刺激响应性金属-多酚胶囊的可控组装及其在药物递送中的应用

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摘要 微胶囊是一类由天然或合成壁材构筑的具有中空结构的微型容器. 通过金属-多酚网络(metal-phenolic networks, MPN)自组装制备的功能微胶囊, 因其具有pH响应性、荧光成像、正电子发射断层扫描成像等特性, 在化学和生物医学等交叉研究领域引起了广泛关注. 本研究以沸石咪唑酯骨架材料(zeolite imidazolate framework-8, ZIF-8)为模板, 制备了pH响应性的MPN胶囊, 并研究了其细胞毒性和细胞相互作用. 通过改变ZIF-8模板的尺寸, 可以在200 nm~1 μ m范围内调控MPN胶囊的大小; 由于ZIF-8纳米颗粒可以利用乙二胺四乙酸(EDTA)溶解, 因此, 在MPN制备过程中可以在温和的条件下去除模板; 调控单宁酸和 Fe^{3+} 浓度及反应时间来控制胶囊壁厚. 该胶囊具有良好的pH响应性, 并能有效封装和递送抗肿瘤药物阿霉素, 在纳米药物领域具有潜在的应用价值.

关键词 金属有机框架材料, 金属-多酚网络结构, 胶囊, 刺激响应, 药物递送

随着纳米技术的发展, 癌症的诊断和治疗已经取得了重大的进步, 但其仍然是威胁人类生命健康的疾病之一^[1,2]. 常见的癌症治疗手段有放射治疗、化学治疗、手术治疗等. 但传统的癌症治疗手段存在多药耐药性、易复发和易转移等问题^[3]. 例如, 阿霉素(doxorubicin, DOX)是临床使用的一种化疗药物, 但在体内给药时存在较大的副作用, 会严重影响心脏等器官的正常功能. 因此, 为了提高抗癌药物的治疗效果并减少对人体的毒副作用, 常用的方法是将药物封装在纳米载体中^[4]. 常见的药物载体有脂质体、聚合物颗粒、微胶囊等. 其中, 微胶囊是一类由天然或合成壁材构筑的具有中空结构的微型容器^[5]. 微胶囊具有众多可控的理化性质, 如尺寸、渗透性、刺激响应性等, 可以实现药物的有效封装与释放, 在药物递送、生物成像等领域引起了广泛的关注^[6-8]. 近年来, 科学家开发了不同结

构和功能的微胶囊, 实现了已包封药物的刺激响应性释放^[9]. 其中, 对内源刺激(例如pH、酶、氧化还原作用等)响应的胶囊备受关注.

多酚是一种广泛存在于自然界中多元酚类化合物, 是千百年来人类生活中必不可少的部分^[10]. 多酚在动植物中都起着重要的作用, 例如作为信号分子可实现激素调节、防紫外线、抗菌抗炎、预防有机体氧化以及抑制肿瘤生长等^[11-14]. 多酚具有良好的生物相容性, 是一种构筑生物功能材料的绿色原料. 近年来, 不同多酚类的功能材料(例如薄膜、颗粒、水凝胶等)被成功制备并广泛地用于生物医学等研究领域^[15-18]. 多酚中存在儿茶酚、没食子酰基等结构, 在基于多酚材料的组装中涉及多种共价键反应(例如迈克尔加成、席夫碱反应)和非共价相互作用(例如氢键、配位作用、静电相互作用、疏水作用、 π - π 堆积和阳离子- π 作用等).

