层层自组装纳米药物载体在癌症治疗中的研究进展

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摘要 层层组装制备过程简单, 适用基材广, 可通过组装基元和超分子组装驱动力等参数的调控和组合, 衍生出众多的超分子组装结构和功能, 发展为最有潜力的表面可控制备、修饰技术. 层层自组装技术近些年来在生物医学领域研究突出, 本文主要概括了层层自组装纳米药物载体共载两种治疗制剂, 以及不同响应型和靶向功能的纳米药物载体的构建及功能, 并在此基础上探讨了其在抗癌治疗的发展前景.

关键词 层层自组装, 纳米材料, 癌症治疗, 靶向, 刺激响应

早在1966年, Iler^[1]就报道了表面带电荷的基片在带相反电荷的胶体溶液中交替沉积获得超薄膜的研究, 当时这种以静电相互作用为推动力的超薄膜制备技术并未引起人们的注意. 直到1991年, Decher和Hong^[2]重新提出静电交替沉积技术, 并将其应用于聚电解质和有机小分子的超薄膜制备中, 随后被广泛应用于生物材料的表面修饰. 除利用静电作用为驱动力外, 层层自组装技术(layer-by-layer self-assembled technology, LbL技术)还有氢键、共价键、疏水作用等驱动力. 此方法与其他表面修饰技术相比具有很多优点: (1) 普适性, 大部分化合物都可以通过调节溶液pH使其带上电荷, 理论上都可作为组装膜; (2) 可控性, 通过组装条件以及聚电解质的结构控制多层膜的厚度、表面电荷、表面形貌; (3) 良好的稳定性, 层层之间存在较强的化学作用, 使多层膜系统具有良好的热稳定性和机械稳定性; (4) 简单性, 操作过程简便, 不需要复杂的合成条件和昂贵的仪器

设备; (5) 多样性, 对基底形状、尺寸没有严格的要求, 可应用于曲面、胶体微粒表面和多孔材料. LbL纳米粒子作为新型的药物载体在结构和功能上的多样性, 使其在治疗癌症方面拥有巨大的前景(图1)^[3~7].

1 LbL纳米药物载体系统负载抗癌药物

在癌症治疗过程中很多因素会阻碍药物的传递, 例如实体瘤组织内渗透性差、纳米药物载体难以到达肿瘤内部、抗癌药物全身分布导致的强烈副作用. LbL技术通过对各种参数的精确控制, 例如膜结构、膜厚度、化学性质和机械性能, 能实现药物进入肿瘤细胞并有序释放^[8,9].

1.1 传递治疗制剂

天然的生物大分子, 包括多肽、蛋白质、核酸以及大多数的多糖分子, 在水溶液中具有较好的相容性, 同时在溶液中带有大量的电荷, 所以通过静电作

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图1 (网络版彩色)负载抗癌药物层层自组装纳米粒子的构建^[7]

Figure 1 (Color online) Structure of layer-by-layer self-assembled anti-cancer drug nanoparticles^[7]

用很容易负载到多层膜中,这一特性可进一步扩展到所有具有电荷的水溶性大分子药物和多电荷的亲水性小分子药物。疏水性药物的负载不能像亲水性药物一样直接吸附在药物载体上,可通过以下几种不同的方法来达到负载疏水药物的目的:(1)利用载体的疏水性内腔,例如油相、介孔二氧化硅、胶束、脂质体;(2)将药物共价键合到能够组装的电解质分子上,得到具有组装能力的大分子前体药物,例如Thierry等人^[10]将前体药物的概念应用到层状功能膜设计中,抗癌药物紫杉醇通过能够自我断裂的酯键与透明质酸结合,与壳聚糖静电组装可获得药物含量可控的多层膜,在生理条件下,由酯键断裂实现药物缓释;(3)利用聚合物的疏水性质,例如超支化聚合物的疏水核心和高密度的末端基、环糊精的疏水内腔。

