综述

不同来源外泌体在创面愈合过程各阶段的治疗作用

滕泳翔[1,2] 宿刚[1] 李兆欣[3] 翟朝晖[4] 李玉莉[5] 陈远征[6]*

([1]山东第二医科大学临床医学院, 潍坊 261053; [2]山东第二医科大学附属临沂市人民医院烧伤整形外科, 临沂 276002; [3]山东第二医科大学附属中医院, 潍坊 261053; [4]山东第二医科大学整形外科学研究所, 潍坊 261053; [5]康复大学康复科学与工程学院, 青岛 266071; [6]应急总医院烧伤整形外科, 北京 100028)

摘要 慢性难愈合创面是全球范围内的公共卫生问题, 愈合过程涉及一系列重叠的空间和时间阶段, 其过程具有复杂性以及多样性, 对于使用现有临床治疗手段难以愈合的慢性难愈合创面, 亟需寻找新的治疗方法。干细胞旁分泌的外泌体能在创面愈合多阶段发挥作用, 在治疗慢性难愈合创面中展现出独特优势。外泌体是调节皮肤细胞生物学行为的细胞间通讯的新型载体, 广泛存在于多种体液中, 而且无免疫反应性、无致瘤性以及较好的生物相容性等优点, 其主要来源于脂肪源性干细胞、骨髓源性干细胞、人脐带干细胞等干细胞和其他类型的细胞。不同类型的外泌体根据其细胞来源和相邻细胞组分的不同, 功能各具特点, 在创面愈合中发挥的作用也不同。因此, 文章对不同外泌体在创面愈合不同阶段中的具体作用和机制进行了探讨, 并对目前的局限性和各种观点进行了综述。

关键词 外泌体; 创面愈合; 慢性创面; 干细胞; 再生

Preclinical Research Progress of Wound Healing Treated by Multiple Exosomes

TENG Yongxiang[1,2], SU Gang[1], LI Zhaoxin[3], ZHAI Zhaohui[4], LI Yuli[5], CHEN Yuanzheng[6]*

([1]*School of Clinical Medicine, Shandong Second Medical University, Weifang 261053, China*; [2]*Department of Burn and Plastic Surgery, Linyi People's Hospital, Shandong Second Medical University, Linyi 276002, China*; [3]*Weifang Hospital of Traditional Chinese Medicine, Shandong Second Medical Universityy, Weifang 261053, China*; [4]*Institution of Plastic Surgery, Shandong Second Medical University, Weifang 261053, China*; [5]*School of Rehabilitation Sciences and Engineering, University of Health and Rehabilitation Sciences, Qingdao 266071, China*; [6]*Department of Burn and Plastic Surgery, Emergency General Hospital, Beijing 100028, China*)

Abstract Chronic refractory wound is a major public health problem in the world. The healing process involves a series of overlapping space and time stages, and its process is complex and diverse. For the use of existing clinical treatment of chronic refractory wounds that are difficult to heal, there is an urgent need to find a new treatment. The exosomes secreted by stem cells can play a role in multiple stages of wound healing, showing a unique advantage in the treatment of chronic refractory wounds. Exosomes is a new type of intercellular communication

收稿日期: 2024-07-10 接受日期: 2024-08-23

山东省自然科学基金(批准号: ZR2022MH211)和山东省自然科学基金重点项目(批准号: ZR2020KE018)资助的课题

*通信作者。Tel: 15963960228, E-mail: 15963960228@163.com

Received: July 10, 2024 Accepted: August 23, 2024

This work was supported by the Shandong Provincial Natural Science Foundation (Grant No.ZR2022MH211), and the Key Program of Shandong Provincial Natural Science Foundation (Grant No.ZR2020KE018)

*Corresponding author. Tel: +86-15963960228, E-mail: 15963960228@163.com

carrier that regulates the biological behavior of skin cells. It widely exists in a variety of body fluids and has the advantages of non-immunoreactivity, tumorigenicity and good biocompatibility. It mainly comes from adipose-derived stem cells, bone marrow-derived stem cells, human umbilical cord stem cells and other types of stem cells. Different types of exosomes have different functions and play different roles in wound healing according to their cell sources and adjacent cell components. Therefore, this paper discusses the specific role and mechanism of different exosomes in different stages of wound healing, and summarizes the current limitations and various viewpoints.

Keywords exosomes; wound healing; chronic wound; stem cell; regeneration

创面是由各种内在病理和外部机械因素损害皮肤组织结构或完整性造成的, 分为急性和慢性[1]。慢性创面主要指由多种因素, 如糖尿病、压力损伤、动脉/静脉功能不全、缺血性损伤、感染性伤口和手术伤口等引起, 持续4~12周仍未愈合或未表现出愈合趋势的创面[2-3]。目前慢性难愈合创面是全球范围内的主要临床治疗难题, 严重的甚至危及生命, 因此促进慢性难愈合创面的早愈合以及抑制皮肤/软组织创伤后的瘢痕形成是目前最为迫切的需求。

间充质干细胞(mesenchymal stem cells, MSCs)是一种非造血的多能成体干细胞, 可以从骨髓、脂肪组织或其他器官中分离得到, 具有黏附性和高分化能力等特性[4]。在过去的研究中, MSCs通过改善肉芽形成和再上皮化、增加再血管化、调节炎症和细胞外基质(extracellular matrix, ECM)修复来帮助缩短修复时间, 证实了MSCs在伤口愈合中的重要作用。基于这些特性, MSCs是创面组织修复和再生的合适候选者。然而, MSCs的细胞治疗存在一定的局限性, 例如不易储存、突变相关的致瘤性、细胞活性低、免疫排斥、移植存活率较低, 以及伦理因素的限制等, 成为了MSCs应用于创面治疗的主要障碍[5-6]。最近研究发现MSCs的组织再生能力主要来源于其旁分泌因子——外泌体(exosomes, Exos), 其被鉴定为MSCs外分泌的主要成分, 既有来源MSCs的特性, 又具自身的优点, 如无免疫反应性或致瘤性等, 进一步研究表明不同干细胞来源的Exos具有不同的治疗重点, 是治疗创面最具潜力的选择[7]。凭借这些特性, MSCs-exos成为了再生医学中MSCs的一种有前途的治疗替代品。