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多酚可与多种聚合物、有机小分子及金属离子等发生相互作用^[19-21]。其中,多酚与不同金属离子通过配位作用可以形成金属-多酚网络(metal-phenolic networks, MPN)结构。该过程需要使羟基去质子化,因此pH会影响金属与多酚的络合,较高的pH有利于配位键的形成,较低的pH可以破坏配位作用。墨尔本大学Caruso课题组^[15]利用模板法构筑了具有pH响应性的MPN微胶囊,当胶囊处于酸性环境中时,有利于胶囊的解离从而释放封装的药物。另外,该课题组^[22]建立了一个功能性MPN胶囊库,胶囊可以由不同的多酚(例如邻苯二酚、邻苯三酚、没食子酸等)和不同的金属离子构成,MPN胶囊的特性可以通过选择不同多酚和金属离子来决定,以此控制胶囊壁层的厚度、降解特性和荧光特性等。

合成MPN胶囊可以选用多种模板,例如碳酸钙颗粒、聚苯乙烯微球和乳液等^[22-25]。虽然可供选择的模板众多,但是现有的模板存在很多缺点。例如聚苯乙烯微球需要有机溶剂去除,并且很难去除干净;CaCO₃颗粒虽然可以使用乙二胺四乙酸(EDTA)去除,但是其尺寸通常在微米范围内^[26-28]。因此,模板去除条件温和、粒径可控是选择模板的重要条件。沸石咪唑酯骨架材料(zeolite imidazolate framework-8, ZIF-8)颗粒由Zn²⁺与2-甲基咪唑(2-MIM)配体连接而成,是一种具有四配位结构的方钠石(SOD)拓扑结构的纳米多孔材料^[29]。ZIF-8在水溶液中特别是酸性条件中极不稳定,容易分解,不利于MPN在其表面的组装。在本课题组^[30]前期工作中,利用聚乙二醇(PEG)制备了在水中稳定性较高的ZIF-8纳米颗粒,并实现了药物和蛋白的可控封装,通过改变实验条件可以控制ZIF-8纳米颗粒的尺寸。另外,ZIF-8纳米颗粒可在EDTA溶液中降解。因此,PEG调控的ZIF-8纳米颗粒是制备小尺寸MPN胶囊的最佳选择。

本工作以PEG调控的ZIF-8颗粒为模板,制备了负载抗肿瘤药物DOX的MPN胶囊,并探索了在细胞内进行药物递送方面的应用。如图1所示,我们首先通过PEG介导矿化的方法一步制备了封装DOX的ZIF-8颗

粒(DOX@ZIF-8)。基于金属-多酚配位作用将TA-Fe³⁺络合物涂覆在ZIF-8颗粒表面,然后用EDTA除去模板可以得到封装有DOX的MPN胶囊(DOX@MPN capsules)。并研究了MPN胶囊的刺激响应性释放以及其细胞毒性和细胞相互作用。

1 实验

(i) 试剂与仪器。八臂聚乙二醇羟基(8-arm-PEG-OH, 20 kDa)、聚乙二醇羟基(PEG-OH, 2 kDa)购买于赛诺邦格(厦门);六水合三氯化铁(FeCl₃·6H₂O)、单宁酸(TA)购买于西格玛奥德里奇(上海)贸易有限公司。六水合硝酸锌(Zn(NO₃)₂·6H₂O)、2-MIM、3-吗啡丙磺酸(MOPS)以及盐酸阿霉素(DOX·HCl)购买于阿拉丁试剂(上海)有限公司。一水合柠檬酸钠、磷酸氢二钠以及乙二胺四乙酸(EDTA)均购自国药集团化学试剂有限公司。细胞培养基(DMEM)、磷酸盐缓冲液(DPBS)、胎牛血清(FBS)以及胰蛋白酶均购买于德国Gibco。

透射电子显微镜(TEM, JEOL JEM-1400)表征ZIF-8颗粒和MPN胶囊的形貌;扫描电子显微镜(SEM, Zeiss G300)表征ZIF-8颗粒的形貌;纳米粒度仪(Malvern Zetasizer, Nano ZS90)表征ZIF-8颗粒的分散性、尺寸和Zeta电位;紫外可见分光光度计(UV-Vis, Shimadzu, UV-2600)表征MPN胶囊的降解以及DOX的包封;原位X射线衍射仪(XRD, PANalytical)分析ZIF-8的晶型结构;通过酶标仪(TECAN, SPARK 10M)进行细胞毒性测定;流式细胞仪(ACEA, NovoCyte 3009)用于分析胶囊与细胞之间的相互作用;激光共聚焦显微镜(CLSM, Leica SP8)用于分析药物分子在细胞中分布情况。通过倒置荧光显微镜(Axio Observer 3),观察细胞的荧光图像。