1.2 提高LbL载体稳定性

在生理环境中,纳米药物载体易被网状内皮系统中的吞噬细胞作为异物摄取,因此,对于降低LbL纳米药物载体的清除率是十分重要的。在多层膜最外层接枝或组装的亲水长链是一件“隐形衣”,可使LbL载体逃避免疫系统的吞噬。Cheng等人^[11]证明了LbL纳米粒最外层结合聚乙二醇后,在生理环境中的清除率降低了,小鼠体内的半衰期也延长到20 h。血清中含有大量带负电荷的蛋白质,倾向于吸附到带

正电荷的表面,LbL载体带负电荷的最外层与之静电排斥有助于在血液中的稳定性。Peng等人^[12]制备的功能性多层膜,分别以白蛋白、肝素和聚乙二醇为最外层,血清蛋白和血小板在白蛋白和聚乙二醇为最外层的多层膜上吸附量较低,显示了这两种多层膜具有良好的血液相容性。

1.3 共载治疗制剂

LbL载体的胶囊结构为负载多种治疗制剂提供了基础,基底核心和多层膜上可负载至少两种治疗制剂。

(1) 传递两种化疗药物。Ramasamy等人^[13]结合LbL技术和脂质体的优势构建LbL脂质体药物载体,聚乙二醇和聚赖氨酸自组装多层膜,核心负载阿霉素(doxorubicin, DOX)和多层膜负载米托蒽醌以实现共载药物。雄性大鼠的药代动力学结果表明,LbL多层膜结构显著降低了两种药物的清除率,并延长了其在体内的循环时间。

(2) 传递两种基因。LbL交替沉积为同时传递两种基因提供了一个简单的方法,Bishop等人^[14]报道了一种共同传递两种基因的LbL纳米系统,基因和聚合物在纳米基底表面交替沉积实现共载。DNA和siRNA共载在金纳米表面,该共载纳米粒子与绿色荧光蛋白基因标记的人乳腺癌细胞培养,此DNA在细胞中顺利表达,且siRNA基因沉默的作用效果比

Lipofectamine 2000(一种市售转染试剂)的效果更好。Bishop等人^[15]还用同样的方法传递两种质粒DNA,并通过不同的组装次序实现了DNA表达时间上的差异。

(3) 共同传递化疗药物和基因。基因治疗对癌症化疗有着很强的增效作用, Yao等人^[16]同样制备共载化疗药物和siRNA纳米LbL系统, 化疗药物索拉菲尼负载在阳离子脂质体核心, siRNA负载在脂质体多层膜上, 并利用多层膜的pH敏感性来控释药物。实验结果表明, 此纳米系统对于肿瘤治疗具有更好的效果。

2 LbL纳米载药系统的缓控释

依据临床应用需要, 有效调控负载药物的控释和缓释, 提高药物有效性, 降低药物剂量过大的毒性, 是药物载体重要的功能之一。从形式上看, 通过多层膜释放药物一般包括3种方式: (1) 药物从多层膜中被动扩散出来; (2) 多层膜水解释放药物; (3) 微环境刺激释放。第1种方式与多层膜渗透性有关, 后两种方式主要通过多层膜解体使药物释放。其中, 微环境刺激包括pH、离子强度、化学、电化学、光和热等, 这些刺激释放既保护了药物, 也降低了药物对机体的毒性, 提高了药物利用率^[17]。LbL超薄膜在实现药物控释行为上具有独特的优势: (1) 环境依赖的多层膜组装特点决定了可通过调控层状膜结构实现药物的控释; (2) 多种聚合物的组装特点引入了包括水解、酶解、刺激响应等特征来调控药物释放; (3) 逐层组装的制备方式使层状膜可以通过对药物进行空间分布上的设计实现释放行为上的调控^[18]。以下将针对多层膜控释药物治疗癌症进行简单总结, 并对LbL技术的应用进行举例。