1 Exos的介绍

Exos是直径为30~150 nm的带负电荷的生物纳米级球形脂质双层囊泡, 多数类型细胞(包括MSCs)均能分泌, 并广泛存在于细胞培养上清液和许多体液如血清、唾液、乳汁、脑脊液、尿液和精液等中[8]。根据其细胞来源和相邻细胞组分的不同, Exos携带的生物活性物质的类型和功能各具特点。近几年的研究显示, Exos是一种具有诊断和治疗价值[9]的新型治疗手段, 是细胞间通信的重要介质, 能广泛地转运遗传物质以及蛋白质, 介导细胞间通讯促进组织再生和伤口闭合[10]。作为细胞间通讯工具, Exos具有以下优势。(1) Exos可以作为介质通过生物屏障, 其脂质双层结构可以保护转运物质, 实现细胞间的广泛转运, 完成多种生物学过程。(2) Exos分泌的细胞因子等活性物质可以促进伤口血管化改善伤口循环、调节炎症应激状态和募集干细胞进一步促进伤口修复[11]。(3) Exos特定的表面蛋白, 可以实现载体的靶向转运从而提高治疗的精确性, 例如作为药物载体, 可以提高药物治疗的有效浓度[12]。(4) 靶向编辑Exos内容、预处理MSCs或人工修饰Exos表面分子受体, 可以提升Exos的修复效果。所以Exos因其对生物过程调控和对受体细胞的特异性靶向作用等多种优势, 成为了再生医学领域和免疫疾病的研究热点。

2 不同Exos在创面愈合中的作用机制研究

皮肤组织损伤后的伤口愈合由重叠在一起的四个独立阶段组成[13], 包括止血、炎症、细胞增殖和组织重塑[14]。快速闭合损伤、预防感染和形成功能性瘢痕是完成创面愈合过程的关键步骤, 每个步骤出现问题都可导致伤口愈合延迟以及慢性伤口形成, 如过度炎症、细胞迁移和增殖受损、胶原蛋白(collagen)形成和沉积异常、上皮再形成被抑制等[15]。不同Exos各具治疗特点, 通过多种治疗机制, 如释放生物活性因子, 激活以及调节信号通路等方

式在创面愈合的不同阶段发挥促进创面愈合的作用(图1)。

2.1 止血

止血阶段主要表现为血小板的聚集和内外源性凝血途径的激活。Exos通过分泌氧化酶和糖蛋白，激活血小板以及促进凝血因子的生成，缩短凝血时间，同时通过影响细胞的生理学过程诱导血液的凝固，从而达到Exos促进止血的效果(图2)。血小板来源干细胞衍生的外泌体(platelet-derived exosomes, PD-exos)在止血阶段的血液循环中富集程度升高，通过自身表面存在的磷脂酰丝氨酸(phosphatidylserine, PS)凝血酶诱导激活的微小RNA[16][miR223(microRNA223, miRNA223)[17]、miR-142-3p[18]、miR-320b[19])]调节内皮细胞的表达，促进细胞的增殖。PD-exos与NADPH氧化酶(nicotinamide adenine dinucleotide phosphate oxidase, NADPH oxidase, NOX)[20]、骨髓细胞(bone marrow cells, BMCs)[21]相互作用，促进纤维蛋白的结合，发挥凝血作用。其他像脐带来源干细胞衍生的外泌体(umbilical cord-MSCs-derived exosomes, UC-MSCs-exos)，通过AIF核易位抑制以及活化聚ADP核糖聚合酶1(poly ADP-ribose polymerase-1, PARP-1)影响活性氧(reactive oxidative species, ROS)基团或分子诱导的细胞凋亡，诱导体外血液凝固[22]。除此之外，Exos还能降低血清免疫球蛋白E(immunoglobulin E, IgE)的水平，上调嗜酸性粒细胞和肥大细胞表达的白细胞介素-4(interleukin-4, IL-4)、IL-23、IL-31以及肿瘤坏死因子-α(tumor necrosis factor-α, TNF-α)的表达，释放组胺，缩短凝血过程，促进创面愈合[23]。

2.2 炎症

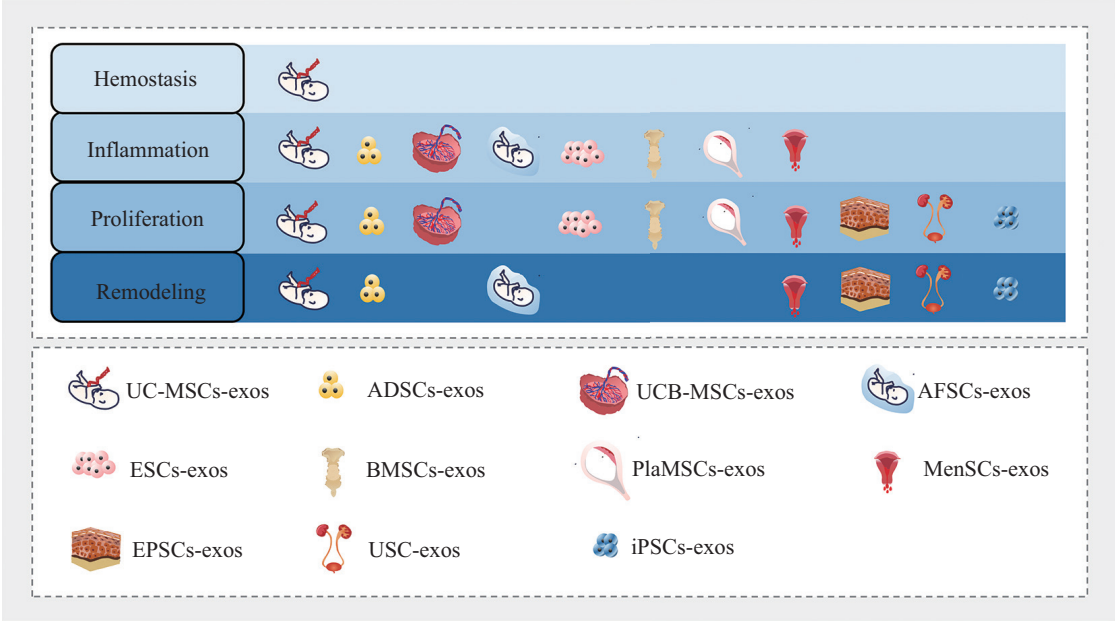
出血停止时，炎症阶段的中性粒细胞和巨噬细胞开始依次募集发挥清除细菌和细胞碎片的作用，伴随着巨噬细胞促炎(classically activated macrophage, M1)/抗炎(alternatively activated macrophage, M2)途径的转变，刺激人真皮成纤维细胞(human dermal fibroblasts-adult, HDF)和上皮细胞生成，促进血管的形成，使炎症阶段过渡至增殖阶段。然而炎性细胞的过度聚集、巨噬细胞的极化促炎、ROS的过度生成，都会导致正常组织的损伤和正常细胞的死亡。Exos可以通过抑制炎症细胞因子的分泌、加快M2/M1的极化速率促进炎症阶段向增殖阶段的转变，抑制ROS相关蛋白的表达，改善创面的愈合情况，在炎症阶段的抗炎、抗氧化、抗凋亡过程中发挥重要作用(图3)。

