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工程化细胞外囊泡作为衰老相关疾病的靶向治疗递送载体

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摘要 随着人口老龄化趋势的加剧,衰老相关疾病带来的健康问题日益突出,对个人健康和社会经济发展造成了严重影响。细胞外囊泡通过传递信号分子、运输细胞内成分以及调节免疫反应,在介导细胞间通信和调控细胞功能中发挥着关键作用。鉴于其低免疫原性、高生物相容性和跨生物屏障能力,细胞外囊泡已成为新一代的衰老相关疾病的靶向治疗递送载体。然而,天然细胞外囊泡存在载药效率低、靶向性不足和体内循环时间短等局限,严重制约其临床应用。近年来,工程化细胞外囊泡凭借其更高的载药效率、靶向能力和体内循环时间,为衰老相关疾病的治疗提供了新策略。本文系统综述了工程化细胞外囊泡的药物装载方法、靶向递送策略和延长体内循环技术,并阐述了工程化细胞外囊泡作为靶向治疗递送载体在衰老相关疾病的应用现状。此外,本文还总结了作为递送载体的工程化细胞外囊泡在应用于不同衰老相关疾病治疗时的共性特征和差异化需求,并探讨了其在临床转化过程中面临的机遇与挑战,旨在为工程化细胞外囊泡治疗衰老相关疾病提供理论依据。

关键词 工程化细胞外囊泡; 药物装载; 靶向递送; 衰老相关疾病

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Engineered Extracellular Vesicles as Targeted Delivery Vehicles for Age-related Diseases

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Abstract As the trend of population aging intensifies, health issues caused by age-related diseases have become increasingly prominent, exerting severe impacts on individual health and socioeconomic development. Extracellular vesicles (EVs) play a crucial role in mediating intercellular communication and regulating cellular functions by transmitting signaling molecules, transporting intracellular components, and modulating immune responses. Owing to their low immunogenicity, high biocompatibility, and ability to cross biological barriers, EVs have emerged as a new generation of targeted therapeutic delivery vehicles for age-related diseases. However, natural EVs exhibit limitations such as low drug-loading efficiency,

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insufficient targeting capability, and short *in vivo* circulation time, which severely restrict their clinical applications. In recent years, engineered EVs, with their enhanced drug-loading efficiency, targeting ability, and prolonged *in vivo* circulation, have provided a novel strategy for the treatment of age-related diseases. This review systematically summarizes the drug loading methods, targeted delivery strategies, and techniques to prolong *in vivo* circulation of engineered EVs, and elaborates on the current application status of engineered EVs as targeted therapeutic delivery vehicles in age-related diseases. Furthermore, this review synthesizes the common characteristics and differential requirements of engineered EVs as delivery vehicles in the treatment of various age-related diseases, and discusses the opportunities and challenges in their clinical translation, aiming to provide a theoretical basis for engineered EV-based therapy for age-related diseases.

Key words engineered extracellular vesicles (engineered EVs); drug loading; targeted delivery; age-related diseases

衰老是指生物体的组织和器官随着年龄的增长而发生不可避免的功能性下降,逐渐趋向死亡且不可逆转的现象[1, 2]。衰老已被确认为多种人类疾病的最主要危险因素之一,例如癌症、神经退行性疾病、心血管疾病和骨退行性疾病[3-6]。衰老相关疾病降低患者的生活质量,导致更高的医疗保健需求,带来沉重的经济负担[7, 8]。目前的研究表明,一些抗衰治疗策略可用于缓解多种与衰老相关的疾病[9, 10]。其中,细胞外囊泡(extracellular vesicles, EVs)作为一种新型的、广泛应用的无细胞再生疗法而备受关注。

EVs 是由细胞分泌的具有脂质双层膜结构的微小囊泡,被认为是细胞间通讯的重要形式[11, 12]。根据其大小、生物学特性和生成途径,EVs 可分为三类:外泌体、微囊泡和凋亡囊泡[13, 14]。外泌体(40~200 nm)的生物发生始于细胞膜内吞作用形成早期内体,其通过选择性包裹机制将特定内容物包裹形成腔内囊泡(intraluminal vesicles, ILVs);ILVs 逐渐转变为晚期内体并最终形成多泡体(multivesicular bodies, MVBs),MVBs 通过与细胞膜融合将其内含的 ILVs 以外泌体形式分泌至细胞外基质[15]。微泡(200~1 000 nm)由质膜向外出芽形成,这一过程涉及膜蛋白质与脂质的局部重构,并依赖于肌动蛋白-肌球蛋白的相互作用以及随后的 ATP 依赖性收缩反应[16]。凋亡小体(1 000~5 000 nm)是细胞程序性死亡过程中产生的内含胞质、细胞器和核碎片的囊泡结构,其生物发生过程经历 3 个阶段:凋亡膜起泡、凋亡膜突起、凋亡细胞碎裂形成凋亡小体[17]。

EVs 携带多种功能性分子,包括蛋白质、核酸(DNA、编码 RNA 及非编码 RNA)和脂质等生物活性成分[18, 19]。作为细胞间信息传递的重要载体,EVs 通过转运生物分子参与多种生理和病理过程的调控,在衰老相关疾病的治疗中展现出巨大潜力[20, 21]。EVs 不仅可递送内源性生物活性物质,也能高效负载外源性治疗剂,进而调控受体细胞功能[22, 23]。基于其天然的生物发生机制、稳定的膜结构及良好的生物相容性,EVs 在药物递送领域展现出显著优势,是一种极具临床应用前景的天然纳米递送载体。

然而,EVs 在来源、组成及功能等方面均表现出显著的异质性特征,这一特性增加其在临床转化中的不确定性[24]。EVs 的来源多样,几乎所有的细胞类型都能分泌 EVs,包括肿瘤细胞、干细胞和免疫细胞等,不同来源的 EVs 在组成和功能上存在显著差异[25]。例如,肿瘤细胞来源的 EVs 通常携带促进肿瘤生长、转移和免疫逃逸的分子,间充质干细胞分泌的 EVs 主要富含促组织修复和再生相关的功能性分子,而免疫细胞来源的 EVs 普遍携带与其免疫功能密切相关的分子,在调节免疫应答和抗肿瘤免疫等方面具有重要作用[26-28]。EVs 的异质性特征使其在细胞间通讯、微环境调控、疾病发生发展等过程中表现出截然不同的生物学效应,这种复杂性既为其临床应用提供广阔的选择空间,也给标准化治疗策略的制定带来重大挑战。