2.1 基于多层膜结构调控的药物控释行为

由于多层膜组装过程是一个依赖于组装环境的过程, 因此可通过调控这些因素来改变多层膜的厚度、溶胀度等一系列结构参数, 而这些参数会直接影响到药物释放, 特别是基于扩散负载和释放的行为。Rubner课题组^[19]通过对一系列强弱聚电解质多层膜的研究, 发现通过对弱电解质组装环境下pH的调控, 可有效调控多层膜孔径和药物的释放行为。层状膜还可通过对药物进行空间分布上的设计实现释放行为的调控, 负载多种药物时, 通过调控药物负载到多层膜的顺序, 可以有效地调控两种药物的释放顺

序^[20]。Deng等人^[21]发现一种化疗药物和siRNA共同传递的纳米载体, DOX负载在带负电的磷脂脂质体核心, siRNA和聚赖氨酸作为LbL纳米系统的多层膜。两种制剂在时间上错开释放, siRNA释放后72 h释放DOX, 此设计治疗三阴性乳腺癌的疗效是裸DOX治疗的4倍。其中, 聚赖氨酸具有蛋白酶降解特点, 使得此纳米系统在特种酶响应环境下的癌症治疗具有很大的应用前景。

2.2 基于多层膜刺激响应特点的药物控释

在层层组装基元中引入刺激响应因素, 可赋予层层组装膜各种智能响应特点, 实现药物具有环境响应功能的释放行为。由于层层组装基元的多样性, 近几年来, 刺激响应型LbL纳米系统在癌症治疗中备受关注。

(1) pH刺激响应型。由于肿瘤组织及细胞内的低pH环境, 基于pH的刺激响应行为成为纳米药物传递中关键的领域。对于一些弱聚电解质, 改变pH可改变其离子化程度和带电程度, 从而改变多层膜正负电荷的相互作用力。对小分子药物而言, 意味着与小分子药物结合的电荷量会随pH的改变而改变, 从而达到pH响应药物控释。另一种达到pH响应的方式是组装具有pH敏感的聚合物, pH改变导致多层膜解体达到控释药物的目的。Du等人^[22]通过修饰介孔二氧化硅纳米粒(mesoporous silica nanoparticles, MSN)使其表面带上羧基作为基底, pH敏感的异硫氰酸荧光素修饰几丁质和海藻酸钠(sodium alginate, ALG)两种聚电解质层层组装, 形成了在高盐浓度中稳定性高且具有pH响应型的荧光纳米药物载体(图2(a))。修饰后的MSN可提高DOX的载药量, 多功能的聚电解质外壳可根据介质的pH调节药物释放, 实现常规pH下药物的稳定包埋, 肿瘤低pH环境下的响应性释放。

(2) 氧化还原刺激响应型。在电位作用下, 由于氧化还原反应的发生, 多层膜内电荷平衡被打破, 离子进入多层膜内导致渗透压增加, 此时聚电解质发生形变以平衡渗透压, 最终导致多层膜解体和药物的释放^[26]。Song等人^[23]用聚二茂铁硅烷(poly(ferrocenylsilanes), PFS)作为聚阳离子, 聚丙烯酸(poly(acrylic acid), PAA)作为聚阴离子层层自组装中空的纳米管(图2(b)), 利用具有电氧化还原活性的PFS, 通过氧化还原响应控制释放荧光分子, 此系统用于负载抗癌药物具有很强的应用性, LbL载体达到肿瘤组

图2 (网络版彩色)不同刺激响应型的LbL载药纳米粒. (a) pH刺激响应型; (b) 氧化还原刺激响应型; (c) 光刺激响应型; (d) 温度刺激响应型^[22-25]

Figure 2 (Color online) Different stimuli-responsive LbL nanocarriers. (a) pH-responsive. (b) Redox-responsive. (c) Light-responsive. (d) Temperature-responsive^[22-25]