2.2.1 抗炎 胎盘间充质干细胞来源外泌体(placenta MSCs-derived exosomes, PlaMSCs-exos)。PlaMSCs-exos通过多个信号通路的调节变化在抗炎以及其他创面治疗过程发挥作用，例如PlaMSCs-exos抑制细胞外调节蛋白激酶(extracellular regulated protein kinase, ERK)、c-Jun氨基末端激酶(c-Jun N-terminal kinase, JNK)、p38丝裂原活化蛋白激酶(p38 mitogen-activated protein kinase, p38 MAPK)、磷脂酰肌醇3-激酶(phosphoinositide 3-kinase, PI3K)和蛋白激酶B(protein kinase B, AKT)的磷酸化，以及核因子-κB(nuclear factor kappa-B, NF-κB)的核转位和凋亡相关蛋白的表达，减轻炎症反应，发挥抗炎作用[24]，为炎症阶段的非细胞治疗提供了新的途径。除此之外，研究表明PlaMSCs-exos能有效促进创面再生，其治疗作用与PlaMSCs相似但又优于PlaMSCs，例如促进血管生成相关基因表达，刺激内皮血管形成以及促进细胞迁移。通过下调Yes相关蛋白(yes-associated protein, Yap)信号转导途径从而抑制同源框1基因(engrailed homeobox 1, *EN1*)的活化来实现伤口再生[25]。当然，PlaMSCs-exos涉及的丝裂原活化蛋白激酶激酶(mitogen-activated protein kinase kinase, MAPKK, 又称MEK)/ERK/环磷腺苷效应元件结合蛋白(cAMP-response element binding protein, CREB)信号转导的激活，还具有促进自主神经恢复的作用，对于创面治疗具有重要的指导价值[26]。

2.2.2 抗氧化 抗氧化方面的内容主要聚集在以下两方面。

(1) 脂肪干细胞来源的外泌体(ADSCs-derived exosomes, ADSCs-exos)。ADSCs-exos是目前研究最多的Exos之一，能改善创面氧化应激和炎性细胞因子的分泌，增加创面周围血管生成，减少创面损伤，改善瘢痕，从而促进创面愈合。

在炎症阶段，ADSCs-exos可以抑制细胞内ROS的生成，通过下调NOX1、NOX4(ROS的主要来源)等炎症和氧化应激相关蛋白的水平，参与炎症过程。LI等[27]发现过表达核因子E2相关因子(nuclear factor erythroid 2-related factor 2, Nrf2)的ADSCs-exos能抑制ROS炎症细胞因子(IL-1β、IL-6和TNF-α)的分泌和表达。另外Nrf2高表达的ADSCs-exos可以提高衰老标记蛋白30(senescence marker protein

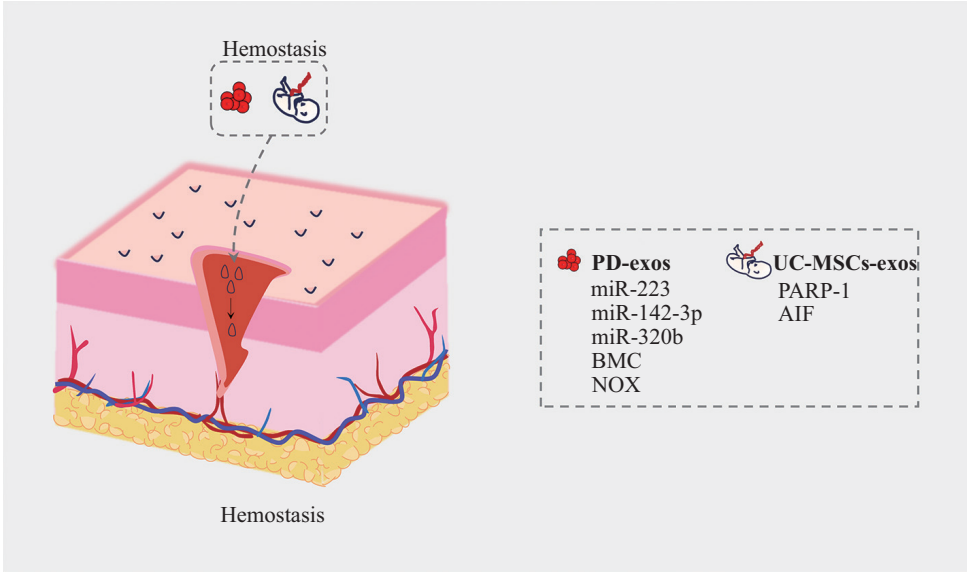


UC-MSCs-exos: 脐带来源干细胞衍生的外泌体; ADSCs-exos: 脂肪干细胞来源的外泌体; UCB-MSCs-exos: 脐带血干细胞来源的外泌体; AFSCs-exos: 羊水干细胞来源外泌体; ESCs-exos: 胚胎干细胞来源外泌体; BMSCs-exos: 骨髓间充质干细胞来源外泌体; PlaMSCs-exos: 胎盘间充质干细胞来源外泌体; MenSCs-exos: 月经血来源的间充质干细胞来源的外泌体; EPSCs-exos: 表皮干细胞来源外泌体; USCs-exos: 尿源性干细胞来源的外泌体; iPSCs-exos: 多功能诱导干细胞来源的外泌体。

UC-MSCs-exos: umbilical cord-MSCs-derived exosomes; ADSCs-exos: adipose-derived stem cells-derived exosomes; UCB-MSCs-exos: umbilical cord blood-MSCs-derived exosomes; AFSCs-exos: amniotic fluid stem cells-derived exosomes; ESCs-exos: embryonic stem cells-derived exosomes; BMSCs-exos: bone marrow-MSCs-derived exosomes; PlaMSCs-exos: placenta MSCs-derived exosomes; MenSCs-exos: menstrual blood-MSCs-derived exosomes; EPSCs-exos: epidermal stem cells-derived exosomes; USCs-exos: urine-derived stem cells-derived exosomes; iPSCs-exos: induced pluripotent stem cells-derived exosomes.

图1 不同外泌体作用于创面愈合多个阶段示意图

Fig.1 The schematic diagram of different exosomes acting on multiple stages of wound healing

