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·综述·

间充质干细胞来源外泌体的工程化及其在口腔医学领域的应用进展

赵云峰[1,2]，刘倩[1]，李萌[1,3]，李露颖[1]，张薇[1]，胡先同[4]，马楚凡[1]
1.空军军医大学特色医学中心口腔科，北京（100142）；2.中国医科大学，辽宁 沈阳（110122）；3.安徽医科大学，安徽 合肥（230032）；4.中国人民解放军总医院第四医学中心，北京（100048）

【摘要】近年来，间充质干细胞来源的外泌体（mesenchymal stem cell-derived exosomes，MSC-EXO）在口腔医学领域受到了越来越多的关注，成为生物医学研究的前沿热点。本文就间充质干细胞来源外泌体的工程化及其在口腔医学领域的应用进行综述，以期对口腔医学的发展提供新的思路。外泌体是一种由细胞分泌的纳米级膜性囊泡，内含多种蛋白质、RNAs、脂质和其他生物分子。它们通过循环系统运输，能够与其他细胞相互作用，调控其生物学行为，参与多种生理和病理过程。在口腔疾病治疗方面，外泌体因其天然的生物活性和多功能性，展现出巨大的潜力。然而研究发现，单纯依赖天然外泌体的功能可能无法完全满足复杂的临床需求。为此，工程化外泌体的概念应运而生。工程化外泌体通过生物工程技术对外泌体进行改造，可以增强其靶向性，使其能够更加精准地到达病灶部位。同时，工程化外泌体还能够通过表面修饰或内部装载，携带特定的治疗性分子，如药物、基因编辑工具或信号分子，从而提高治疗效果。此外，这种工程化处理还可以赋予外泌体更强的稳定性，使其在体内循环时能够更好地抵抗免疫系统的清除，延长其半衰期，提高治疗的有效性。尽管工程化外泌体在口腔医学等领域引起了广泛关注，但当前其应用仍主要停留在基础研究阶段。为了推动工程化外泌体进入临床应用阶段，需要提供更充分的生物相容性证据，明确其治疗作用及机制。

【关键词】间充质干细胞；工程化外泌体；外泌体修饰；口腔医学；牙髓再生；牙周再生；骨再生；口腔癌；组织再生
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Engineering of mesenchymal stem cell-derived exosomes and their application progress in the field of oral medicine

ZHAO Yunfeng[1,2], LIU Qian[1], LI Meng[1,3], LI Luying[1], ZHANG Wei[1], HU Xiantong[4], MA Chufan[1]. 1. Department of Stomatology, Air Force Medical University Air Force Medical Center, PLA, Beijing 100142, China; 2. China Medical University, Shenyang 110122, China; 3. Anhui Medical University, Hefei 230032, China; 4. The Fourth Medical Center of the General Hospital of CPLA, Beijing 100048, China
Corresponding author: MA Chufan, Email: machufan_fmmu@163.com

【Abstract】In recent years, mesenchymal stem cell-derived exosomes (MSC-EXO) have garnered increasing attention in the field of stomatology and have become an established research area in biomedical research. This article reviews the engineering of exosomes derived from mesenchymal stem cells and their application in the field of stomatology, in order to provide new ideas for the development of stomatology. Exosomes are nanoscale membrane vesicles secreted by cells and contain a variety of proteins, RNAs, lipids, and other biomolecules. They are transported through the circulatory system and can interact with other cells to regulate their biological behavior and participate in a variety of physiological and pathological processes. In the treatment of oral diseases, exosomes have shown great potential due to their