(ii) ZIF-8纳米颗粒的制备。ZIF-8纳米颗粒根据本课题组^[30]之前报道的方法制备。将10 mg的PEG(8-arm-PEG-OH, $M_w = 20$ kDa或PEG-OH, $M_w = 2$ kDa)溶解于2-MIM水溶液(160 mmol/L, 2 mL)中。随后,加入Zn(NO₃)₂·6H₂O溶液(40 mmol/L, 2 mL),混合30 min。将

图1 (网络版彩色)DOX@MPN胶囊的制备过程示意图

Figure 1 (Color online) Scheme of DOX@MPN capsules preparation

得到的ZIF-8(8-arm-PEG-OH@ZIF-8; PEG-OH@ZIF-8)纳米颗粒离心去除上清液,并用Milli-Q水洗涤2遍,甲醇清洗3遍。最后,将其置于2 mL甲醇溶液中保存。通过将ZIF-8纳米颗粒与药物分子共沉淀来合成封装DOX的纳米颗粒。将8-arm-PEG-OH($M_w = 20$ kDa, 10 mg)与DOX(4 mg)加入2-MIM溶液(2 mL, 160 mmol/L)中,待其完全溶解后,加入等体积的 $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 溶液(40 mmol/L, 2 mL)。随后,离心去除上清液,将收集得到的纳米颗粒用Milli-Q水清洗2遍,甲醇清洗3遍。最后,将得到的单分散DOX@ZIF-8纳米颗粒置于2 mL甲醇溶液中保存。制备及保存均在避光条件下进行。

(iii) MPN胶囊的制备。分别向0.5 mL TA溶液(23.5、9.4、4.7和3 mmol/L)中加入1 mL Milli-Q水,随后分别与0.5 mL $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 溶液(37、14.8、7.4和5 mmol/L)(TA: Fe^{3+} 的摩尔比始终保持为1:1.6)混合并使用涡旋混合器混合30 s。将所得的 Fe^{3+} -TA混合液离心(5340 r/min, 5 min),随后用孔径为0.22 μm 的过滤器过滤除去溶液中的沉淀物。取出1 mL(约7 mg)预先制备的ZIF-8纳米颗粒,离心除去上清液,并加入Milli-Q水(300 μL),通过超声涡旋等方式加以分散。在涡旋的条件下,将ZIF-8纳米颗粒分散液加入到纯化的 Fe^{3+} -TA溶液(1 mL)中,持续涡旋2 min。随后,离心去除上清液。使用MOPS缓冲液(2 mL, 20 mmol/L, pH 8)调节pH,并对其进行孵育不同时间(0 min、30 min、12 h和24 h),以使金属离子与酚类分子牢固交联。用MOPS水溶液(2 mL)洗涤颗粒3次,以去除多余的 Fe^{3+} 和TA。加入EDTA水溶液(1.5 mL, 50 mmol/L, pH 8.0)溶解ZIF-8纳米颗粒,并持续涡旋2 min。随后,离心(5960 r/min, 5 min),除去上清液,并用Milli-Q水离心洗涤3遍。最后,将所得到的MPN胶囊分散在MOPS缓冲溶液中保存。DOX@MPN微胶囊的制备与该方法类似。

(iv) MPN胶囊pH响应性的测试。为测试MPN胶囊在不同pH条件下胶囊的降解能力,将等量的MPN胶囊分散到等体积的缓冲溶液中,pH分别为7.4、6.5、5.5、4.5,并将其置于37°C, 4300 r/min的金属震荡浴中震荡3 h。通过UV-vis测试配体-金属电荷转移(LMCT)谱带强度随时间的变化情况,以监测每个样品的分解情况。