织后可在电脉冲变化下实现脉冲释放药物。

(3) 光刺激响应型. 光照的波长和强度可以轻松而精确地进行调节, 同时光照的方向、时间和照射区域容易控制, 因此选用光敏型聚合物组装多层膜控释药物引起了人们的广泛关注. Fan等人^[24]用卟啉和吡啶青绿分别作为光敏剂制备层层自组装纳米粒子(图2(c)), 在可见光照射下, 光敏剂产生单重态氧, 氧化含碲聚合物转变成亲水性的Te=O基团, 导致药物释放, 且药物释放后多层膜的结构依然保持完整, 降低了治疗过程中载体对机体的副作用. Wajs等人^[27]将偶氮苯和环糊精(cyclodextrins, CD)键接在葡聚糖聚合物上, 利用CD具有疏水的内腔和亲水的外部特殊结构, 通过主客体作用包裹偶氮苯实现层层自组装, 以金为基底自组装后溶解得到中空的纳米载体, 利用偶氮苯分子在紫外光下构象的改变, 导致多层膜解体实现药物释放。

(4) 温度刺激响应型. 具有温度响应型的亲水-疏水相转化聚合物可以用来实现多层膜中药物分子的释放. Li等人^[28]通过将温度敏感的聚N-异丙基丙烯

酰胺(poly(*N*-isopropylacrylamide), PNIPAM)与聚-D-乳酸共聚成聚电解质, 用来组装Fe₃O₄@SiO₂纳米粒子, 形成具有温度敏感的LbL系统, 并考察了其通过温度响应控释化疗药物在癌症治疗上的效果. Zhou等人^[25]用强聚电解质聚二烯丙基二甲基氯化铵(poly(diallyldimethylammonium chloride), PDAC)和聚苯乙烯磺酸钠(poly(styrenesulfonate), PSS)层层组装地塞米松纳米粒子得到具有温敏性的LbL纳米粒子, 考察了层数、离子强度、温度和最外层等参数对药物释放的影响, 以及LbL系统的最佳热转变条件, 研究了PDAC/PSS LbL系统温度敏感型的机制(图2(d)).

3 LbL纳米载药系统靶向肿瘤

癌症药物传递的主要目的是在特定肿瘤位置控释抗癌药物, LbL系统多层膜基元为连接靶向配体提供了键合基团。

3.1 被动靶向

被动靶向指药物载体通过正常生理过程运送至

特定器官,相较于正常组织,50~200 nm范围内的大分子和颗粒更趋向于聚集在肿瘤组织的性质,即实体瘤的高通透性和滞留效应(enhanced permeability and retention effect, EPR)^[29,30], LbL纳米药物载体均符合该条件。

3.2 主动靶向

与正常细胞组织相比,肿瘤细胞组织区域存在一些高表达的分子,成为靶向药物载体的靶向受体,如肿瘤细胞膜表面高表达的叶酸受体(folate receptor, FR)^[31]、表皮生长因子受体(epidermal growth factor receptors, EGFR)^[32]、CD44糖蛋白^[33]和 $\alpha\beta 3$ 整合素^[34]等,以及肿瘤脉管系统高表达的血管内皮生长因子受体(vascular endothelial growth factor, VEGFR)^[35]、血管细胞黏附因子-1(vascular cell adhesion molecule-1, VCAM-1)^[36,37]等。LbL载体系统最外层膜修饰上相应的靶向配体,即可靶向癌细胞。

(1) 叶酸受体靶向。细胞膜上的FR是目前常见的分子靶点之一,FR在卵巢癌、肾癌和乳腺癌等上皮系肿瘤细胞表面高水平表达。Zhou等人^[38,39]将PAA/聚乙烯亚胺和壳聚糖/Alg在聚乳酸-羟基乙酸纳米粒子表面组装后,共价结合的PEG-Folate组装在最外层,HepG2肿瘤细胞对于连接上叶酸的LbL纳米粒子具有更高的吸收率,表明纳米粒子更易被肿瘤细胞内吞,即具有良好的靶向性。