此外,天然 EVs 存在稳定性差、靶向效率低和体内滞留时间短等问题[29, 30]。为了克服天然 EVs 的局限性,通过生物技术对其进行工程化改造可以充分利用 EVs 的递送载体功能,增强其对受体细胞的选择性,提高病变部位的药物浓度,减少非靶向部位的毒副作用,最大限度提高治疗效果[31, 32]。近年来,已有多项临床前研究报道了工程化 EVs 作为药物靶向递送系统在治疗衰老相关疾病中的潜力[33, 34]。研究通过多种工程化策略优化 EVs 的抗衰老作用:一方面,采用供体细胞改造或直接导入技术,将抗衰药物高效装载至 EVs 内;另一方面,通过化学修饰、基因工程或膜融合等方法,在 EVs 表面

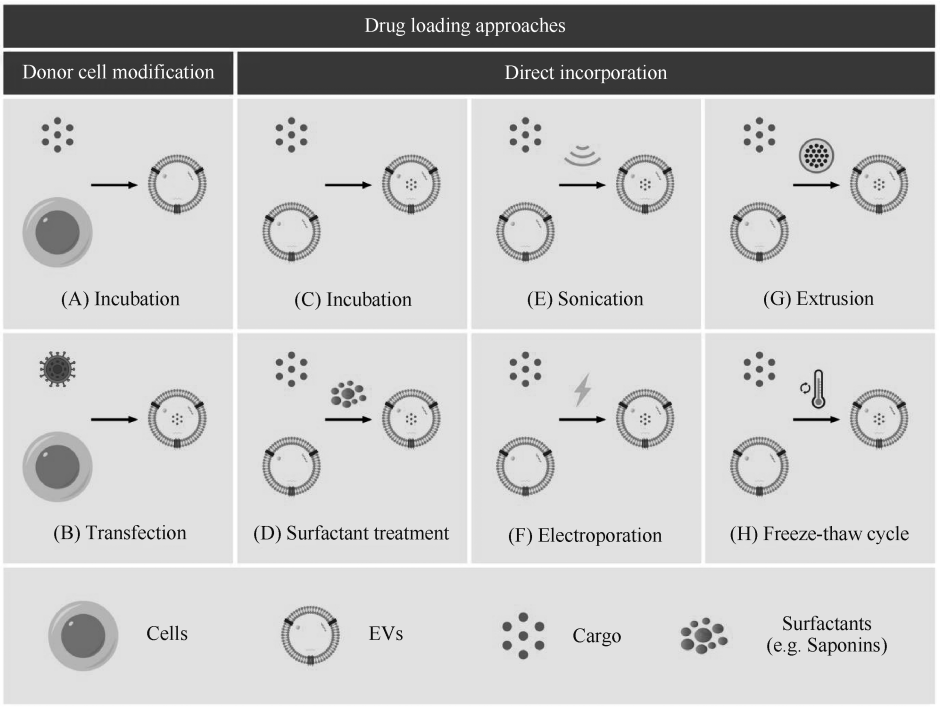

Fig. 1　Drug loading approaches of engineered extracellular vesicles donor cell modification and direct incorporation can be used to load cargo into EVs　(A) Incubation with donor cells: incubating the therapeutic agent with the donor cells of EVs results in the secretion of EVs encapsulated with the drug. (B) Transfection: the donor cells of EVs overexpress specific cargo that are consequently either encapsulated within the EVs or expressed on their membrane upon transfection. (C) Incubation with EVs: co-incubation of the drug with EVs facilitates the entry of the drug into the EVs. (D) Surfactant treatment: incubation with surfactants enhances membrane permeability, thereby substantially augmenting the loading capacity of EVs. (E) Sonication: sonication creates mechanical shear forces that disrupt the integrity of the membrane, thereby enabling the diffusion of drugs into EVs. (F) Electroporation: electroporation uses a long electric pulse to transiently disrupt the phospholipid bilayer, creating temporary pores that allow entry of molecules into EVs. (G) Extrusion: this method entails loading a mixture of EVs and drugs into a syringe extruder equipped with a filter for extrusion. (H) Freeze-thaw cycle: the freeze-thaw cycle involves combining EVs with drugs at −80 ℃ or in liquid nitrogen, followed by thawing at room temperature

展示特异性靶向配体,提升其对衰老组织、器官及细胞的识别能力[35]。另外,将 EVs 与新型生物材料复合构建递送系统,不仅显著增强其抗衰老功效,还能有效延长体内循环时间,从而提升整体治疗效果[36]。此综述系统总结了工程化 EVs 的药物装载方法、靶向递送策略和延长体内循环技术,阐述了其作为靶向治疗递送载体在衰老相关疾病治疗中的研究进展,重点探讨其在临床转化过程中面临的主要挑战与发展前景。

1　工程化细胞外囊泡作为药物靶向递送系统

天然 EVs 凭借其低免疫原性、高生物相容性及跨生物屏障能力,已成为新一代药物靶向递送系统的研究热点[37]。然而,其临床应用仍面临关键挑战:药物装载效率低下、靶向递送效率不足及体内循环时间短暂[38]。针对这些技术瓶颈,研究通过开发多种药物装载方法、优化靶向递送策略及创新体内循环延长技术等工程化策略,显著提升 EVs 的载药效率、靶向精度和治疗效果。

1.1　药物装载方法

药物装载是工程化 EVs 研究的核心方向,其优势在于利用 EVs 固有的生物相容性、结构稳定性及靶向归巢特性,实现治疗药物的高效递送[39]。目前,已发展出多种载药技术体系,主要致力于解决载药效率与载药稳定性等关键技术难题(**Fig. 1**)。

1.1.1　*供体细胞改造*　供体细胞改造是指通过生物技术手段改造亲代细胞,使其分泌携带特定治疗分子的 EVs。基于浓度梯度的被动载药是将治疗药物与供体细胞直接共孵育,使药物自发装载入 EVs[40]。Pascucci 等人通过将紫杉醇与间充质基质细胞共孵育,获得负载紫杉醇的 EVs[41]。共孵育方法操作简便且能保持药物和 EVs 的生物活性,但载药效率较低。转染是供体细胞改造的重要方法,主

要包括化学转染、电穿孔和病毒载体转染等方式。Zhang 等人采用转染试剂使 HEK293T 细胞过表达 miR-29,其分泌的工程化 EVs 可显著抑制胃癌血管生成[42]。Shi 等人通过慢病毒载体将 pre-miR-214 转染至间充质干细胞,发现 miR-214 富集的 EVs 具有增强的神经保护作用[43]。尽管转染方法能有效增强 EVs 的治疗功能,但其作用机制尚未完全阐明。此外,该方法需注意病毒载体和转染试剂可能带来的安全风险。