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【作者简介】赵云峰，住院医师，硕士，Email：qykqzyf@163.com
【通信作者】马楚凡，教授，博士，Email：machufan_fmmu@163.com

natural biological activity and versatility. However, studies have found that relying solely on the function of natural exosomes may not fully meet the complex clinical requirements. Therefore, the concept of engineered exosomes has emerged. Engineered exosomes can be modified by bioengineering technology to enhance their targeting, allowing them to reach the lesion site more accurately. At the same time, engineered exosomes can also be surface modified or loaded internally to carry specific therapeutic molecules, such as drugs, gene editing tools or signaling molecules to improve the therapeutic effect. In addition, this engineered treatment can also confer greater stability to exosomes, making them better able to resist clearance by the immune system when circulating in the body, extending their half-life, and improving the effectiveness of treatment. Although engineered exosomes have attracted extensive attention in the fields of stomatology and other fields, their application is still mainly in the stage of basic research. To promote the clinical application of engineered exosomes, it is necessary to provide more sufficient evidence of biocompatibility and clarify their therapeutic effect and mechanism.

【Key words】 mesenchymal stem cell; engineered exosomes; exosome modification; stomatology; dental pulp regeneration; periodontal regeneration; bone regeneration; oral cancer; tissue regeneration

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外泌体是一组由细胞分泌的双层脂质膜结构的囊泡，可携带来自亲代细胞的各种物质，如DNA、RNA、脂类和蛋白质等[1-2]。间充质干细胞来源的外泌体（mesenchymal stem cell-derived exosomes, MSC-EXO）具有低免疫原性、稳定性、易于量产、功能可控性等优点，在口腔医学领域显示出巨大的应用潜力。然而，天然外泌体仍存在提取制备困难、靶向性低、半衰期短、功能性内容物的成分和含量不确定等问题[3-4]，严重限制了其临床应用。近年来，工程化外泌体逐渐成为研究热点。工程化外泌体是一类通过生物工程技术修饰改造的外泌体，具有强靶向性、抵抗机体免疫系统清除以及可装载特定内容物等特点。工程化外泌体不仅具有天然外泌体优异的生物相容性，并且具有优于天然外泌体的特性，如高生物活性、稳定性、靶向性、载药效率高、载药浓度可控、系统高效递送等[5-7]。这些特点显著提高了基于外泌体治疗的有效性和安全性，从而可以实现全面和多样化的治疗。因此，本文就MSC-EXO的工程化及其在口腔医学领域的最新研究进展做一综述。

1　外泌体生物学特点

外泌体是一种由细胞分泌的直径在30～150 nm的纳米级脂质双层囊泡，通过细胞的内吞途径形成，可携带蛋白质、脂类、RNA、代谢产物、生长因子和细胞因子等多种生物分子，在细胞间信息传递中起关键作用。

外泌体的生成过程可大致分为：内陷、内吞、融合与分泌。早期细胞质膜内陷捕获某些细胞外成分及膜蛋白，形成早期内涵体；这些内涵体随后通过内吞作用进一步发展为成熟的晚期内涵体，晚期内涵体凹陷最终演变为细胞内具有动态亚细胞结构的多泡小体（multivesicular bodies, MVB）。其中部分MVB会被溶酶体降解，而另一部分则与细胞膜融合，从细胞中释放形成外泌体[8]。释放后的外泌体可以经由旁分泌作用于特定靶细胞，产生相应的作用。外泌体在体液中相对稳定，并易于通过生物标志物技术检测。在疾病或损伤进程中，外泌体内成分会随之发生改变，使其成为各类疾病诊断、预测病情进展及治疗效果的有力工具[9-10]。

MSC-EXO具有与MSC相似的多种治疗功能，包括修复受损组织、抑制巨噬细胞极化以及促进血管生成等效果。MSC-EXO作为一种高效的天然药物递送纳米载体，具有更高的生物活性、靶向性、载药效率高、载药浓度可控、系统高效递送等优点，确保了其在诊治多种疾病中的前景和潜力。作为细胞间通信的关键媒介，MSC-EXO在包括骨形成在内的多种生物学过程中发挥着重要的作用，在骨再生领域有着显著的治疗潜力。MSC-EXO能够靶向骨组织，并诱导成骨分化，从而加速骨再生的过程，因此在骨缺损处发挥了显著的治