(2) 核酸适配体靶向配体。核酸适配体(apptamer)是利用指数富集的配体系统进化技术的体外筛选技术,从核酸分子库中得到的寡核苷酸片段。核酸适配体折叠成明确的三维结构,可以特异性地结合靶向分子,在某些情况下与抗体竞争甚至超过它们。Gao等人^[40]制备了修饰有凝血酶核酸适配体脂质体包裹的介孔二氧化硅纳米粒子,并载药多西他赛用于癌症的治疗。Xie等人^[41]在牛血清蛋白纳米粒子表面组装聚烯丙胺盐酸盐(poly(allylamine hydrochloride)), PAH)/PSS多层膜,之后组装PAH-g-PEG-COOH单层,通过与碳二亚胺的缩合反应接枝核酸适配体AS1411,可靶向癌细胞膜中过量表达的核仁素。

(3) RGD靶向配体。短肽由于能够被细胞识别以及随后一系列细胞行为,因此受到特别关注,成为研究热点之一。精氨酸-甘氨酸-天冬氨酸(Arg-Gly-Asp, RGD)短肽是很多细胞外基质蛋白和细胞膜中所共有的、能被细胞识别的最小序列,可以同 $\alpha\beta 3$ 整合

素特异性结合。Li等人^[42]利用层层自组装技术提高了FOLFIRINOX化疗方案的疗效,与聚合物纳米粒子相比,此LbL纳米药物载体具有体内半衰期长和血清中稳定性高的优良性能。RGD共价结合在LbL纳米载体表面上,与人胰腺癌细胞共培养和注射入荷瘤小鼠后的荧光成像结果都表明接上RGD的LbL纳米粒子具有良好的靶向性。

(4) CD44蛋白靶向。肿瘤细胞高表达的CD44使其能作为靶向受体,CD44与透明质酸(hyaluronan, HA)形成复合物被肿瘤细胞内吞。Freag等人^[43]用Chi和HA这两种天然可生物降解的聚合物,层层组装得到具有靶向作用的纳米药物载体,并考察了LbL纳米粒的理化性质、生物相容性和血清中的稳定性,以及对两种人乳腺癌细胞(MCF-7和MDA-MB-231)的靶向研究,证实了其良好的靶向性及生物相容性。

4 多功能LbL纳米载药系统

一个成功的药物传递系统需要能在正确的时刻和位点释放,在癌症治疗中,多功能载药系统的构建是十分重要的,LbL技术可以很容易地制备多功能载体。Han等人^[44]设计了双靶向、多重响应共载DOX和siRNA的LbL纳米载体,穿膜肽TAT修饰的MSN作为阳离子纳米核心且负载化疗药物DOX,聚烯丙胺盐酸盐-柠康酸酐(poly(allylamine hydrochloride-citraconic anhydride), PAH-Cit)作为聚阴离子,半乳糖修饰的三甲基半胱氨酸壳聚糖(galactose-modified methyl chitosan-cysteine, GTC)作为聚阳离子,最后负载内皮血管生长因子siRNA。该LbL纳米系统最外层GTC上的半乳糖基特异性识别肝癌细胞过量表达的无唾液酸糖蛋白实现一级靶向,胞吞进入癌细胞后,在癌细胞酸性条件(pH 5.0)下,PAH-Cit的侧链水解使多层膜瓦解,癌细胞质内谷胱甘肽使GTC间二硫键断裂,实现氧化还原响应释放siRNA。另外,穿膜肽TAT可以协助MSN进入细胞核实现二级靶向,并在细胞核内持续释放。通过小剂量的静脉注射考察其抗癌效果,结果表明该LbL系统作为高效和安全的药物载体能够最大化化疗和基因治疗的增效作用。