取1 mL DOX@MPN胶囊分散液于9 mL不同pH(7.4、4.5)的柠檬酸钠-磷酸氢二钠缓冲溶液中,其中DOX浓度为534 $\mu\text{g/mL}$,在不同时间点(0、0.5、1、2、4、8、12和24 h)取1 mL样品进行离心过滤,并稀释至3 mL,通过紫外-可见吸收光谱检测DOX的释放量。

DOX的紫外吸收峰为480 nm。

(v) MPN胶囊与细胞相互作用实验。将人宫颈癌细胞(HeLa细胞)以每孔 5×10^4 个细胞的密度接种到24孔板中,并置于 CO_2 培养箱中过夜,细胞贴壁。将DOX@MPN胶囊与细胞共培养2、4、6、8 h。孵育后,加入DPBS冲洗细胞2次,随后加入100 μL 胰蛋白酶,消化3 min。补加400 μL DPBS分散细胞并用流式细胞仪进行定量分析。

激光共聚焦小皿中接种 1×10^5 个HeLa细胞,随后培养过夜,细胞贴壁。加入DOX@MPN胶囊与细胞共培养([DOX] = 5 $\mu\text{g/mL}$),孵育时间为2、4、8 h。孵育完成后,移除旧培养基,并加入500 μL DPBS清洗两次。之后加入500 μL 4%的多聚甲醛固定15 min,并用Hoechst 33342(2 $\mu\text{g/mL}$, 500 μL , 20 min)和WGA-AF488(1 $\mu\text{g/mL}$, 500 μL , 6 min)染色剂对细胞核和细胞膜进行染色。最后,通过CLSM观察HeLa细胞对胶囊的摄取情况。荧光显微镜通道: E_x -DOX = 488 nm, PE通道成像; E_x -WGA-AF488 = 495 nm, FITC通道成像; E_x -Hoechst = 358 nm, DAPI通道成像。

(vi) MPN胶囊的细胞毒性测定。使用MTT实验检测胶囊的细胞毒性。96孔板中接种 1×10^4 个HeLa细胞,细胞孵育过夜、贴壁生长。细胞与不同浓度的DOX@MPN以及MPN胶囊于37°C, 5% CO_2 培养箱中分别培养24和48 h, DOX浓度梯度设置为(10、5、2.5、1.25、0.625 $\mu\text{g/mL}$)。随后向每个孔中加入10 μL MTT溶液(5 mg/mL),继续孵育4 h。将上层培养基移除后,每孔加入100 μL DMSO,待甲臞完全溶解后,用酶标仪测试每孔在570 nm处的吸光度。

(vii) 细胞活/死染色实验。24孔板中接种 1×10^5 个HeLa细胞并培养过夜,细胞贴壁。加入不同浓度的MPN胶囊和DOX@MPN胶囊([DOX] = 2.5, 10 $\mu\text{g/mL}$)与细胞共培养24和48 h。之后去除旧培养基,并加入活/死染料对细胞进行染色处理,孵育10 min后,使用倒置荧光显微镜观察样品(碘化丙啶和钙黄绿素的浓度分别为4和2 $\mu\text{mol/L}$)。

2 结果与分析

2.1 ZIF-8纳米颗粒以及MPN胶囊的制备及表征

由SEM(图2(a)~(c))、TEM(图2(d)~(f))以及动态光散射(dynamic light scattering, DLS)数据(图2(g)~(i))可以看出,包封不同分子的ZIF-8纳米颗粒均为正十二面

图2 (网络版彩色)ZIF-8纳米颗粒和MPN胶囊的SEM和TEM图及DLS分布图. 8-arm-PEG-OH@ZIF-8(a, d, g)、PEG-OH@ZIF-8(b, e, h)和DOX@ZIF-8(c, f, i)的SEM(a~c)、TEM(d~f)图片以及粒径分布图(g~i); 8-arm-PEG-OH@MPN(j)、PEG-OH@MPN(k)和DOX@MPN(l)的TEM图片. 内嵌图为颗粒分散液照片