1.1.2 直接导入 直接导入法是对分离后的 EVs 进行直接改造的技术。相较于供体细胞改造,该方法具有批次间变异小、更适用于规模化生产等优势[44]。Tian 等人将姜黄素与骨髓间充质干细胞共孵育,制备的工程化 EVs 可显著抑制病变组织的炎症反应和细胞凋亡[45]。表面活性剂(例如皂苷、Triton X-100 等)处理能增加 EVs 膜通透性,显著提高载药效率[46]。Fuhrmann 等人研究表明,皂苷处理可使工程化 EVs 的亲水性吖啉的载药量较共孵育法提高 11 倍[47]。尽管皂苷处理不影响 EVs 的粒径分布和 zeta 电位,但潜在的溶血风险可能限制其临床应用[48]。电穿孔技术利用高压电脉冲瞬时破坏 EVs 膜的磷脂双分子层结构,形成临时性孔隙以促进药物装载[49]。Liu 等通过电穿孔将白介素-12(interleukin-12,IL-12)mRNA 载入 EVs,制备的 IL-12-EVs 对原发性肿瘤病灶具有显著治疗效果[50]。电穿孔具有操作可控性强的优势,便于人工干预。超声法通过机械剪切力破坏 EVs 膜结构,促进药物扩散进入囊泡[51]。Fu 等人采用超声技术成功构建了封装 miR-193a-3p 的 EVs,可有效抑制肿瘤细胞增殖、迁移和侵袭[52]。超声法能够显著提高载药效率,但超声处理可能导致 EVs 聚集、生物活性降低等副作用[53]。挤压法利用机械压力驱动 EVs-药物混合体系通过限定孔径的纳米级滤膜,从而实现高效载药[54]。Haney 等人将过氧化氢酶与 EVs 混合体系通过挤出器进行循环挤压处理,获得具有神经保护作用的负载过氧化氢酶的外泌体[55]。该技术具有较高载药效率,但机械剪切力可能导致 EVs 膜结构的完整性受损。冻融循环法通过反复冷冻(-80 ℃或液氮)和解冻实现药物装载[56]。Tran 等人通过冻融循环制备的负载阿司匹林的 EVs 可显著抑制乳腺癌和结直肠癌细胞增殖[57]。该方法操作简便,但载药效率通常低于超声法和挤出法。

1.2 靶向递送策略

天然 EVs 靶向性低,进入体内后通常会被单核吞噬系统迅速清除,导致治疗效果相对较低,严重限制其临床应用[58]。为了应对这些挑战,已经开发了许多针对性的靶向修饰策略,以提高其靶向递送效率(**Fig. 2**)。

1.2.1 基因工程 EVs 膜由跨膜蛋白质(例如 Lamp 2b、GPI 和四跨膜蛋白质,例如 CD63、CD9、CD81 等)组成,这些蛋白质能够与特定配体结合,从而增强 EVs 的靶向递送效率[59]。一种广泛应用于制备靶向 EVs 的技术是利用质粒载体对供体细胞进行基因修饰,该质粒编码的靶向配体可融合到上述跨膜蛋白质之一。Yu 等人通过用编码狂犬病毒糖蛋白(rabies viral glycoprotein,RVG)-Lamp2b 的质粒转染 HEK293T 细胞,制备脑神经靶向肽 RVG 功能化的 EVs,体内成像结果显示,RVG-EVs 在大脑区域中的荧光信号强度显著高于 EVs 对照组[60]。Liang 等人通过基因工程技术增强 EVs 对人表皮生长因子受体-2 阳性癌细胞的靶向性,从而显著提升肿瘤治疗效果[61]。基因工程改造的 EVs 能有效提升其靶向递送效率,但在 EVs 膜中引入靶向部分可能会干扰膜蛋白质的正常功能。

1.2.2 疏水插入 疏水插入法利用疏水性材料(例如聚乙二醇化的合成磷脂)偶联的靶向配体修饰 EVs,通过选择性识别特定受体提升其体内靶向性[62]。Wu 等人采用疏水插入法将纤维蛋白靶向肽 CREKA 修饰于 EVs 表面,使其在纤维蛋白高表达的组织缺损部位中富集[63]。Liang 等人通过脂锚连接将血管肽-2 插入 EVs 膜,构建了可穿越血脑屏障并靶向胶质母细胞瘤的靶向递送系统[64]。研究表明,将配体偶联的聚乙二醇化合成磷脂整合至 EVs 膜不仅能增强 EVs 的靶向递送效率,还可延长其循环半衰期[65]。需要注意的是,该技术需精确调控配体负载密度,以避免 EVs 理化性质改变、功能丧失及潜在细胞毒性。

1.2.3 点击化学 点击化学是表面修饰 EVs 的一种新技术,炔基通过 1-乙基-3-(3-二甲基氨基丙基)碳二酰亚胺-N-羟基琥珀酰亚胺(EDC-NHS)缩合反应连接到 EVs 表面,在铜的存在下与叠氮基团的靶向连接部分共价结合[66]。点击化学反应高效,可以在有机溶剂和水性缓冲液中以较短的反应时间进行。Jia 等人利用点击化学技术,将神经纤毛蛋白-1 靶向肽 RGE (RGERPPR)连接至 EVs 膜,成功构建胶质瘤靶向 EVs[67]。相较于铜催化反应,无铜点击化学具有反应速率快、细胞毒性低等优势[68]。Kang 等人采用无铜点击化学方法,将心血管靶向肽(car-