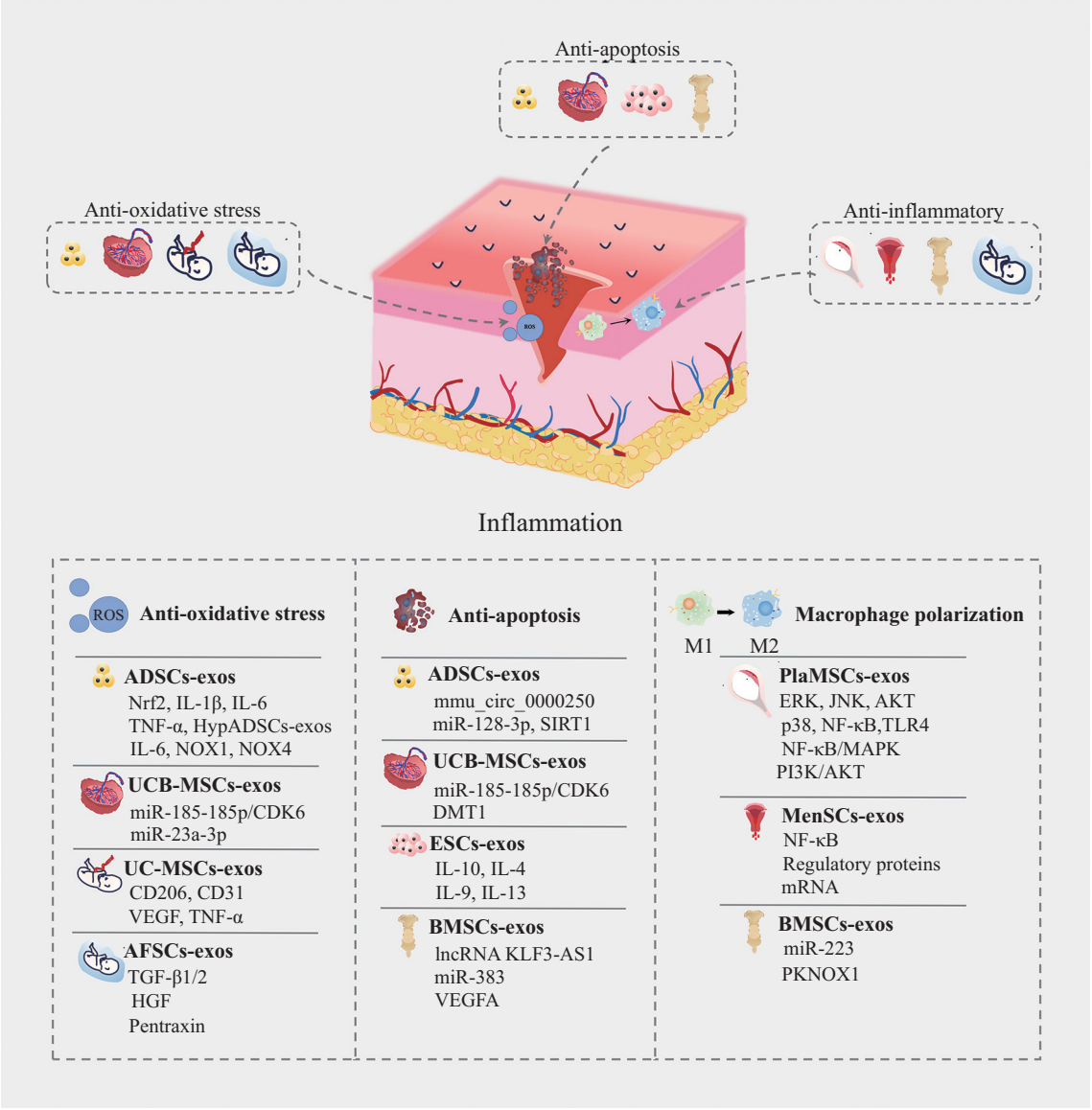


PD-exos: 血小板来源干细胞衍生的外泌体; UC-MSCs-exos: 脐带来源干细胞衍生的外泌体; BMCs: 骨髓细胞。

PD-exos: platelet-derived exosomes; UC-MSCs-exos: umbilical cord-MSCs-derived exosomes; BMCs: bone marrow cells.

图2 不同外泌体在创面愈合止血阶段的不同作用机制

Fig.2 Different mechanisms of various exosomes in the hemostasis stage of wound healing



ADSCs-exos: 脂肪干细胞来源的外泌体; UCB-MSCs-exos: 脐带血干细胞来源的外泌体; UC-MSCs-exos: 脐带来源干细胞衍生的外泌体; AFSCs-exos: 羊水干细胞来源外泌体; ESCs-exos: 胚胎干细胞来源外泌体; BMSCs-exos: 骨髓间充质干细胞来源外泌体; PlaMSCs-exos: 胎盘间充质干细胞来源外泌体; MenSCs-exos: 月经血来源的间充质干细胞来源的外泌体。

ADSCs-exos: adipose-derived stem cells-derived exosomes; UCB-MSCs-exos: umbilical cord blood-MSCs-derived exosomes; UC-MSCs-exos: umbilical cord-MSCs-derived exosomes; AFSCs-exos: amniotic fluid stem cells-derived exosomes; ESCs-exos: embryonic stem cells-derived exosomes; BMSCs-exos: bone marrow-MSCs-derived exosomes; PlaMSCs-exos: placenta MSCs-derived exosomes; MenSCs-exos: menstrual blood-MSCs-derived exosomes.

图3　不同种外泌体在创面愈合炎症阶段的不同作用机制

Fig.3　Different mechanisms of various exosomes in the inflammation stage of wound healing

30, SMP30)、血管内皮生长因子(vascular endothelial growth factor, VEGF)和血管内皮细胞生长因子受体2(vascular endothelial growth factor receptor 2, VEGFR2)的磷酸化水平，促进内皮祖细胞增殖以及血管生成。WU等[28]通过低氧预处理的ADSCs-exos(hypoxia adipose stem cells-derived exosomes, HypADSCs-exos)下调糖尿病小鼠炎症因子的表达，抑制过度炎症的发生。预处理的ADSCs-exos还能发挥在炎症阶段之外的创面修复作用，mmu_circ_0000250修饰的ADSCs-exos可以逆转高糖环境下血管生成的抑制作用，SHI等[29]发现mmu_circ_0000250的过表达，可以下调miR-128- 3p的表达水平，增强沉寂信息调节因子1(silent information regulator 1, SIRT1)的表达能力，通过自噬激活抑制

细胞凋亡, 加速全层皮肤伤口愈合。炎症期之后, ADSCs-exos抑制高糖环境下内皮细胞的损伤、促进内皮祖细胞的分化以及真皮细胞和角质形成细胞(keratinocyte, KC)的增殖, 促进创面愈合[30-31]。同时ADSCs-exos高表达的血管生成素1(angiopoietin 1, ANG1)、胎肝激酶-1(fetal liver kinase-1, FILK1)和VEGF, 能协同增强血管内皮细胞的活性, 提高血管生成的能力[32]。除此之外WU等[33]研究发现, 激活NF-κB通路, ADSCs-exos可以增强脂多糖(lipopolysaccharides, LPS)刺激的人脐静脉内皮细胞(human umbilical vein endothelial cells, HUVECs)的血管生成能力, 另外AKT和ERK信号通路, 也有相似的作用。在创面愈合后期, ADSCs-exos通过抑制转化生长因子-β2(transforming growth factor-β2, TGF-β2)表达下调神经源性基因Notch同源蛋白1(neurogenic locus notch homolog protein 1, Notch-1), 可以抑制瘢痕疙瘩成纤维细胞中细胞外基质的产生[34]。ADSCs-exos还可以被HDF内化, 增加受体中的神经钙黏蛋白(neural-cadherin, N-cadherin)、细胞周期蛋白-1(cyclin-1)、增殖细胞核抗原(proliferating cell nuclear antigen, PCNA)、collagen I、collagen III、水通道蛋白3(aquaporin, AQP3)等的表达, 下调TNF-α的表达, 减少瘢痕形成[35-36]。

