic copper sulfide nanocrystals exhibiting near-infrared photothermal and photodynamic therapeutic effects. *ACS Nano*, 2015, 9: 1788–1800

- 24 Ding X, Liow C H, Zhang M, et al. Surface plasmon resonance enhanced light absorption and photothermal therapy in the second near-infrared window. *J Am Chem Soc*, 2014, 136: 15684–15693
- 25 Xie Y, Carbone L, Nobile C, et al. Metallic-like stoichiometric copper sulfide nanocrystals: Phase- and shape-selective synthesis, near-infrared surface plasmon resonance properties, and their modeling. *ACS Nano*, 2013, 7: 7352–7369
- 26 Yang T, Wang Y, Ke H, et al. Protein-nanoreactor-assisted synthesis of semiconductor nanocrystals for efficient cancer theranostics. *Adv Mater*, 2016, 28: 5923–5930
- 27 Liu X, Wang X, Zhou B, et al. Size-controlled synthesis of Cu_{2-x}E (E = S, Se) nanocrystals with strong tunable near-infrared localized surface plasmon resonance and high conductivity in thin films. *Adv Funct Mater*, 2013, 23: 1256–1264
- 28 Chen W, Zhong P, Meng F, et al. Redox and pH-responsive degradable micelles for dually activated intracellular anticancer drug release. *J Control Release*, 2013, 169: 171–179
- 29 He J, Ai L, Liu X, et al. Plasmonic CuS nanodisk assembly based composite nanocapsules for NIR-laser-driven synergistic chemo-photothermal cancer therapy. *J Mater Chem B*, 2018, 6: 1035–1043
- 30 Hong S, Na Y S, Choi S, et al. Non-covalent self-assembly and covalent polymerization co-contribute to polydopamine formation. *Adv Funct Mater*, 2012, 22: 4711–4717
- 31 Dreyer D R, Miller D J, Freeman B D, et al. Elucidating the structure of poly(dopamine). *Langmuir*, 2012, 28: 6428–6435
- 32 Liu L, Shao B, Yang F. Polydopamine coating—Surface modification of polyester filter and fouling reduction. *Sep Purif Technol*, 2013, 118: 226–233
- 33 Miao W, Shim G, Lee S, et al. Safety and tumor tissue accumulation of PEGylated graphene oxide nanosheets for co-delivery of anticancer drug and photosensitizer. *Biomaterials*, 2013, 34: 3402–3410
- 34 Park B J, Whichard Z L, Corey S J. Dasatinib synergizes with both cytotoxic and signal transduction inhibitors in heterogeneous breast cancer cell lines—Lessons for design of combination targeted therapy. *Cancer Lett*, 2012, 320: 104–110

Summary for “具有高光热转化率和环境pH响应的载药硫化铜纳米粒子”

Drug-loaded copper sulfide nanoparticles with high photothermal conversion and pH-stimuli response for drug delivery

Yujie Qin, Yu Han, Hang Jin, Ying Gao & Xiaoqian Shan*

School of Materials Science and Engineering, Shanghai Institute of Technology, Shanghai 201418, China

* Corresponding author, E-mail: shanxiaoqian@sit.edu.cn

In recent years, the incidence of malignant tumors is increasing year by year. Therefore, new antitumor drugs and treatment modalities are urgently needed. While, the biggest challenge to oncology drugs is to treat tumors in a safe and effective manner. Currently, most of the cancer patients in treatment are suffering due to side effects of oncology drugs. In order to reduce negative effects of tumor drugs, formulations of multifunctional nanocarriers have attracted attention because of their significant biological activity without damage to normal cells. These include formulations such as drug-loaded silica nanoparticles, photothermal treatment of gold nanoparticles, photodynamic therapy of copper sulfide nanoparticles and other multifunctional nanocomposites.

In this study, a novel kind of high photothermal conversion CuS NPs as a pH-sensitive drug release system is going to be introduced. Firstly, CuS NPs with a uniform particle size was prepared by copper ions and sulfur ions in solution. Secondly, the PDA was modified on the surface of CuS NPs and DOX was adsorbed on the outside of PDA via non-covalent self-assembly. Finally, a drug-loaded core-shell nanoparticle DOX@CuS/PDA was obtained, with the core of CuS NPs and the shell of PDA. Further studies on DOX@CuS/PDA were performed to verify its anti-cancer effect.

Surface morphology of DOX@CuS/PDA was observed by transport electronic microscope (TEM). TEM images show that DOX@CuS/PDA is uniform with a size of 70 ± 10 nm and appears as a spherical shape with a CuS NPs core. After comprehensive consideration of absorbance test, wavelength penetration capability and equipment, 808 nm near-infrared light was considered to be the most suitable light source. The 200 $\mu\text{g/mL}$ of CuS NPs solution was heated to 54°C in 120 s under the irradiation of 808 nm near-infrared light with a power of 1 W/cm^2 , and the photothermal conversion rate was as high as 34.2%. The temperature of DOX@CuS/PDA was raised to 41.4°C in the same condition. *In vitro* release assay, the cumulative release of DOX at 72 h in the environment of pH 5.0 was 6 times higher than that of pH 7.4, which proves that DOX@CuS/PDA possesses a significant pH-stimulation response mechanism. Interestingly, DOX is adsorbed on the surface of the PDA through non-covalent self-assembly. When DOX@CuS/PDA stimulated by weak acid environment, this state of non-covalent self-assembly is undermined and the DOX is released. The mechanism of drug loading and pH response release was introduced in details. In the cancer cell experiments of DOX@CuS/PDA *in vitro*, the survival rate of cancer cells is 27%–33%, which effectively enhances the killing effect of tumor cells. It shows that the combination of advertising heat therapy and chemotherapy has good synergy, through the calculation of coordination coefficient.

CuS NPs converts light energy into heat energy under 808 nm near-infrared lights to kill tumor cells by thermal effect and the DOX in DOX@CuS/PDA will accelerate release in a weak acid environment of tumor site. Tumor cells can be effectively killed by combining photothermal therapy with drug therapy. From the above, a novel research is put forward in this study for the combination of photothermal therapy and chemotherapy and provides a new understanding of drug-loaded by adsorption and pH-stimuli response.

copper sulfide nanoparticles, polydopamine, near-infrared photothermal conversion, pH-stimuli response

doi: [10.1360/TB-2019-0214](https://doi.org/10.1360/TB-2019-0214)