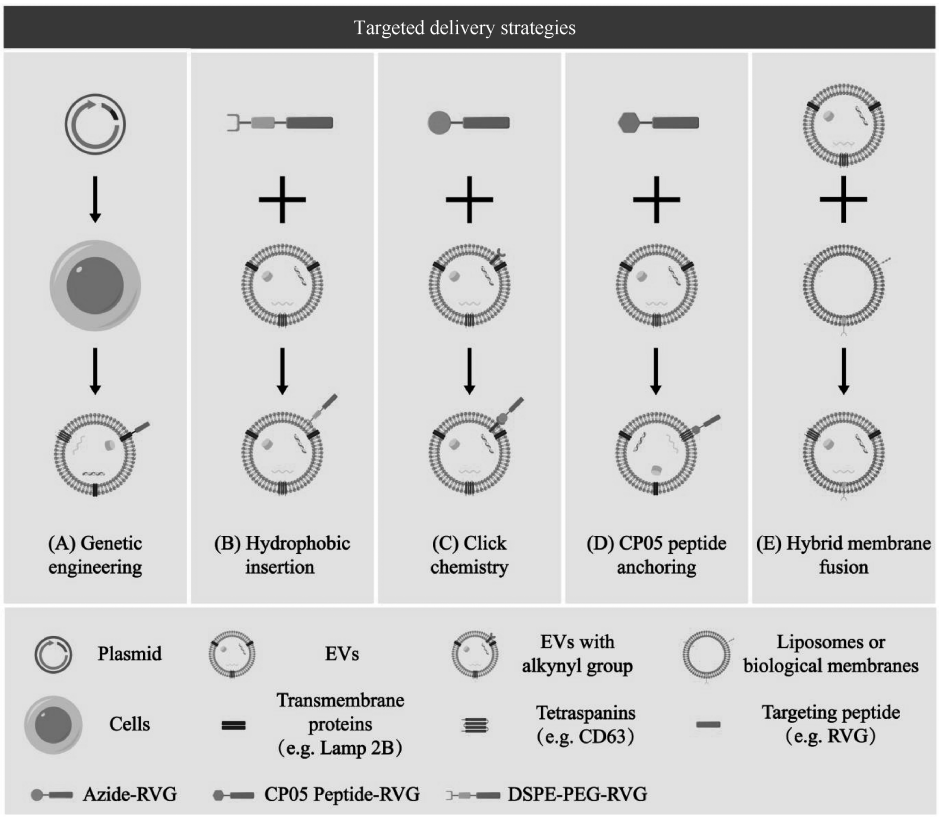

Fig. 2　Targeted delivery strategies of engineered extracellular vesicles　(A) Genetic engineering: this process genetically modifies donor cells by using plasmid vectors that encode targeting ligand fused with transmembrane proteins so that the secreted EVs can carry the targeting molecule. (B) Hydrophobic insertion: a targeting ligand is conjugated with PEG-phospholipids to modify the EV membrane. (C) Click chemistry: targeting moiety with the azide group is linked to the EVs with alkyne group on the membrane through the click chemistry reaction. (D) CP05 peptide anchoring: CP05 peptide acts as a pivotal link between the targeting peptide and the EV-associated membrane protein CD63. (E) Hybrid membrane fusion: liposomes or biological membranes endowed with targeting moieties fuse with EVs, resulting in the display of targeting molecules on the surface of the hybrid membrane nanovesicles

diac-targeting peptide, CTP)共价偶联至 EVs 表面,研究证明,CTP-EVs 的心血管靶向效率显著提升[69]。尽管点击化学具有操作简便和反应快速的优势,但仍可能引发氨基酸残基氧化和偶联物活性降低等副反应。

1.2.4　CP05 肽锚定　CP05 肽(CRHSQMTVTSRL)对 EVs 膜蛋白质 CD63 的第 2 个胞外环具有高亲和力,可作为 EVs 表面修饰的关键连接分子[70]。Li 等人设计了一种融合肽修饰系统,包含钛结合肽(titanium-binding peptide, TBP)和 CP05 肽,能同时特异性结合钛种植体表面并捕获 EVs[71]。Ma 等人利用 CP05 肽将 I/III 型胶原蛋白的胶原结合域偶联至 EVs 膜,提高了 EVs 在颅骨缺损部位的滞留性和稳定性[72]。CP05 肽锚定技术能在维持 EVs 粒径和形态的前提下实现精准靶向。未来研究应聚焦于开发具有更高结合效率、稳定性和 EVs 亲和力的新型功能肽。

1.2.5　膜融合　目前,研究通过膜融合技术构建了多种纳米材料-EVs 杂交系统[73]。该技术主要采用孵育、挤压和冻融等方法实现膜融合。当靶向修饰的脂质体或生物膜与 EVs 融合后,杂交囊泡表面可呈现特异性靶向分子。Li 等人将表达 CD47 的肿瘤细胞 EVs 与环肽 RGD(cyclic arginine-glycine-aspartate acid, cRGD)修饰的脂质体融合,构建了共载 miR-497 和雷公藤甲素的仿生杂交纳米颗粒,体内研究显示,该颗粒能有效富集在肿瘤部位[74]。Wu 等人设计的中性粒细胞膜-EVs 杂交囊泡,可特异性靶向损伤肾组织[75]。与天然 EVs 相比,膜融合杂交 EVs 在生理环境中具有增强的稳定性,延长的循环时间和特定的靶向能力。然而,由于各种未解决的问题,膜融合杂交 EVs 的临床转化仍有较长的路要走,包括纯化工艺的优化和表征技术的发展,以及脂质体与 EVs 最佳比例的确定。

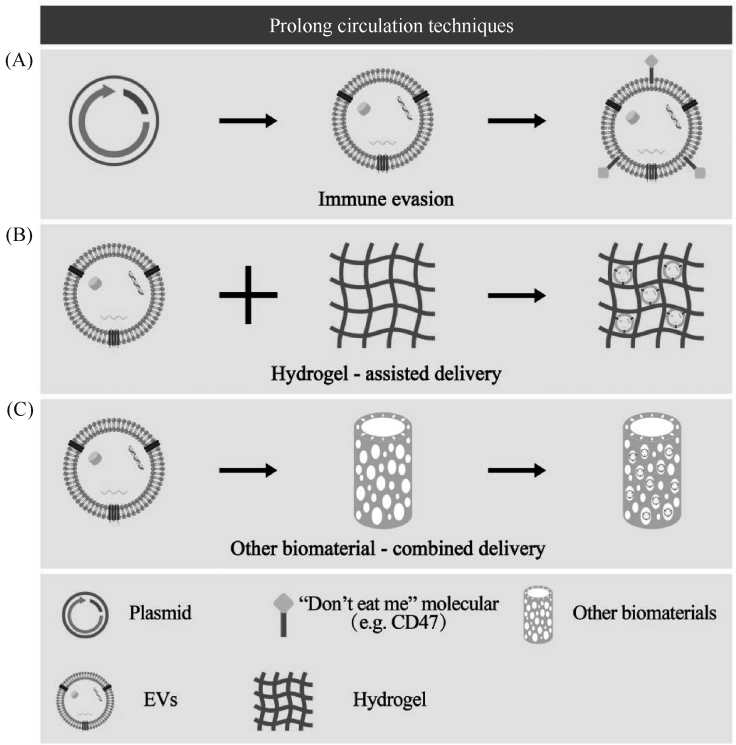

Fig. 3 Prolonged circulation techniques of engineered extracellular vesicles (A) Immune evasion: the overexpression of "don't eat me" molecules on the surface of EVs help avoid macrophage-mediated phagocytosis. (B) Hydrogel-assisted delivery: incorporating EVs with hydrogels creates an appropriate supporting niche for sustained EV release. (C) Other biomaterial-combined delivery: the combination of other biomaterials with EVs facilitates the stable and sustained release of EVs

1.3 延长体内循环技术

全身给药后,EVs 在血液中迅速被清除,这种快速清除机制主要涉及循环吞噬细胞(例如巨噬细胞和中性粒细胞)的吞噬作用[76]。针对 EVs 体内快速清除的挑战,目前已有多种技术可延长 EVs 体内循环时间或持续高效递送 EVs,进而增强其治疗效果(**Fig. 3**)。