(2) 脐带血干细胞来源的外泌体(umbilical cord blood-MSCs-derived exosomes, UCB-MSCs-exos)。UCB-MSCs-exos在皮肤创伤愈合的细胞方面发挥重要的调节作用。UCB-MSCs-exos中检测到大量的miRNA, 这些miRNA通过翻译抑制或mRNA降解胚胎相关的关键调节因子, 例如miR-185-185p(其下调会导致内皮血管生成受损, 上调则能避免HUVECs受到的高氧诱导损伤), 抑制细胞凋亡并促进细胞迁移, 通过miR-185-185p/细胞分裂蛋白激酶6(cyclin-dependent kinase 6, CDK6)级联反应提高细胞的抗氧化能力[37-38]。研究还发现, 表达miR-23a-3p的UCB-MSCs-exos可以降低细胞内ROS水平, 通过靶基因二价金属转运蛋白1(divalent metal transporter 1, DMT1)抑制铁凋亡减轻细胞的损伤[39], 同时UCB-MSCs-exos作为刺激HDF中的ECM蛋白生长和分泌的关键因素, 可用于皮肤再生材料, 更进一步显示出了UCB-MSCs-exos作为无细胞治疗剂的潜力。

2.2.3 抗凋亡 胚胎干细胞来源于外泌体(embryonic stem cells-derived exosomes, ESCs-exos)。胚胎干细胞(embryonic stem cells, ESCs)具有无限的自我更新能力和多向分化潜能, 其特异性miRNA、mRNA和蛋白质, 为ESCs提供了再生的潜力。ESCs-exos含有除碱性成纤维细胞生长因子(basic fibroblast growth factor, Bfgf)之外的许多蛋白质和miRNA, 可以显著促进血管形成, 促进细胞增殖[40]。ESCs-exos可以抑制炎症和细胞凋亡, 上调抗炎细胞因子IL-10、IL-4、IL-9和IL-13的表达。在体外, ESCs-exos下调促纤维化标志物的表达, 调节细胞表型, 并且通过miR-6766-3p显著下调TGF-β及其下游SMADs蛋白(drosophila mothers against decapentaplegic protein, SMADs), 尤其是磷酸化蛋白p-SMAD2/3(phospho-SMAD2/3)的表达, 促进血管新生, 恢复衰老内皮细胞的活力, 进而促进创面愈合[41-43]。因此, ESCs-exos有望成为治疗衰老相关疾病的天然纳米生物材料, 这为损伤或疾病后的再生医学和组织替代提供了更好的选择。

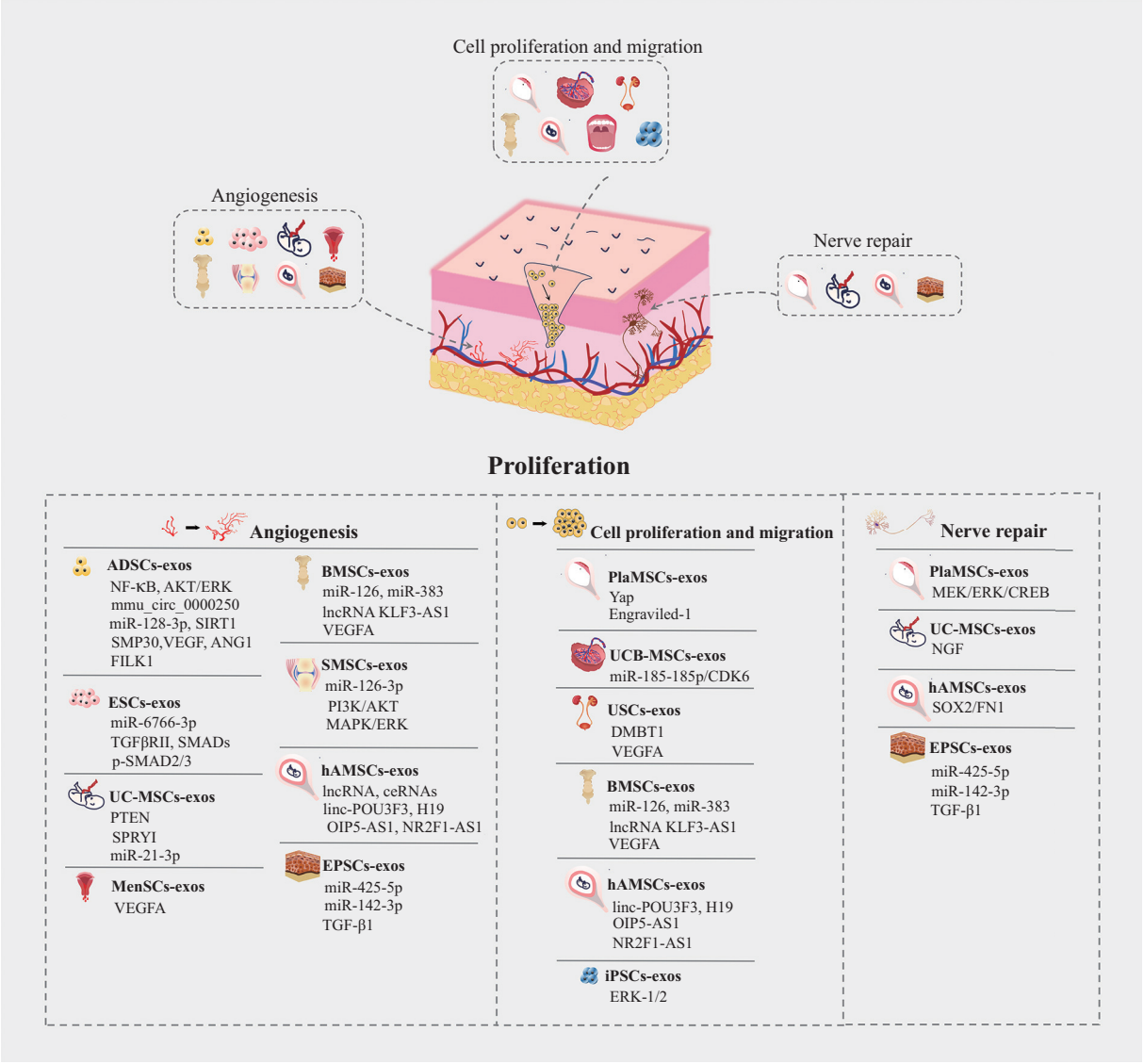
2.3 增殖

增殖阶段重要的是肉芽组织的形成以及新生血管的生成。HDF分泌细胞外基质[纤连蛋白(fibronectin)和collagen I/III], 促进细胞的迁移和增殖。细胞活性因子作用于血管, 促进血管的再生, 加快创面的覆盖。Exos可以分泌多种细胞因子如TGF-β和血小板源性生长因子(platelet-derived growth factor, PDGF), 协同刺激细胞外基质和新生血管的生成, 刺激HDF、上皮细胞等向损伤部位迁移, 增强受损部位的上皮化, 改善创面的血液供应, 发挥治疗作用, 影响创面愈合的增殖期(图4)。