Figure 2 (Color online) The SEM, TEM images and DLS (dynamic light scattering) result of ZIF-8 particles and capsules. SEM (a~c), TEM (d~f) images and the size distribution (g~i) of 8-arm-PEG-OH@ZIF-8 (a, d, g), PEG-OH@ZIF-8 (b, e, h) and DOX@ZIF-8 (c, f, i) particles respectively; TEM images of 8-arm-PEG-OH@MPN (j), PEG-OH@MPN (k) and DOX@MPN (l) capsules, respectively. The insets are the particle suspensions

体结构, 具有良好的分散性, 尺寸均一. 8-arm-PEG-OH@ZIF-8和DOX@ZIF-8颗粒的尺寸在200 nm左右(图2(g), (i)), 而PEG-OH@ZIF-8的尺寸约为1 μm (图2(h)), 表明ZIF-8纳米颗粒的尺寸可以通过改变包封的PEG来进行调控, 而DOX对ZIF-8颗粒尺寸的影响可忽略不计. ZIF-8纳米颗粒的分散液颜色为乳白色(图2(a), (b)内嵌图), 包封DOX的ZIF-8纳米颗粒分散液为红色

(图2(c)内嵌图), 初步表明DOX的成功封装. 如图2(j)~(l)所示, 以ZIF-8为模板, 一步法制备的MPN胶囊可以保持原有模板的尺寸, 而由于干燥过程中失水, 因此胶囊产生褶皱和折痕.

此外, XRD结果(图3(a))表明, 制备所得包封生物分子的ZIF-8颗粒衍射图谱与标准样品的ZIF-8晶体衍射图谱相符, 证明封装PEG或DOX后, ZIF-8的晶体结

图 3 (网络版彩色)ZIF-8纳米颗粒和MPN胶囊的表征。(a) ZIF-8纳米颗粒的XRD谱图。(b) DOX溶液、ZIF-8纳米颗粒、MPN胶囊、DOX@ZIF-8纳米颗粒和DOX@MPN胶囊的紫外-可见吸收光谱。(c) ZIF-8纳米颗粒进行MPN涂覆前后的Zeta电位图(i, 8-arm-PEG-OH@ZIF-8; ii, PEG-OH@ZIF-8; iii, DOX@ZIF-8)。(d) TA溶液(绿色实线)、Fe³⁺溶液(黄色实线)以及MPN溶液(紫色实线)的紫外-可见吸收光谱。紫色区域为多酚配体-金属电荷转移带(570 nm处)

Figure 3 (Color online) Characterization of ZIF-8 particles and MPN capsules. (a) XRD patterns of ZIF-8 particles. (b) UV-Vis spectra of DOX solution, suspensions of ZIF-8 nanoparticles, MPN capsules, DOX@ZIF-8 nanoparticles and DOX@MPN capsules. (c) Zeta potential of ZIF-8 particles before and after MPN coatings (i, 8-arm-PEG-OH@ZIF-8; ii, PEG-OH@ZIF-8; iii, DOX@ZIF-8). (d) UV-Vis spectra of TA solution (solid green line), Fe³⁺ solution (solid yellow line), and MPN capsule suspension (solid purple line) showing the LMCT band around ~570 nm (purple area)

构未被破坏。同时,由紫外-可见光谱(图3(b))可以观察到DOX的特征峰,表明DOX已被成功封装到ZIF-8纳米颗粒中。将ZIF-8纳米颗粒与MPN溶液孵育后,测试颗粒的Zeta电位(图3(c))。涂覆MPN后,颗粒的Zeta电位值由20 mV降低至-35 mV左右,可以表明MPN在纳米颗粒表面的成功涂覆。MPN胶囊的紫外-可见光谱(图3(d))结果显示在570 nm处存在LMCT带,而Fe³⁺溶液和TA溶液的紫外光谱中并不存在,表明MPN溶液中的Fe³⁺和TA之间存在配位的作用。