1.3.1 *免疫逃避* 巨噬细胞表面的信号调节蛋白 α(signal regulatory protein alpha, SIRPα)通过识别 EVs 表面的 CD47 配体,激活"别吃我"信号,进而负调控巨噬细胞对 EVs 的吞噬作用[77]。研究发现,CD47 的表面功能化使 EVs 能够有效逃避单核吞噬系统,促进其在肿瘤组织中的积累[78]。另一项研究证实,CD47 修饰的 EVs 能有效逃避免疫监视,延长其在肝组织中的半衰期[79]。CD47 在 EVs 表面的过表达在调控 EVs 生物分布和延长循环时间方面展现出重要潜力。除了 CD47,其他一些分子,包括 CD24、CD44 和 PD-L1 等,已被确认为有前景的候选分子[80-82]。这些分子可能有助于阻止巨噬细胞介导的吞噬作用,从而提高治疗效果。

1.3.2 *水凝胶封装* 水凝胶是一种三维网络结构凝胶,可作为局部药物递送的理想载体。目前,用于封装 EVs 的水凝胶主要采用天然聚合物材料,可分为多糖类(例如壳聚糖、海藻酸盐、透明质酸及琼脂糖)和蛋白质类(例如胶原蛋白、明胶与丝素蛋白)[83]。将 EVs 负载于水凝胶中可构建持续释放系统,这种递送方式能有效克服天然 EVs 的局限性[84]。大量研究表明,EVs 负载水凝胶在组织修复和再生方面具有巨大的潜力,例如皮肤组织损伤、心血管损伤和骨损伤[85]。Han 等人开发聚乙烯醇/甘油/氯化钠复合水凝胶用于大鼠心肌梗死治疗,该体系显著提高 EVs 的稳定性并改善心血管功能[86]。Shi 等人构建基于没食子酸缀合壳聚糖和氧化透明质酸的多功能水凝胶,实现 EVs 的缓释递送[87]。EVs-水凝胶复合体系的储存稳定性和生物活性保持,是临床转化需要解决的关键问题。

1.3.3 *其他生物材料负载* 除水凝胶外,EVs 与其他生物材料的复合体系也受到广泛关注。生物材料不仅能稳定 EVs、实现控释,还可赋予其新功能,为工程化 EVs 研究提供新思路[88]。目前研究的 EVs-生物材料复合系统主要包括 3D 打印支架、负载贴片和纤维蛋白粘合剂等,这些体系均表现出良好的生物相容性和 EVs 滞留性[89]。Yuan 等人开发的明

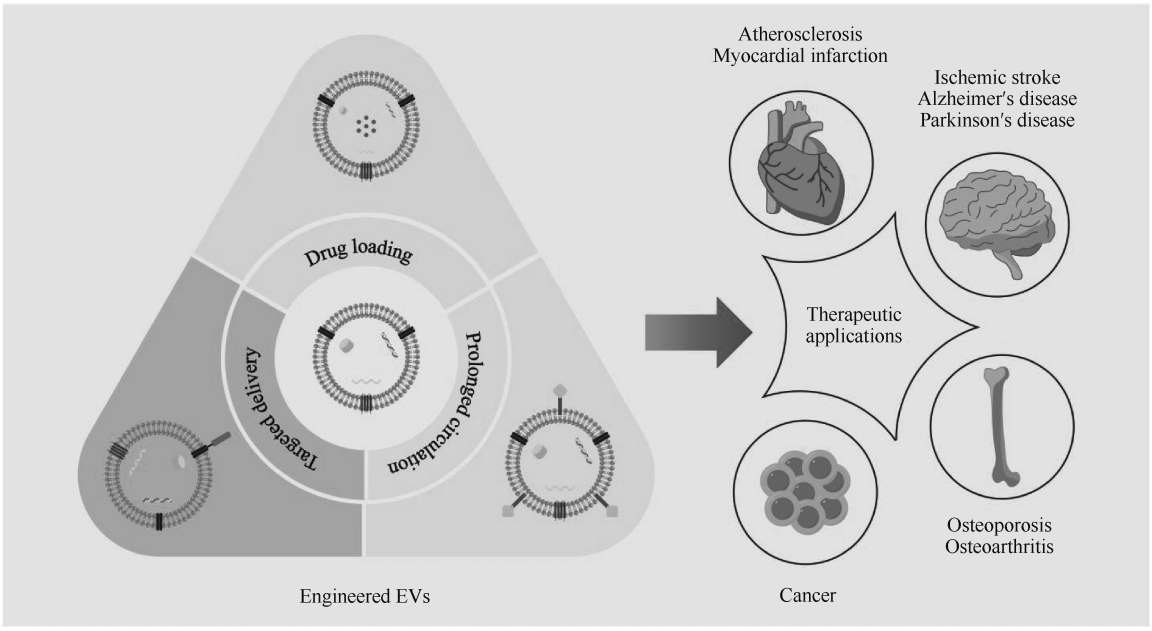

Fig. 4　Therapeutic applications of engineered extracellular vesicles for age-related diseases　Engineering extracellular vesicles through drug loading approaches, targeted delivery strategies, and prolonged circulation techniques facilitates their therapeutic application in age-related diseases, including cardiovascular diseases, neurodegenerative diseases, osteodegenerative diseases, and cancer

胶基微针贴片,能高效负载含 miR-29b 模拟物的 EVs,显著提高 EVs 在梗死心肌中的滞留率[90]。为了提高钛植入物的生物功能,Wei 等人通过骨形态发生蛋白 2/EVs 共修饰钛纳米管植入体以促进成骨分化[91]。在临床转化过程中,生物材料的选择需综合考虑低免疫原性、制备简易性以及与生物活性物质的相容性。

2　工程化细胞外囊泡作为衰老相关疾病的靶向治疗递送载体

目前,工程化 EVs 作为衰老相关疾病的靶向治疗递送载体的研究仍主要以基础研究为主,大多数研究尚处于体外实验或动物实验阶段[92]。部分研究项目,例如缺血性脑卒中(NCT03384433)和乳腺癌(NCT04592484)的治疗虽已成功进入Ⅰ/Ⅱ期临床试验阶段,工程化 EVs 的临床转化仍面临标准化生产体系的建立、靶向递送效率的优化和长期安全性的评估等关键挑战,这些因素显著制约了其临床应用进程。尽管如此,现有研究数据表明,工程化 EVs 作为靶向递送载体在心血管疾病、神经退行性疾病、骨退行性疾病及癌症等领域已展现出显著的治疗潜力(**Fig. 4**, **Table 1**)。