疗效果[11]。此外，MSC-EXO通过其表面受体与配体的相互作用特定地结合靶细胞，这种靶向机制不仅促进了细胞间信号传递，还可以通过传递细胞内成分来刺激成骨细胞的增殖与分化[12]。

尽管外泌体在多个领域展现出巨大的应用潜力，但是天然外泌体提取制备困难、靶向性低、半衰期短、功能性内容物的成分和含量不确定等问题，严重限制了其临床应用。为了克服以上局限性，并实现更精确的治疗效果，研究者通过工程化手段对外泌体进行修饰改造，以增强其功能并扩大应用范围。

2　MSC-EXO工程化改造

为克服天然外泌体的局限性，对于MSC-EXO的工程化改造成为研究热点。研究表明[13-15]通过工程化手段对外泌体进行改造，可以有效提高外泌体的产量。此外，外泌体通过工程化改造也可以提高其稳定性[16-17]。外泌体的工程化改造途径主要分为两个方面：亲本细胞预处理和外泌体修饰（图1）。

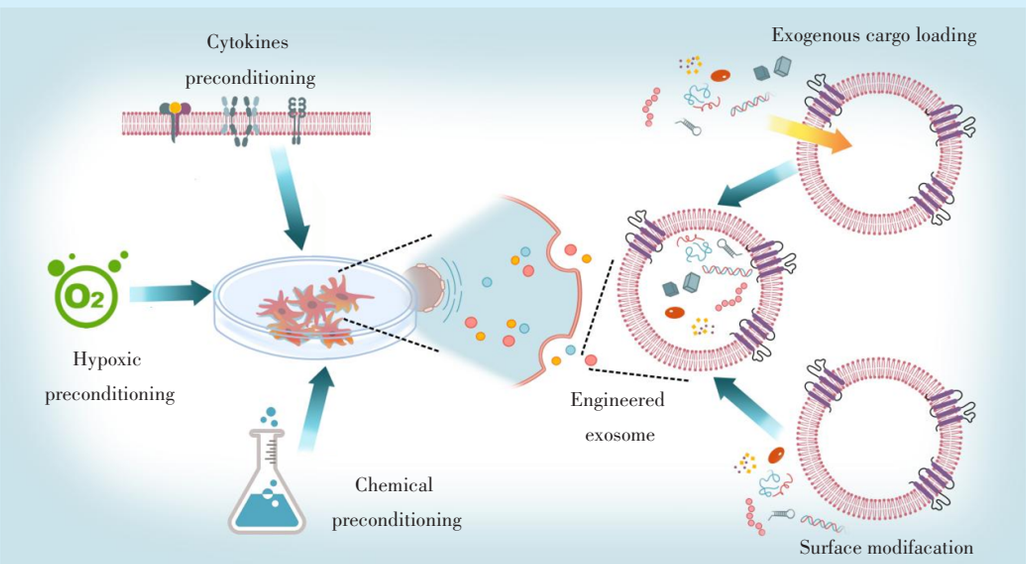

Figure 1　Methods of engineering transformation of mesenchymal stem cell-derived exosomes

图1　间充质干细胞来源外泌体工程化改造的方法

2.1　亲本细胞预处理

亲本细胞预处理是指在提取外泌体之前，通过一系列操作对亲本细胞进行处理，以调整或增强外泌体的性质、功能或产量。这些处理可以是物理、化学、基因或营养等方面的干预，目的是优化细胞分泌的外泌体的质量和特性，使其在后续应用中更加有效。MSC的预处理主要有低氧预处理、细胞因子预处理和化学预处理三种方式。