选取8-arm-PEG-OH@ZIF-8为模板,研究不同浓度的TA与Fe³⁺(图4(a)~(e))以及孵育时间(图4(f)~(j))对胶囊厚度和形貌的影响。首先,固定孵育时间为0.5 h,改变TA及Fe³⁺的浓度,除去模板后得到MPN胶囊。MPN胶囊的尺寸约为200 nm,分散性较好,且膜厚随着TA和Fe³⁺浓度的增加而增大,最厚可达36.01 nm([TA] = 23.5 mmol/L, [Fe³⁺] = 37 mmol/L, 图4(e))。当TA和Fe³⁺浓度分别为4.7和7.4 mmol/L时,制备的MPN胶囊(图4

(c))形貌相对较好,因此随后的实验选用该浓度胶囊进行研究。随后,探究了孵育时间对胶囊膜厚的影响。固定TA和Fe³⁺浓度分别为4.7和7.4 mmol/L,研究不同孵育时间(0、0.5、12和24 h)对胶囊膜厚和形态的影响。如图4(j)所示,随着孵育时间的延长,MPN胶囊的膜厚随之增加,当孵育时长为24 h时,厚度为41.81 nm。

2.2 MPN胶囊的DOX负载量以及pH响应性能

使用紫外-可见吸收光谱对MPN胶囊的DOX负载量以及pH响应性进行研究。通过测量上清液在480 nm处的吸收值,计算MPN胶囊中DOX的负载率(DOX的负载率 = 胶囊中DOX的质量/胶囊质量×100%)以及封装率(DOX的封装率 = 胶囊中DOX的质量/DOX初始质量×100%)。通过计算,胶囊中DOX的负载率为3.77%,封装率为3.91%。如图5(a)显示,当溶液pH降低时,MPN分散液的吸光度随之降低,且在pH 5.5时,LMCT带消失,表明胶囊几乎完全分解。为了进一步验证胶囊

图4 (网络版彩色)不同浓度的TA和Fe³⁺制备得到胶囊的TEM图。(a) [TA] = 23.5 mmol/L, [Fe³⁺] = 37 mmol/L; (b) [TA] = 9.4 mmol/L, [Fe³⁺] = 14.8 mmol/L; (c) [TA] = 4.7 mmol/L, [Fe³⁺] = 7.4 mmol/L; (d) [TA] = 3 mmol/L, [Fe³⁺] = 5 mmol/L; (e) 不同浓度的TA和Fe³⁺制备得到胶囊厚度柱状图。不同沉积时间MPN胶囊的TEM图((f)~(i), 沉积时间分别为0、0.5、12、24 h)以及胶囊厚度柱状图(j)

Figure 4 (Color online) TEM images of the capsules prepared from different concentrations of TA and Fe³⁺. (a) [TA] = 23.5 mmol/L, [Fe³⁺] = 37 mmol/L; (b) [TA] = 9.4 mmol/L, [Fe³⁺] = 14.8 mmol/L; (c) [TA] = 4.7 mmol/L, [Fe³⁺] = 7.4 mmol/L; (d) [TA] = 3 mmol/L, [Fe³⁺] = 5 mmol/L; (e) the thickness histogram of MPN capsules prepared from different concentration TA and Fe³⁺; TEM images of capsules with different incubation time ((f)~(i), incubation times are 0, 0.5, 12 and 24 h respectively); (j) the capsule thickness histogram

图5 (网络版彩色)TA-Fe³⁺胶囊pH响应性。(a) MPN胶囊分散液在不同pH下的紫外-可见吸收光谱; (b) MPN胶囊中DOX随pH变化的释放曲线; (c) TA-Fe³⁺络合作用的pH相应性变化

Figure 5 (Color online) The pH responsiveness of TA-Fe³⁺ capsules. (a) UV-Vis spectra of MPN capsules suspensions at different pH buffer; (b) DOX release profiles from DOX@MPN capsules at different pH buffer; (c) scheme of pH-dependent structure transition of TA-Fe³⁺

的pH响应性, 将DOX@MPN胶囊与不同pH的缓冲溶液共孵育, 并通过紫外-可见吸收光谱监控DOX的释放。如图5(b)所示, 在pH 7.5的条件下, DOX的释放量较少, 且释放速度缓慢; 而在pH 4.5的条件下, DOX释放量较