2.3.1 细胞增殖、迁移 细胞增殖和迁移方面包括以下三方面的内容。

(1) UC-MSCs-exos。脐带间充质干细胞(UC-MSCs)以其多能性而闻名, UC-MSCs是典型的自我更新和多潜能成体干细胞, 可分化为终末细胞。其分泌的UC-MSCs-exos具有良好的稳定性和免疫原性, 能转运多种蛋白和生长因子, 其治疗方式涉及炎症、增殖、重塑多个阶段, 是研究较多的一类Exos[44]。

UC-MSCs-exos的体内外实验表明其具有调节内皮细胞抗氧化应激损伤的能力, 创面检测到CD206、CD31、VEGF相关促进愈合标志物的上调, 以及TNF-α的下调, 同时UC-MSCs-exos促进HUVECs和小鼠胚胎成纤维细胞(NIH 3T3)的增殖, 减轻氧化



ADSCs-exos: 脂肪干细胞来源的外泌体; ESCs-exos: 胚胎干细胞来源外泌体; UC-MSCs-exos: 脐带来源干细胞衍生的外泌体; MenSCs-exos: 月经血来源的间充质干细胞来源的外泌体; BMSCs-exos: 骨髓间充质干细胞来源外泌体; SMSCs-exos: 滑膜间充质干细胞来源外泌体; hAMSCs-exos: 人羊膜间充质干细胞来源的外泌体; EPSCs-exos: 表皮干细胞来源外泌体; PlaMSCs-exos: 胎盘间充质干细胞来源外泌体; UCB-MSCs-exos: 脐带血干细胞来源的外泌体; USCs-exos: 尿源性干细胞来源的外泌体; iPSCs-exos: 多功能诱导干细胞来源的外泌体。

ADSCs-exos: adipose-derived stem cells-derived exosomes; ESCs-exos: embryonic stem cells-derived exosomes; UC-MSCs-exos: umbilical cord-MSCs-derived exosomes; MenSCs-exos: menstrual blood-MSCs-derived exosomes; BMSCs-exos: bone marrow-MSCs-derived exosomes; SMSCs-exos: synovial-MSCs-derived exosomes; hAMSCs-exos: human amniotic membrane-MSCs-derived exosomes; EPSCs-exos: epidermal stem cells-derived exosomes; PlaMSCs-exos: placenta MSCs-derived exosomes; UCB-MSCs-exos: umbilical cord blood-MSCs-derived exosomes; USCs-exos: urine-derived stem cells-derived exosomes; iPSCs-exos: induced pluripotent stem cells-derived exosomes.

图4　多种外泌体在创面愈合增殖阶段的不同作用机制

Fig.4　Different mechanisms of various exosomes in the proliferation stage of wound healing

应激损伤, 加速皮肤的伤口愈合。在体内, UC-MSCs-exos能减少炎症浸润、加快血管重塑、促进胶原纤维的增殖, 可在多个阶段促进创面的修复[45]。值得注意的是, 富集在UC-MSCs-exos中的miR-21-3p能够抑制PTEN(phosphatase and tensin homolog deleted on chromosome ten)和软脂酰化磷蛋白(sprouty homolog 1, SPRYI)的表达, 有助于加速上皮再形成、减少瘢痕宽度和促进血管生成[46], 同时PTEN还可以通过和过表达miR-150-5p的UC-MSCs-exos协同激活PI3K/AKT通路促进皮肤创面愈合, 建立起一种PTEN/PI3K/AKT和PTEN/miR-21-3p的相互作用关系[47]。在神经方面, 现有研究证明了失神经是创

面难愈合的重要因素之一, 如糖尿病创面的周围神经损伤已经被认定为糖尿病创面难愈合的主要原因之一, ZHU等[48]研究发现, UC-MSCs-exos可以体外刺激HDF分泌神经生长因子(nerve growth factor, NGF), 从而促进皮肤神经再生、皮肤再生和全层皮肤伤口愈合, 不仅为神经相关治疗打开了一个新的治疗方向, 也进一步体现了UC-MSCs-exos的治疗价值。

(2) 尿源性干细胞来源的外泌体(USCs-derived exosomes, USCs-exos)。尿源性干细胞(urine-derived stem cells, USCs)与其他干细胞相比在获取、安全、培养和适用性等方面具有不可取代的优势, 正在成为Exos提取的优良来源。USCs-exos可以增强伤口愈合的相关细胞功能, 包括KC、HDF和HUVEC等细胞的增殖和迁移能力, 以及内皮细胞的血管生成能力。同时USCs-exos富含参与调节伤口愈合相关生物过程的蛋白质, 如VEGFA、Sushi重复蛋白X连锁2(sushi repeat containing protein X-linked 2, SRPX2)、血管生成素相关4蛋白(angiopoietin like 4, ANGPTL4)等, 这些蛋白促进髓核细胞(nucleus pulposus cell, NPC)增殖和ECM合成, 激活TGF-β, 提高SMAD和AKT的磷酸化水平, 促进基质蛋白3(matrilin 3, MATN3)的表达, 帮助调节ECM的稳态[49-50], 尤其, 促血管生成蛋白1(DMBT1)在USCs-exos中极高表达, 显著诱导VEGFA蛋白高表达以及显著促进AKT的磷酸化(PI3K活化的指标), 且当DMBT1表达被抑制时则会出现逆转现象, 进一步证明了DMBT1在USCs-exos参与的创面治疗过程中的重要性[49,51]。

(3) 月经血来源的间充质干细胞来源的外泌体(menstrual blood-MSCs-derived exosomes, MenSCs-exos)。MenSCs提供了一种既无痛又没有BM-MSCs捐赠所引起的伦理问题的替代方式, MenSCs表达的八聚体结合转录因子4(octamer transcription factor 4, OCT-4), 这是ESCs的标志物, 与其他来源的MSCs相比是一种独特的标志物[51], 间接反映出MenSCs-exos具有独特的治疗优势。例如在创面的修复过程中, MenSCs-exos通过激活NF-κB诱导巨噬细胞极化抑制炎症的发生、下调I/III型胶原比值减少瘢痕的形成、上调*collagen I*的mRNA促进再上皮化提高伤口闭合率、上调VEGFA促进新生血管生成等多个阶段促进创面的愈合[52]。同时MenSCs-exos通过递送大量调节蛋白和mRNA(let-7、miR21)改善受损细胞和组织的再生修复[53]。在MenSCs-exos治疗优势的基础之上, 通过对MenSCs-exos相关的新技术, 例如工程分子、低氧处理条件、靶向药物、癌症免疫疗法和水凝胶等的研究, MenSCs-exos的治疗潜力有望进一步被发掘。