2.1.1　低氧预处理　低氧条件，即细胞在0%～10%的氧分压下培养，虽可能引致细胞死亡，但低氧预处理通过增强生存信号的表达，有效减轻了因低氧引起的细胞凋亡[18]。人体内的MSC处于低氧状态，因此低氧更接近生理状态，有助于保持它们的特性，并提高治疗潜能。例如，受损区氧供应减少是一种常见表现，MSC迁移到该区域后就会产生大量治疗性旁分泌因子来修复组织[19]。有研究表明低氧预处理可减少体外培养的骨髓间充质干细胞凋亡[20]。此外，低氧状态可激活糖酵解，从而改善MSC的增殖和遗传稳定性[21]。低氧还可以提升MSC的分化能力，表现为八聚体结合转录因子4、Nanog和Sox2等关键分子的表达上调[22]，并且氧分压的降低可激活缺氧诱导因子1α，该因子可进一步与血管生成相关的记忆表达，如血管内皮生长因子和C-X-C基序趋化因子受体4，从而增强了MSC的旁分泌能力和迁移能力[23]。

2.1.2　细胞因子预处理　细胞因子和炎性刺激可提高旁分泌效率，并调节包括外泌体在内的多种潜在治疗因子的生成与分泌[24-25]。肿瘤坏死因子-α（tumor necrosis factor, TNF-α）作为广泛研究的炎性细胞因子之一，应用该分子预处理可有效增强

MSC-EXO对包括牙周炎在内的多种疾病的治疗效果[26]。转化生长因子－β1预处理MSC-EXO可上调miRNA-135b以减轻骨关节炎导致的大鼠软骨损伤[27]。

2.1.3　化学预处理　MSC可以检测到化学信号，从而做出表型的改变[28]。最近的研究表明，二甲双胍通过促进自噬作用显著增强了MSC-EXO的释放。此外，二甲双胍还介导了α-胰蛋白酶抑制物重链H4在MVB中的转移，并使其在释放的外泌体中积聚。这些经过处理的MSC-EXO在体外能有效延缓髓核细胞的衰老，并提高椎间盘退行性变的治疗效果[29]。来自姜黄素预处理的MSC-EXO通过调节miRNA-124/NF-kB和miRNA-143/ROCK1/TLR9信号通路可以减轻骨关节炎[30]。

研究表明[31-32]，电修饰、声波引导、冲击波、机械力学、温度、辐照等亲本细胞预处理方式均可以提高外泌体产量。而电修饰、疏水与膜融合、磁引导可以增强外泌体的靶向性。特定的情况下[33-34]，疏水与膜融合、磁引导、金属粒子可较好地增强外泌体靶向性；而电修饰和声波修饰则可以提高外泌体的数量和质量。

2.2　外泌体修饰

2.2.1　“货物”装载至外泌体　装载策略主要分为内源性和外源性两种方式。内源性装载依赖于对外泌体供体细胞的工程化修饰，主要通过转染或共孵育等方法将目标分子或药物转入细胞内，“货物”进入亲本细胞，亲本细胞在产生外泌体的过程中将细胞内的“货物”装载到细胞内的多泡小体[35-36]。此方法适用于载入有治疗作用的内源性蛋白质和核苷酸。而外源性装载是将药物通过电穿孔、超声或共孵育等方法直接装载到已提取的外泌体中，常用于装载小分子药物[37]，虽效率更高，但可能会破坏外泌体结构的完整性[38]。