2.1　动脉粥样硬化

动脉粥样硬化是一种与衰老密切相关的疾病,年龄增长是其进展的独立危险因素[93]。动脉粥样硬化斑块存在明显的细胞衰老特征,表现为增殖能力下降、生长停滞、DNA 损伤增加、端粒缩短及功能异常[94]。在动脉粥样硬化的实验模型中,不同来源的工程化 EVs 可以减轻炎症、缓解动脉粥样硬化进程。Xie 等人利用血小板膜表面糖蛋白介导的靶向炎症细胞特性,通过将 M2 型巨噬细胞来源的 EVs 与血小板膜融合,成功构建了具有动脉粥样硬化斑块靶向性和抗炎作用的杂化囊泡系统,显著抑制动脉粥样硬化的进展[95]。Wei 等人开发的负载空心氧化铈纳米颗粒的巨噬细胞 EVs,依赖巨噬细胞膜蛋白的靶向作用,特异性富集于动脉粥样硬化斑块,有效改善炎症免疫微环境并修复内皮细胞损伤[96]。动脉粥样硬化保护性 EVs 主要来源于耐受性树突状细胞、调节性 T 细胞、天然态血管内皮细胞、收缩型平滑肌细胞及间充质干细胞等[97]。通过工程化改造(例如治疗分子装载和靶向配体修饰)动脉粥样硬化保护性 EVs 可进一步增强其治疗效果。

2.2　心肌梗死

心肌梗死是指由于冠状动脉血流减少导致心肌细胞缺氧及缺血性坏死[98]。在衰老过程中,心肌细胞展现出不同水平的细胞内改变(包括细胞器、大分子和离子稳态),损害了其结构和功能完整性[99]。研究发现,老年人群心肌梗死发病率及死亡率显著

Table 1 Engineered extracellular vesicles as targeted therapeutic delivery vehicles for age-related diseases

Application	EV source	Cargo	Drug loading approach	Targeting molecule	Targeted delivery strategy	Outcome	Reference
Atherosclerosis	M2 macrophages	miR-99a-5p	N/A	Platelet membrane proteins	Hybrid membrane fusion	Prevented the formation of foam cells and mitigated the progression of atherosclerosis	[95]
	RAW264.7 cells	hollow cerium oxide nanoparticles	Extrusion	Macrophage membrane proteins	N/A	Remodeled the immune microenvironment in the lesion area and repaired endothelial cell damage	[96]
Myocardial infarction	Cardiosphere-derived cells	N/A	N/A	CMP peptide	Genetic engineering	Decreased cardiomyocyte apoptosis and enhanced cardiac retention	[101]
	Bone marrow mesenchymal stem cells	Curcumin	Incubation	CTP peptide	Hydrophobic insertion	Diminished reactive oxygen species over-accumulation in ischemic myocardium	[102]
	Bone marrow mesenchymal stem cells	Functional miRNAs	N/A	Platelet membrane proteins	Hybrid membrane fusion	Realized cardiac repair	[103]
Ischemic stroke	Adipose-derived stem cells	Fxr2	N/A	M2 macrophage targeting peptide	Genetic engineering	Improved neurofunction recovery in cerebral ischemia/reperfusion	[106]
	BV2 microglia cell lines	NR2B9c	Sonication	RVG29 peptide	Click chemistry	Restored the neurological deficit, infarct size and neuronal apoptosis	[107]
Alzheimer's disease	HT22 hippocampus neuron cells	Corynoxine-B	Sonication	Fe65	Genetic engineering	Ameliorated the cognitive decline and pathogenesis	[112]
	Bone marrow mesenchymal stem cells	N/A	N/A	RVG peptide	Hydrophobic insertion	Alleviated memory deficits and effectively decreased the amyloid-β level and amyloid plaque	[113]
Parkinson's disease	Umbilical cord mesenchymal stem cells	Zwitterionic nanoparticles	Extrusion	Guanidine groups in the L-arginine derivative	Hybrid membrane fusion	Promoted the growth of neuronal cells and participated in the process of nerve repair	[119]
	Dendritic cells	shRNA minicircles	Electroporation	RVG peptide	Genetic engineering	Decreased alpha-synuclein aggregation and reduced the loss of dopaminergic neurons	[120]
Osteoporosis	NIH3T3 cells	Liposomes encapsulating antagomir-188	Extrusion	C-X-C motif chemokine receptor 4	Genetic engineering	Promoted osteogenic differentiation and reversed the age-related bone loss	[122]
	Induced pluripotent stem cells	si-Shn3	Electroporation	SDSSD peptide	Hydrophobic insertion	Enhanced osteogenic differentiation and inhibited osteoclast formation	[123]
	Red blood cells	Anti-miR-214	Electroporation	Tartrate-resistant acid phosphatase binding peptide	CP05 peptide anchoring	Inhibited osteoclast activity and improved bone density	[124]
	Bone marrow stem cells	si-STING	Electroporation	CAP peptide	Hydrophobic insertion	Reduced the excessive friction and the accumulation of aged chondrocytes	[128]
Osteoarthritis	Tonsil-derived mesenchymal stem cells	Transforming growth factor -β1	Transfection	Collagen type II alpha 1antibody	Hybrid membrane fusion	Demonstrated excellent chondroprotective and anti-inflammatory effects	[129]
	Dendritic cells	Kartogenin	Electroporation	E7 peptide	Genetic engineering	Promoted the chondrogenesis of synovial fluid-derived mesenchymal stem cells	[130]
Breast cancer	HBE cell lines	Doxorubicin	Sonication	AS1411 aptamer	Hydrophobic insertion	Inhibited migration, proliferation and induced apoptosis of cancer cells	[134]
Lung cancer	Chimeric antigen receptor-T cells	Paclitaxel	Incubation	Lung-targeting liposomes	Hybrid membrane fusion	Enhanced the antitumor effects	[135]
Prostate cancer	HEK293T cells	si-SIRT6	Electroporation	E3 aptamer	Hydrophobic insertion	Resulted in a lower proliferation rate of tumor and less metastatic area	[136]

高于年轻群体[100]。临床前研究已证明,工程化 EVs 在心肌梗死动物模型中的治疗潜力。Mentkowski 等人通过基因工程在心肌球来源细胞 EVs 表面表达心肌细胞靶向肽(cardiomyocyte specific peptide,CMP),显著增强工程化 EVs 的心肌内吞效率,减少心肌细胞凋亡[101]。Chen 等人通过疏水插入法将心脏靶向肽(cardiac targeting peptide,CTP)展示在 EVs 膜表面,所构建的负载姜黄素的 CTP-EVs 能特异性靶向缺血心肌,加速心肌梗死后的心脏恢复[102]。Li 等人采用膜融合技术构建血小板膜修饰 EVs(P-EVs),模拟血小板-单核细胞相互作用机制[103]。在心肌缺血-再灌注损伤模型中,P-EVs“劫持”外周血中活化的单核细胞,通过单核细胞的趋化作用被募集到心肌损伤区域,促进心肌修复。此外,与传统单一疗法相比,EVs-生物支架复合系统作为新兴研究方向,通过空间限制和缓释调控,延长心肌梗死治疗的时效性,展现出良好的临床应用前景。