2.3.2 血管生成 血管生成方面主要包括以下三方面的内容。

(1) 骨髓间充质干细胞来源外泌体(bone marrow-MSCs-derived exosomes, BMSCs-exos)。BMSCs-exos是首个被研究的干细胞Exos, 主要通过调控炎症阶段的巨噬细胞表型转变发挥创面治疗作用。通过输注没有Exos的BMSCs, 只有低水平的M2型巨噬细胞表达并延迟伤口修复, 同时表达miR-223的Exos通过靶向PKNOX1(pbx/knotted 1 homeobox 1)调节巨噬细胞极化, 促进了机体的抗炎能力都证实了上述观点[54]。除了促进巨噬细胞的表型转变外, 近期非编码RNA与BMSCs-exos的应用, 为创面愈合领域提供了许多新的治疗思路。在血管生成方面, miR-126是一种内皮特异性miRNA, 与血管的稳态以及生成有关, 过表达miR-126的BMSCs-exos可以促进HUVECs的增殖、迁移和血管生成[55], 而表达长链非编码RNA KLF3-AS1(klf3 antisense RNA 1, lncRNA KLF3-AS1)的BMSCs-exos也促进了高糖环境下HUVECs的增殖、迁移和管腔形成, BMSCs-exos表现出了在血管生成方面的潜力。不仅如此, 表达lncRNA KLF3-AS1的BMSCs-exos还能抑制细胞凋亡, 下调miR-383促进其靶点VEGFA的表达, 促进创面愈合, 研究发现体重减轻可以抑制其促进创面愈合的作用[56], 故通过影响体重可以进一步改善创面的愈合情况。BMSCs-exos除了参与炎症阶段的巨噬细胞表型转变的主要作用外, 与非编码RNA的联合应用以及通过干涉体重等相关因素改善创面愈合效果, 都体现出了BMSCs-exos在创面治疗领域还有更多提高治疗效益的新方式。

(2) 滑膜间充质干细胞来源外泌体(synovial-MSCs-derived exosomes, SMSCs-exos)。 来源于滑膜的MSCs(synovial MSCs), 具有组织特异性, 能促进结缔组织生成和HDF的增殖[57], 具有高效的修复性。但SMSCs-exos不具备促血管生成的能力, 研究发现利用基因过表达技术修饰SMSCs, 通过过表达miR-126-3p(micro-RNA-126-3p), 可以将内皮祖细胞的血管生成能力转移到SMSCs-exos之中, 能显著激

活PI3K/AKT和促分裂素原活化蛋白激酶(mitogen-activated protein kinases, MAPK)/ERK通路, 从而加速上皮再生, 激活血管生成能力, 并促进体内胶原成熟, 对于缺血性疾病以及创面的新生血管生成和组织修复来说具有重要的治疗意义[58-59]。这种通过基因过表达修饰改善Exos功能的方式, 赋予SMSCs-exos更多的治疗能力以及治疗优势, 从而参与更多的创面愈合过程, 在发挥SMSCs-exos治疗优势的同时, 进一步提升治疗效率。

(3) 人羊膜间充质干细胞来源的外泌体(human amniotic membrane-MSCs-derived exosomes, hAMSCs-exos)。人羊膜间充质干细胞(human amniotic membrane-MSCs, hAMCs)是来源于上胚层的多能祖细胞, 具有胚胎干细胞和间充质干细胞的特征, 具有分化为所有三个胚层的潜力[60], 有抗纤维化、抗炎等优点。hAMSCs-exos含有多种生物活性分子, 在处理损伤组织时可以分泌抗凋亡、抗纤维化、抗炎和促血管生成相关的多种细胞因子, 如与血管生成相关的lncRNA包括PANTR1(也称为linc-POU3F3)、H19(long noncoding RNA H19)、OIP5-AS1(long noncoding RNA OIP5-AS1)和NR2F1-AS1(long noncoding RNA NR2F1-AS1)富集在hAMSCs-exos中, 可以调节内皮细胞的迁移、增殖和血管形成[61]。与UC-MSCs-exos类似, 羊膜间充质干细胞来源的施万细胞样细胞可以通过Exos诱导的SRY-box转录因子2[SRY(sex determining region Y)-box transcription factor 2, SOX2]/FN1通路促进坐骨神经修复[62], 对神经损伤同样具有治疗作用, 为未来联合UC-MSCs-exos和hAMSCs-exos治疗神经方面的损伤提供了更多的可能性。

2.4 重塑

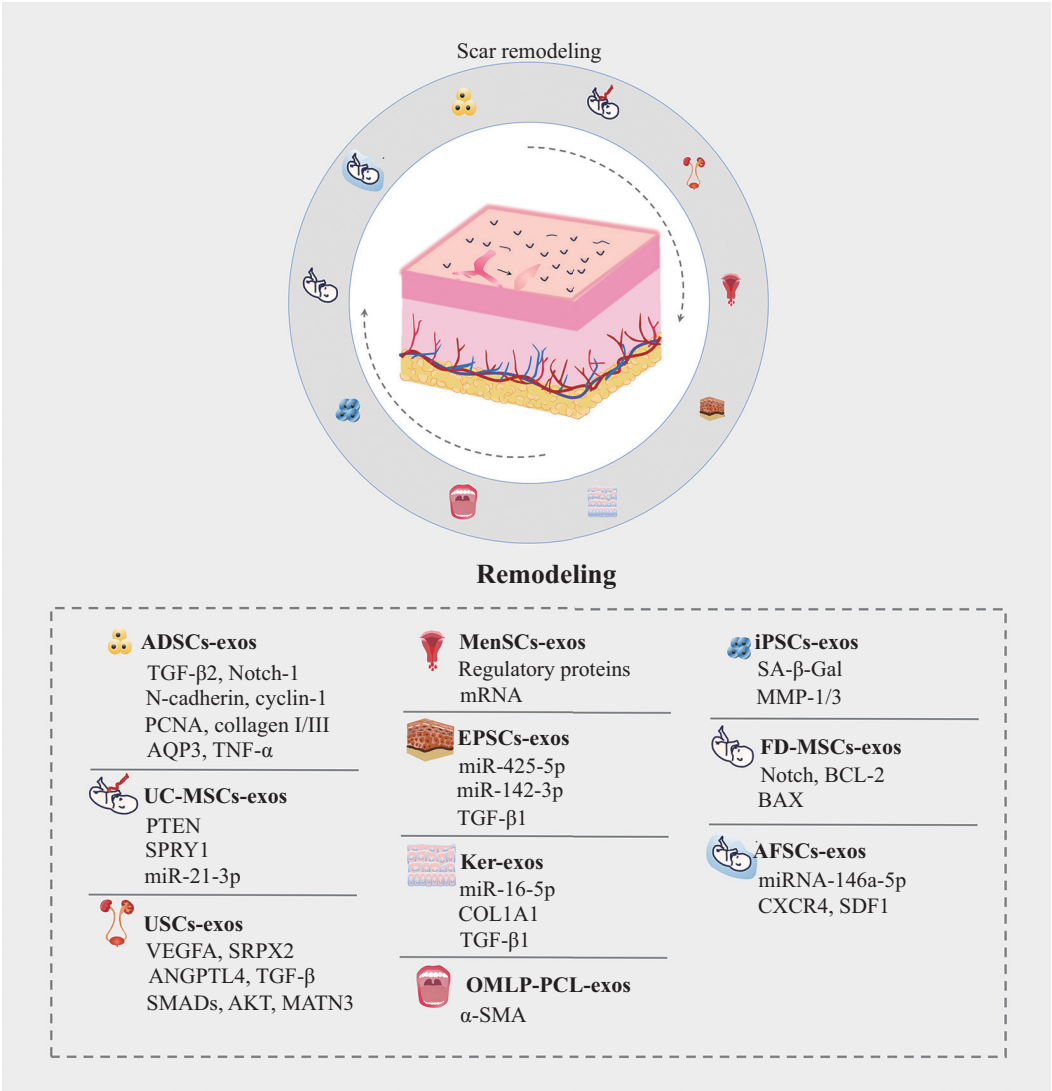
重塑阶段主要涉及到瘢痕的抑制以及增殖期的创面重塑, collagen III被更加强韧的collagen I取代, 细胞外基质产生和分解的平衡对于瘢痕形成发挥重要的作用。Exos通过作用于胶原交联以及收缩、促进细胞的运动和上皮再生、加快collagen I、collagen III以及fibronectin和细胞外基质组分的产生, 激活弹性蛋白和fibronectin的表达, 从而改善创面的瘢痕形成(图5)。