2.2.2　外泌体的表面修饰　外泌体表面修饰是指通过直接修饰外泌体的表面分子，以此来提高MSC外泌体的靶向性与稳定性。表面修饰主要分为基因工程技术和化学修饰。基因工程技术是在基因组的特定位置促进位点特异性的插入、缺失或修饰，从而间接改善外泌体功能。这是通过质粒DNA或mRNA转染亲本细胞来实现的，这些亲本细胞表达与外泌体膜成分融合的靶向部分的基因[39]。将MSC结合肽E7与外泌体膜蛋白溶酶体相关膜蛋白2融合，形成具有靶向特异性的外泌体，修饰后的外泌体相比对照组能诱导更高程度的软骨分化[40]。化学修饰主要包括共价修饰和非共价修饰。“点击化学反应”是最常用的化学修饰方法，点击化学利用炔烃和叠氮化物残基之间的共价相互作用形成稳定的三唑键，可将靶向部分连接到外泌体表面。神经皮素-1靶向肽是一种针对胶质瘤细胞的特异性靶向肽，有研究基于点击化学反应的原理，通过磺酰叠氮化物的环加成反应将神经皮素-1靶向肽固定在外泌体上。结果表明，神经皮素-1靶向肽修饰的载药外泌体能很好地穿过血脑屏障，有效富集于肿瘤区域，对胶质瘤的靶向显像和治疗取得了良好的效果[41]。非共价修饰最常用的方法是受体-配体结合法和基于高阳离子物质与外泌体膜上带负电荷官能团之间相互作用的多价静电法[42]。

3　工程化外泌体在口腔疾病中的应用

3.1　组织再生与修复

骨缺损修复最常用的是自体骨移植，但是存在移植后感染、组织来源不足等问题[43]，现已发现工程化MSC-EXO可有效促进组织再生与修复。工程化MSC-EXO通过多种机制促进组织修复，其中调节局部微环境、促进血管生成和成骨是其关键作用之一。这一过程主要依赖于骨形态发生蛋白（bone morphogenetic protein, BMP）-Smad和磷脂酰肌醇3-激酶（phosphatidylinositol 3-kinase, PI3K）/蛋白激酶B（protein kinase B, Akt）信号通路的调控[44]。血运重建是骨再生的关键基础，有研究[45]构建了负载血管内皮生长因子（vascular endothelial growth factor，VEGF）的工程化外泌体可同时发挥成骨基质诱导MSC成骨分化及作为基因载体可控释放VEGF以重塑血管系统的双重作用，从而有效促进节段性骨缺损的血管化骨再生。此外，MSC-EXO还可通过调控细胞因子的表达，发挥抗炎作用，改善骨缺损修复的微环境，从而减少炎症相关并发症，进一步提升骨组织再生的效果[46]。另有研究发现，神经生长因子刺激的MSC-EXO可增强神经细胞功能和神经营养作用，从而提高骨修复细胞的成骨潜能。其机制涉及神经生长因子诱导的miRNA组分调控，并进一步激活促分裂原活化蛋白激酶（mitogen-activated protein kinase, MAPK）和PI3K/Akt信号通路[47]。

颞下颌关节炎是指累及颞下颌关节和（或）咀嚼肌的一种退行性病变，以进行性软骨破坏、髁突改建和滑膜炎为主要特征，患者通常伴有关节疼

痛、功能障碍[48]。滑膜来源的间充质干细胞(synovial fluid-derived mesenchymal stem cell, SF-MSC)移植是治疗骨关节炎的有效手段,但是如何控制其在软骨关节中的分化是目前存在的问题。研究表明Kartogenin(KGN)(一种软骨样组织形成的诱导剂)可以诱导其分化,通过工程化外泌体包裹KGN,靶向递送至SF-MSC,可以提高其在胞质中的浓度,促进SF-MSC的软骨分化,进而促进软骨修复[40]。Liu等[49]研究发现,炎症刺激可使脂肪MSC产生的外泌体中miR-27b-3p高表达,而miR-27b-3p可通过靶向巨噬细胞集落刺激因子-1促进抗炎型巨噬细胞分化,进而促进颞下颌关节髁突再生。

MSC-EXO可以通过促进牙源性分化、促进血管再生及促进神经再生等方式促进牙髓组织再生。研究发现[50],MSC-EXO可以对牙髓细胞功能发挥多方面的影响,包括迁移、增殖和牙源性分化,以促进牙髓再生。低氧预处理的人落叶乳头干细胞分泌的外泌体通过转运let-7f-5p和miR-210-3p促进血管生成,有潜力成为牙髓再生过程中促血管生成的治疗方法之一[51]。