5 总结与展望

本文从药物传递、刺激响应、靶向以及多功能等方面总结了LbL药物载体体系在癌症治疗上的研究进展,LbL药物载体相较于其他类型药物载体所具有

更好的控释效果, LbL纳米粒和胶囊可在基底和多层膜上载至少两种药剂并且实现其可控释放, 药物可以作为LbL多层膜中的一层, 这样的设计可以精确控制药物负载量, 并对组装成分进行精确调控, 体现出该系统在下一代药物载体中的巨大潜能. 当然, LbL药物载体系统的应用还有很长的路要走, 如何提高

系统的机械性能以保证其在体内长时间循环的稳定性, 以及在LbL药物载体与细胞作用的信号通道等问题还有待进一步的探索. 我们相信围绕实现多种药物可调的持续释放, LbL药物载体系统为临床癌症治疗提供了一种新型手段, 未来将具有极大的应用前景.

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Summary for “层层自组装纳米药物载体在癌症治疗中的研究进展”

Research progress of layer-by-layer self-assembled nanocarriers for cancer treatment

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In the course of cancer treatment, strong side effects of drugs will bring great suffering to the patients, and the drug is difficult to be enriched in the lesion site and greatly reduce the effect of drugs. It is very important to design a suitable drug carrier for the treatment of cancer.

The preparation method of ultrathin films by alternating electrostatic adsorption was reported in 1966. In 1991, the technology of alternating electrostatic deposition was presented, and applied in the preparation of ultrathin film of polyelectrolyte and small organic molecules. Then it has been widely used for surface modification of biomaterials in the field of chemistry and biomedicine. In recent years, the studies of the layer-by-layer self-assembled (LbL) technology in the field of cancer therapy are highlighted, and it has potential as a new drug carrier in the treatment of cancer.

LbL has the advantages of simple preparation process, wide applicable material, regulation and combination of driving force and other parameters through a variety of assembled primitives and a variety of supramolecular assemblies, which are benefit for producing numerous supramolecular assembled structures. It has become the most promising surficial controllable preparation and modification technology. Most of the compounds could be used as an assembled film, which could be charged by adjusting the pH. The drug release behavior can be controlled by combining the different properties of multilayer films.

This article mainly summarized the recent research progress of LbL drug delivery system delivering drug, controllable release, targeting and multifunction in the treatment of cancer. The different loading methods for hydrophilic and hydrophobic drugs were introduced, as well as the structural advantages of co-delivering two kinds of therapeutic agents, including two chemotherapeutic drugs, two genes, one chemotherapy drug and one gene. LbL nano-drug carriers achieve drug controllable release by utilizing the structures and assembling stimuli-responsive polyelectrolyte, including pH, temperature, light and other stimuli-responsive polyelectrolyte. The targeted function of LbL nanoparticles could be divided into active and passive target. The passive target has been determined by the size of carriers, and the active target has been determined by connecting the target group, including folate, aptamer, RGD peptide and so on. The multifunctional LbL nano-drug carrier system is more reasonable for releasing drugs in structure, so as to achieve the purpose of maximizing the therapeutic effect.

LbL drug carriers have unique advantage in providing a more controllable release compared to other delivery methods. LbL nanoparticle and nanocapsule could load at least two kinds of drugs into the substrate and the multilayer film. The drug-loading rate could be accurately controlled by using drug as an assembled component, and it could precisely regulate assembled components, showing great potential for next generation drug-delivery systems. There are some challenges and overcome, for example for further applications, for example, how to improve the mechanical performance in order to ensure the stability for long time *in vivo* circulation. The downstream cellular responses and signaling pathways during the interactions of LbL systems with cells or organs require further investigation. We believe that the LbL nano-drug delivery system will provide a novel approach for clinical therapeutic applications, with tunable sustained release of multiple drugs.

layer-by-layer self assembly, nanomaterials, cancer therapy, targeting, stimuli-responsive

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