多, 且释放速度较快。这主要是由于低pH下, 酚羟基被质子化, 从而导致金属与多酚配位交联的不稳定和薄膜的分解, 致使DOX的快速释放。图5(c)从原理上解释了胶囊的pH响应性。

2.3 细胞相互作用与细胞毒性

MPN胶囊具有pH响应性, 因此可用于药物递送. 我们在合成模板时包封了抗癌药物DOX, 制备了DOX@MPN胶囊. 由TEM图(图2(f))可以看出DOX的封装并未影响胶囊的形貌结构与分散性. 紫外-可见吸收光谱也证明了DOX被成功地封装到了MPN胶囊中(图3(b)).

DOX@MPN胶囊(DOX浓度为2.5 $\mu\text{g/mL}$)与HeLa细胞孵育2、4、6、8 h后, 通过流式细胞仪评估胶囊与HeLa细胞的相互作用量. 图6(a), (b)结果显示, 随着孵育时间的延长, HeLa细胞的荧光强度逐渐升高, 对DOX@MPN胶囊的摄取量逐渐增多. 激光共聚焦显微镜数据(图6(c))也证明在2 h时, 细胞内DOX红色荧光较

少; 而随着时间的延长, (4和8 h)HeLa细胞内的DOX荧光信号逐渐增强, 细胞对DOX@MPN胶囊的吞噬效果明显.

随后, 研究了不同浓度的DOX@MPN胶囊对肿瘤细胞的杀伤作用, 采用MTT实验测试了HeLa细胞与胶囊共培养后的细胞存活率. 如图7(a), (b)所示, 与HeLa细胞共孵育24和48 h后, MPN胶囊对细胞存活率的影响较小, 可忽略不计. 将DOX装载于胶囊后, DOX@MPN胶囊对HeLa细胞的致死率更高, 且显示出剂量依赖性以及时间依赖性的细胞毒性. 细胞活死染色实验则用来进一步表征DOX@MPN胶囊对肿瘤细胞的杀伤作用. 从图7(c)的细胞活/死染色实验结果中可以看出, DOX@MPN胶囊组([DOX] = 10 $\mu\text{g/mL}$)的细胞都被标

图6 (网络版彩色)胶囊与细胞相互作用. (a), (b) DOX@MPN胶囊与HeLa细胞相互作用的流式细胞仪结果([DOX] = 2.5 $\mu\text{g/mL}$); (c) DOX@MPN胶囊与HeLa细胞共培养的激光共聚焦照片, 标尺为10 μm

Figure 6 (Color online) Cell association of capsules to HeLa cells. (a), (b) Flow cytometry to demonstrate the interactions between DOX@MPN capsules and HeLa cells; (c) CLSM images of HeLa cells incubated with DOX@MPN capsules at different time scales. Scale bar is 10 μm

图7 (网络版彩色)胶囊的细胞毒性实验. HeLa细胞与MPN胶囊和DOX@MPN胶囊共培养24(a)和48 h(b)后的细胞存活率; (c) 细胞活/死染色实验的激光共聚焦显微镜照片(绿色活细胞由钙黄绿素染色, 红色死细胞由碘化丙啶染色), 标尺为50 µm

Figure 7 (Color online) Cytotoxicity assay of the capsules. HeLa cell viability after incubation with MPN capsules and DOX@MPN capsules for 24 (a) and 48 h (b), respectively; (c) CLSM images show the live/dead assay (green live cells and red dead cells were stained with calcein and propidium iodide respectively). Scale bar is 50 µm

记上红色荧光, 说明细胞已被杀死, 而相对于空白组以及MPN胶囊组来说, 98%细胞都被标记绿色荧光, 表示细胞正常存活, 材料无细胞毒性.