MSC-EXO可以诱导牙周缺损组织的再生。研究表明经P2X7受体基因修饰后的干细胞分泌的外泌体可以对其周围细胞产生积极作用,并逆转炎症介导的牙周膜干细胞损伤[52]。而来自炎性微环境的外泌体可通过BMP信号促进牙周膜干细胞的成骨和成牙分化[53]。在动物模型中,MSC-EXO治疗显著减少了牙槽骨丧失,促进了牙周组织的再生,突出了它们在恢复牙周完整性方面的潜力。体外研究进一步验证这些效应,显示了外泌体作用后增强的细胞行为,如迁移、成骨分化和血管生成[54]。

3.2　口腔肿瘤的治疗

工程化MSC-EXO通过修饰和载药,在口腔癌治疗中展现出优异的靶向性、稳定性及存储性,同时在生物相容性和调节肿瘤微环境方面具有巨大潜力[55-56]。研究表明,这些外泌体可携带多种抗肿瘤分子(如miRNA和抑瘤蛋白),直接作用于肿瘤细胞,抑制其增殖并诱导凋亡[57-58]。此外,工程化MSC-EXO还可作为高效药物载体,显著增强药物的稳定性和靶向性,提高药物在肿瘤部位的富集度,从而增强化疗和免疫治疗效果[59-60]。在抑制肿瘤转移方面,工程化MSC-EXO通过重塑肿瘤微环境,干扰血管新生并降低肿瘤细胞的迁移能力[61]。研究发现,通过调节Notch和Wnt等信号通路,外泌体能够降低肿瘤细胞的迁移潜力,有效减少转移灶的发生[62]。Xie等[63]发现,miR-101-3p可通过外泌体途径从人骨髓间充质干细胞转移至TCA8113细胞内,从而显著抑制其侵袭和迁移能力。此外,在小鼠移植瘤模型中,接受miR-101-3p负载外泌体注射的裸鼠,其肿瘤体积和重量均显著减少。有研究[64]采用间充质干细胞,通过基因工程获得高表达CXCR4的外泌体作为靶向基因药物递送的载体,再通过电转化加载Survivin基因,构建全新的基因药物递送系统,新的递送系统可以有效地聚集在肿瘤部位并将siRNA释放到肿瘤细胞中,从而在体内敲低肿瘤细胞中的Survivin基因,抑制肿瘤生长。

肿瘤坏死因子相关凋亡诱导配体(TNF-related apoptosis-inducing ligand, TRAIL)能够诱导多种癌细胞发生凋亡,然而,由于肿瘤耐药性及TRAIL半衰期较短,其在临床靶向治疗中的应用受到限制。卡巴他赛(Cabazitaxel, CTX)作为一种新型紫杉烷类化疗药物,通过抑制PI3K/Akt/mTOR信号通路的磷酸化,促进细胞凋亡或自噬,对部分耐药性肿瘤表现出较高的敏感性。有研究[65]探讨了基于MSC-EXO载体递送CTX/TRAIL组合治疗口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)的潜力,结果表明,MSC-EXO作为纳米级药物递送系统,不仅能够提高CTX的稳定性和生物利用度,还可协同TRAIL诱导癌细胞凋亡,从而增强抗肿瘤效果。该研究揭示了基于MSC-EXO的CTX/TRAIL递送系统在口腔癌精准治疗中的应用前景,有望成为一种有效的抗癌策略。

3.3　抗炎作用

炎症是许多疾病的共同途径,导致组织破坏和功能受损。工程化MSC-EXO由于其固有的抗炎特性,在口腔疾病中可以调节异常反应[66]。

细胞因子是炎症反应中的一个关键因素,MSC-EXO内容物可以调节细胞因子的活性。例如,研究表明MSC-EXO能够抑制促炎细胞因子如TNF-α和IL-1β,同时增强抗炎细胞因子如IL-10的分泌[67]。MSC-EXO还可诱导巨噬细胞表型转换,促进M1型促炎巨噬细胞向M2型抗炎巨噬细胞极化,这一过程主要由miRNA等小分子介导,并通过调节细胞因子信号通路相关基因来实现[68-69]。在牙周组织中,MSC-EXO可以下调炎症反应中的关键转录因子NF-κB的表达,从而减少炎症,促进愈合[66]。研究表明[26]TNF-α预处理牙龈组织来源的