2.3　缺血性脑卒中

缺血性脑卒中的主要病理改变是脑梗死,其发生机制为脑组织血液供应不足,初期表现为可逆性神经功能损伤;若缺血持续,则进展为不可逆性梗死,导致神经元及其支持结构坏死[104]。衰老是该疾病最重要的危险因素之一,老年患者表现出更高的病死率、致残率及更差的神经功能预后[105]。研究表明,工程化 EVs 在缺血性脑卒中治疗中展现出显著潜力。Wang 等人通过基因工程技术,构建的 M2 型小胶质细胞靶向 EVs,可抑制细胞铁死亡并改善缺血性脑卒中小鼠神经功能[106]。Haroon 等人通过生物正交点击化学将 RVG29 肽偶联至 EVs 表面,并负载神经保护肽 NR2B9c(KLSSIESDV),该工程化 EVs 在体内研究中表现出增强的脑靶向性和延长的半衰期,能有效递送 NR2B9c 至缺血大脑并显著减轻脑卒中损伤[107]。尽管工程化 EVs 治疗缺血性脑卒中的疗效显著,其临床应用仍需系统评估给药方案(包括最佳剂量、给药途径和给药时间),并通过大规模临床试验验证其安全性与有效性。

2.4　阿尔茨海默症

阿尔茨海默病是一种以记忆障碍和认知功能损害为主要特征的神经退行性疾病[108]。在 65 岁及以上人群中,阿尔茨海默病的患病率达 10%,且随年龄增长呈持续上升趋势[109]。该疾病治疗面临的关键挑战在于血脑屏障(blood-brain barrier,BBB)对中枢神经系统药物递送的严格限制,而 EVs 因其天然的 BBB 穿透能力,为疾病治疗提供了新策略[110]。研究发现,淀粉样前体蛋白(amyloid-β precursor protein,APP)表达上调可促进其胞内结构域与 Fe65 蛋白结合,进而触发淀粉样蛋白-β(amyloid-β,Aβ)的分泌,这一机制在阿尔茨海默病的病理进程中发挥关键作用[111]。Iyaswamy 等人通过基因工程构建 Fe65 过表达的工程化 EVs,能有效递送自噬诱导剂 Corynoxine-B 至阿尔茨海默病模型小鼠脑内 APP 过表达的神经元,显著改善认知功能和疾病进程[112]。Cui 等人将 RVG 肽偶联至 EVs 表面,该工程化 EVs 可特异性靶向阿尔茨海默病模型小鼠大脑的皮质和海马体,导致 Aβ 水平和斑块沉积减少,学习和记忆能力得到显着改善[113]。尽管有关 EVs 治疗阿尔茨海默病的体外和动物研究数量呈增长趋势,但临床评估仍十分匮乏,这一局限主要源于长期治疗、重复给药及下游效应等方面的不确定性[114]。未来研究应进一步优化 EVs 的靶向递送能力,使其能精准作用于星形胶质细胞和小胶质细胞等,实现缓解神经炎症、促进斑块清除和发挥神经保护作用等多重效应。

2.5　帕金森病

帕金森病是一种多见于 65 岁以上人群的进行性发展的神经退行性疾病[115]。其发病机制涉及多巴胺能神经元的核基因突变抑制 α-突触核蛋白(α-synuclein,α-syn)水解,促进其在线粒体内聚集[116]。这一过程导致活性氧(reactive oxygen species,ROS)水平升高,进而上调诱导型一氧化氮合酶(inducible nitric oxide synthase,iNOS)表达,引发神经炎症反应并最终导致神经元细胞损伤[117]。研究表明,L-精氨酸衍生物的胍基团可与帕金森病病理微环境中高表达的 iNOS 和 ROS 反应生成一氧化氮(nitric oxide,NO)[118]。基于这一特点,Wang 等人将间充质干细胞来源的 EVs 与携带 L-精氨酸衍生物的纳米颗粒杂交,构建了 NO 驱动型纳米马达,该纳米马达可清除 ROS、促进 α-syn 聚集体降解并修复神经元[119]。Izco 等人利用 RVG-EVs 作为 shRNA 小环递送载体,显著减少了 α-syn 聚集和多巴胺能神经元丢失,并改善临床症状[120]。工程化 EVs 通过抑制 α-syn 合成、发挥抗炎效应及改善运动功能等多重机制,为帕金森病的治疗提供了新策略,其疗效与安全性可通过优化给药途径(例如鼻内、静脉和植入装置)进一步提升。

2.6　骨质疏松症

骨质疏松症是老年人群中最常见的临床骨病,其特征为骨量减少、骨微结构破坏、骨脆性增加和骨

折风险升高[121]。基于工程化 EVs 的治疗策略在该领域取得显著进展。Hu 等人研究表明,C-X-C 基序趋化因子受体 4(C-X-C motif chemokine receptor 4,CXCR4)阳性的 EVs 与 miR-188 antagomir 修饰的脂质体杂交后,可在骨髓中特异性聚集,有效逆转小鼠年龄相关性骨质丢失[122]。Cui 等人开发了骨靶向肽修饰的 EVs,可特异性递送 siRNA 至成骨细胞,沉默锌指蛋白 3(Schnurri3,*Shn*3)基因后促进成骨分化、抑制破骨细胞形成并增强血管生成[123]。Xu 等人采用 CP05 肽将抗酒石酸酸性磷酸酶结合肽修饰于 EVs 表面,使其靶向递送 anti-miR-214 至破骨细胞,显著抑制破骨细胞活性,并改善骨质疏松小鼠的骨密度[124]。随着生物纳米材料与合成技术的发展,类 EVs 纳米囊泡已成为新的研究热点,未来关键研究方向是通过综合工程策略提升囊泡性能,以建立安全、可靠且具成本效益的骨质疏松治疗体系。

2.7 骨关节炎

骨关节炎是一种常见的退行性骨关节病,在老年人群中尤为高发[125]。该疾病的临床特征表现为关节疼痛、僵硬、肿胀、屈伸活动受限及关节畸形[126]。目前,骨髓干细胞(bone marrow stem cells,BMSCs)是骨关节炎治疗研究中应用最广泛的 EVs 来源,其对骨关节炎的治疗作用已得到证实[127]。Feng 等人研发的软骨细胞亲和肽(chondrocyte affinity peptide,CAP)-EVs 复合水凝胶递送系统可高效负载靶向干扰素基因刺激因子(stimulator of interferon genes,*STING*)的 siRNA,显著减轻骨关节炎大鼠模型的关节磨损并缓解关节衰老[128]。Kim 等人研究发现,将胶原蛋白 II 型 α1 抗体修饰的脂质体与过表达转化生长因子-β1 的 EVs 杂交构建的膜融合囊泡,在骨关节炎病变中表现出显著的靶向性和持久性,可抑制炎症反应并保护软骨[129]。Xu 等人构建的间充质干细胞结合肽 E7 修饰的 EVs,能特异性递送小分子化合物 kartogenin 至滑液来源的间充质干细胞,有效诱导其向软骨细胞分化,进而产生修复病损软骨的治疗效果[130]。然而,现有疗法需多次局部给药以维持治疗浓度,增加了疼痛和不良反应风险。因此,基于生物材料的 EVs 局部控释系统成为新的研究热点,通过调控载体降解速率维持有效药物浓度,从而延长治疗效果[131]。