3 总结

综上所述, 本研究以ZIF-8纳米颗粒为模板, 通过TA与Fe³⁺的快速配位制备了MPN胶囊. 所制备的胶囊

分散性良好, 尺寸可控. 通过改变TA和Fe³⁺的浓度以及孵育时间, 可以实现对胶囊膜厚的有效控制. 该胶囊具有较好pH响应性能, 在低pH下可实现药物分子的快速释放, 对于药物在肿瘤细胞的响应释放具有重要意义. 同时, 所制备的MPN胶囊具有良好的生物相容性, 负载药物的MPN胶囊表现出良好的抗肿瘤细胞活性, 在纳米药物传输领域中具有潜在的应用价值.

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Summary for “刺激响应性金属-多酚胶囊的可控组装及其在药物递送中的应用”

Assembly of responsive metal-phenolic network capsules for drug delivery

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Microcapsules are a kind of semipermeable or closed containers prepared by physical and chemical methods from natural or synthetic materials. Since Messersmith reported the mussel-inspired surface chemistry for multifunctional coatings in 2007, polyphenols have been widely used in biomedical, food processing, and cosmetic fields because of their antioxidant and antibacterial properties. Metal-phenolic networks (MPN) capsules as a kind of functional polyphenol materials have been attracted attention in various fields from catalysis and separation to biomedicine, which was due to their biocompatibility, pH responsibility, and multifunctionality. Besides, MPN capsules with different functions (e.g., drug delivery, catalysis, magnetic resonance imaging, fluorescence imaging, and positron emission tomography) can be fabricated by metal ions and polyphenol ligands. Although preparation strategies of MPN capsules are convenient and different templates (e.g., mesoporous silica particles, calcium carbonate particles, polystyrene microspheres, metal-organic frameworks (MOFs) nanoparticles, drug molecular crystals, biological cells and emulsions) have been used, controllable preparation of MPN capsules are still challenging.

Herein, we reported a novel strategy to construct MPN capsules exhibiting size controllable and thickness adjustable characteristics. Zeolite imidazolate framework-8 (ZIF-8) with regular morphology and variable sizes (200 nm to 1 μm) were prepared by tuning the molecular weight or structure of poly(ethylene glycol) (PEG), which endow ZIF-8 as an ideal candidate to fabricate microcapsules. In addition, functional molecule (e.g., DOX) can be encapsulated in a one-pot synthesis into the template for cytotoxicity investigations. From the SEM, TEM and DLS data, it can be seen that the morphology of DOX@ZIF-8 NPs after co-encapsulation with DOX has been maintained. Furthermore, on mixing TA-Fe³⁺ clear solution with DOX@ZIF-8 particles DOX@ZIF-8@MPN particles were harvested. Template removal process could be easily achieved by dissolution of DOX@ZIF-8@MPN particles in EDTA aqueous solution. Thus the DOX@MPN capsules were fabricated with this facile strategy. Physicochemical properties (e.g., size, morphology, and thickness) of MPN capsules can be tuned by adjusting the template size and incubation time. The effect of TA and Fe³⁺ concentration and incubation time on the thickness and morphology of the capsules was studied systematically. The capsule thickness was tuned with the increase of TA-Fe³⁺ concentration, while the maximum thickness of 36.01 nm was obtained with TA and Fe³⁺ concentration are 23.5 and 37 mmol/L respectively. Then, the effect of incubation time on the capsules thickness was also explored, showing that the thickness of MPN capsules increased with the addition of incubation time. When the incubation time was 24 h, the thickness was 41.81 nm. MPN capsules can be assembled by the pyrogallol groups on TA with Fe³⁺ at pH 8.0. However, the pH sensitivity of MPN capsules could lead to capsules disassemble in acidic aqueous solution at pH 4.5.

In conclusion, we reported a facile strategy to assemble MPN capsules with ZIF-8 particles as template. On co-encapsulation of a functional molecule DOX into the ZIF-8 particles, after template removal with EDTA, DOX@MPN capsules were obtained exhibiting higher toxicity to cancer cells (e.g., HeLa cells) compared with the MPN capsules. This study provides a strategy to prepare functional MPN capsules for therapeutic delivery.

metal-organic frameworks (MOFs), metal-phenolic networks (MPN), capsules, stimuli-responsive, drug delivery

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