MSC（gingival mesenchymal stem cells, GMSC）是治疗牙周炎的理想方法，TNF-α刺激不仅能提高GMSC-EXO的分泌水平，还能增强其内含CD73和miR-1260b的表达，从而促进M2巨噬细胞极化，并抑制破骨细胞形成。另有研究发现[70]，GMSCs-EXO通过同时激活在成骨分化中发挥关键作用的Wnt/β-catenin信号通路，并抑制介导炎症反应的NF-κB信号通路，从而有效改善炎性微环境，显著促进炎症条件下牙周膜干细胞的成骨分化。在免疫调节方面，GMSC-EXO通过IL-17RA-Act1-TRAF6-NF-κB信号通路调节Th17/Treg细胞平衡，从而增强免疫抑制作用，在胶原诱导的关节炎模型中显著缓解炎症反应和骨侵蚀，表明其在炎症性疾病治疗中的潜在应用价值[71]。此外，研究还发现，GMSC-EXO能有效抑制滑膜成纤维细胞异常活化，减少其增殖与迁移，并降低其对关节软骨的破坏作用，从而抑制软骨基质降解，维持关节稳态，并在关节保护方面发挥重要作用[72]。

在牙周炎小鼠模型中，脂肪来源的间充质干细胞和牙乳头干细胞分泌的外泌体能够显著促进牙周膜和牙槽骨的再生[73]。在颞下颌关节骨性关节炎中，外泌体疗法展现出抗炎和促进软骨修复的双重作用。腺苷介导的AKT、ERK和AMPK信号通路激活，促进软骨再生[74]。在口腔溃疡治疗方面，研究开发了一种工程化MSC-EXO递送系统，MSC-EXO经过脂多糖预处理，并与ZIF-8复合，制备成丝素蛋白微针贴剂。该贴剂具有卓越的抗炎和抗菌特性，同时促进溃疡愈合[75]。

综上，MSC-EXO疗法可能成为治疗口腔炎症性疾病的主要手段。MSC-EXO介导的持续信号传递也可以降低疾病发作的频率和严重程度，从而改善患者的预后和生活质量[76]。

4　总结与展望

外泌体作为细胞间通信的重要工具，通过携带RNA和蛋白质等生物活性物质，激活相关信号通路，介导细胞微环境中一系列生物学反应，从而达到治疗效果。MSC-EXO治疗作为一种安全、可行、易管理的无细胞疗法在未来的口腔组织再生领域具有广阔的前景。工程化外泌体弥补了天然外泌体的不足，并且可以根据人类的特定意愿以多种方式进行修饰，从而增强甚至赋予一些新的特性，但是工程化MSC-EXO在临床治疗中的应用仍面临一些挑战。尽管间充质干细胞是目前外泌体生产效率较高的来源，但是大规模生产、分离和纯化外泌体仍然是一个难题[77]。外泌体的质量、纯度和储存条件都会影响其修饰效果[78]。其次，外泌体存在异质性。外泌体的功能受到间充质干细胞来源、修饰方法等因素的影响，许多外泌体的工程化方式可能会改变其原有的形态和大小，工程化后的靶向外泌体在形态上呈现多样性，缺乏统一标准。而这种形态变化对外泌体功能的潜在影响仍需进一步深入研究。再者，外泌体的体内机制尚未完全明确[79]。最后，外泌体长期稳定性和安全性评估仍然不足[80]。相信随着工程化外泌体的深入研究，以上问题会逐步解决，真正实现工程化外泌体的临床转化，为口腔医学的发展提供新的契机。

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