2.8 肿瘤

衰老是癌症发生的重要危险因素[132]。恶性细胞的产生通常源于衰老相关的基因组和表观基因组的稳定性丧失,这种年龄相关的功能失调在免疫监视功能受损、慢性炎症及代谢异常等因素共同作用下,最终促进肿瘤发生[133]。老年人群高发肿瘤包括乳腺癌、肺癌、前列腺癌、结直肠癌和黑色素瘤等。基于 EVs 的药物递送系统展现出卓越的肿瘤穿透能力,其治疗效果显著优于游离药物。Zhang 等人开发的“三重组合”纳米囊泡系统整合了 CD82 过表达、适配体 AS1411 偶联及阿霉素递送,可特异性靶向三阴性乳腺癌细胞并抑制肿瘤增殖[134]。Zhu 等人通过融合靶向间皮素和程序性死亡配体-1 的双特异性嵌合抗原受体-T 细胞来源 EVs 与肺靶向脂质体,成功构建负载紫杉醇的免疫化疗平台,显著延长 CT-26 转移性肺癌模型小鼠的生存期[135]。Han 等人通过 E3 适配体修饰的 EVs 递送靶向沉默调节蛋白 6(Sirtuin 6,*SIRT*6)的 siRNA,可显著抑制前列腺癌细胞的增殖和转移[136]。不同来源 EVs 具有高度可改造特性,可与放射治疗、光动力治疗、光热治疗及声动力治疗等疗法协同应用,在肿瘤治疗领域展现出广阔的临床转化前景。

3 问题与展望

近十年来,EVs 因其独特的生物学特性和功能在药物递送领域展现出显著优势。作为天然来源的纳米载体,EVs 具有良好的生物相容性和较低的免疫原性,从而显著降低了其在体内应用的排斥风险[137]。与此同时,EVs 具有优越的生物屏障穿透能力,表面携带的亲本细胞来源膜蛋白质赋予其天然的归巢性,能够选择性富集于特定组织或病变部位[138]。此外,EVs 的脂质双层膜结构可以有效保护其所负载的内容物在血液循环中不被降解,提高了递送的稳定性和效率[139]。得益于 EVs 高度的可工程化改造特性,通过药物装载、靶向递送和延长体内循环等多种工程化策略,可显著增强其载药效率、靶向能力、治疗效果和用药安全,从而满足多样化的疾病治疗需求。基于上述优势,工程化 EVs 已成为一种理想的靶向治疗递送载体,在衰老相关疾病治疗领域展现出重要应用价值。然而,鉴于不同疾病在病理生理微环境及靶向组织特异性等方面存在显著差异,这对工程化 EVs 作为递送载体的设计提出了疾病特异性的差异化需求。在心血管疾病治疗中,工程化 EVs 需具备针对心血管组织或血管内皮细胞的高效靶向能力,并能在高剪切力的血流环境中维持结构完整性和内容物稳定性,同时递送促血管生成、抗凋亡及抗炎药物以修复损伤组织或稳定斑块。对于神经退行性疾病,工程化 EVs 需突破血

脑屏障,精准靶向与病理进程紧密关联的小胶质细胞和星形胶质细胞递送抗炎药物以减轻神经炎症,或定向输送神经营养因子至退化神经元保护突触结构,还可结合 ROS 刺激响应型材料实现病理微环境依赖的精准药物释放。针对骨退行性疾病,工程化 EVs 需适应骨骼血供少、药物渗透差的特点,通过特异性修饰增强其对骨组织及骨病标志物的靶向性,实现骨吸收抑制剂、骨形成促进剂或骨代谢调节剂的精准递送,并可与生物材料协同应用以延长生物活性成分在骨损伤部位的滞留时间。在肿瘤治疗领域,工程化 EVs 应能实现肿瘤组织的特异性富集,具备单核吞噬系统逃避及溶酶体逃逸的能力,并能整合肿瘤微环境响应因素(例如 PH、酶、氧化还原等)或外源刺激因素(例如光、热、磁等)实现抗肿瘤药物的时空可控释放,从而增强抗肿瘤效应和调节肿瘤微环境。EVs 凭借其共性优势奠定了作为药物载体的基础,同时通过个性化工程改造实现了精准治疗,提升了其在衰老相关疾病治疗中的临床转化潜力。

然而,EVs 的大规模生产和临床转化仍面临挑战:分离纯化技术可扩展性不足、质量控制标准不完善以及保存方案待确立。尽管差速超速离心被视为分离 EVs 的"金标准",但其操作繁琐且易导致囊泡聚集,难以满足量产需求[140]。切向流过滤和阴离子交换色谱等新兴技术虽然能够高效分离富集 EVs,但不同方法获得的 EVs 在纯度、理化性质和生物学功能等方面存在明显差异[141-143]。因此,为确保 EVs 产品的质量标准,必须建立严格的质量控制体系,重点监测重要参数的一致性,包括粒径分布、蛋白质含量、颗粒数/μg 蛋白质等。同时,必须严格控制 EVs 来源(例如物种来源、细胞类型和培养条件等),并采用高通量蛋白质组学和基因组学技术对其内容物进行全面表征。研究表明,不同的存储条件会显著影响 EVs 的浓度、物理特性和生物功能[144]。目前,最常用的保存方案是将 EVs 分装于磷酸盐缓冲液中并冻存于-80 ℃(保存时间不超过 6 个月),但该方案可能会改变 EVs 的特性和功能[145]。现有研究对 EVs 的最佳存储条件尚未达成共识,需通过系统性比较评估不同方案的优劣,确保 EVs 的结构完整性和功能稳定性。通过全面优化 EVs 的分离纯化工艺、质量控制体系以及存储条件,将有效推动工程化 EVs 临床转化进程。

总而言之,EVs 凭借其低毒性、低免疫原性及高度可工程化特性,展现出卓越的药物递送优势。国内外临床前研究证实,工程化 EVs 在多种衰老相关疾病模型中均具有显著治疗效果,提示其广泛的临床转化潜力。因此,工程化 EVs 可作为一个可定制的无细胞治疗平台,为衰老相关疾病的治疗提供新策略。

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