2.4.1 *表皮干细胞来源外泌体*(epidermal stem cells-derived exosomes, EPSCs-exos) 表皮干细胞(EPSCs)是附着在表皮基底层的重要组分, 在表皮的再生、维持、适应等方面都发挥重要作用[63]。EPSCs-exos可以促进全层创面的血管生成和表皮层的再生。研究发现, 表皮干细胞的临床应用可以显著促进慢性伤口愈合, 减少瘢痕形成, 因为EPSCs-exos中富含很多特异性的miRNA, 其中, 表达miR-425-5p和miR-142-3p的EPSCs-exos, 通过下调TGF-β1的表达抑制肌纤维和胶原沉积, 改善collagen的自然分布, 从而提升皮肤附件、血管和神经相关的再生水平, 表现出在抗瘢痕方面的临床潜力[64-65]。

2.4.2 *角质形成细胞来源外泌体*(keratinocyte-derived exosomes, Ker-exos) KC作为皮肤中最主要的细胞类型, 不仅作为结构细胞, 维持机械屏障, 还具有重要的免疫功能, 在创伤修复中起关键作用。像烧伤创面局部应用表达miR-16-5p的Ker-exos可以减少胶原沉积[66], 除此之外, 过表达miR-16-5p的Ker-exos也可以显著抑制TGF-β1对HDF增殖迁移和I型胶原α1(collagen type I alpha 1, COL1A1)表达的促进作用, 表现出了miR-16-5p过表达的Ker-exos在皮肤增生性瘢痕以及纤维化疾病方面的治疗潜力[67]。

2.4.3 *口腔黏膜前体细胞来源的外泌体*(oral mucosa lamina propria-progenitor cell line-derived exosomes, OMLP-PCL-exos) 口腔黏膜固有层(oral mucosa lamina propria, OMLP)的细胞起源于胚胎神经嵴, 与口腔中结缔组织的外胚间充质起源相似[68]。OMLP-PCL-exos具有多能、免疫抑制和抗菌作用, 在干细胞介导下, OMLP-PCL-exos可以在不同环境中发挥重要的修复作用, 包括促进细胞增殖和伤口再生以及下调肌成纤维细胞的形成。在小鼠的创伤模型中, OMLP-PCL-exos还显示出抑制αSMA阳性肌纤维母细胞形成的能力, 显著减少创伤后的胶原沉积[69], 改善创面的瘢痕增生情况。综上所述, OMLP-PCL-exos将来可作为一种新型的可移植治疗产品, 提高患有严重纤维化和疤痕的患者的生活质量, 为临床上的无疤痕伤口愈合提供新的治疗途径。

2.4.4 *多功能诱导干细胞来源的外泌体*(induced pluripotent stem cells-derived exosomes, iPSCs-exos) iPSCs-exos已被证明在创面修复的增殖、免疫和生物活性因子的分泌等方面都具有不可替代的优势, 在理论上可降低对免疫抑制药物的依赖[70]。iPSCs-exos含有多种人类iPSCs衍生蛋白, 还有能影响细胞生理学的各种mRNA、miRNA和lncRNA,



ADSCs-exos: 脂肪干细胞来源的外泌体; UC-MSCs-exos: 脐带来源干细胞衍生的外泌体; USCs-exos: 尿源性干细胞来源的外泌体; MenSCs-exos: 月经血来源的间充质干细胞来源的外泌体; EPSCs-exos: 表皮干细胞来源外泌体; Ker-exos: 角质形成细胞来源外泌体; OMLP-PCL-exos: 口腔黏膜前体细胞来源的外泌体; iPSCs-exos: 多功能诱导干细胞来源的外泌体; FD-MSCs-exos: 胎儿真皮间充质干细胞来源的外泌体; AFSCs-exos: 羊水干细胞来源外泌体。

ADSCs-exos: adipose-derived stem cells-derived exosomes; UC-MSCs-exos: umbilical cord-MSCs-derived exosomes; USCs-exos: urine-derived stem cells-derived exosomes; MenSCs-exos: menstrual blood-MSCs-derived exosomes; EPSCs-exos: epidermal stem cells-derived exosomes; Ker-exos: keratinocyte-derived exosomes; OMLP-PCL-exos: oral mucosa lamina propria-progenitor cell line-derived exosomes; iPSCs-exos: induced pluripotent stem cells-derived exosomes; FD-MSCs-exos: fetal dermal-MSCs-derived exosomes; AFSCs-exos: amniotic fluid stem cells-derived exosomes.

图5 多种外泌体在创面愈合重塑阶段的不同作用机制

Fig.5 The different mechanisms of various exosomes in the remodeling stage of wound healing

例如ERK-1/2的磷酸化, 促进皮肤细胞的增殖和迁移[71]。KIM等[72]通过iPSCs-exos显著下调衰老HDF中衰老相关的β-半乳糖苷酶(senescence-associated β-galactosidase activity, SA-β-Gal)和基质金属蛋白酶(matrix metalloproteinase-1/3, MMP-1/3)的表达水平, 从而提高人角质形成细胞(human immortalized keratinocytes, HaCaT)和HDF的活力, 加快细胞周期进程以及collagen的分泌、介导老化皮肤中基质的再平衡过程, 促进真皮基质的重建, 显示出了iPSCs-exos用于治疗皮肤老化的治疗潜力。除此之外, 干扰素γ(interferon γ, IFN-γ)预处理的iPSCs-exos也可使调节IL-25和IL-33的相关mRNA表达下调, 促进角蛋白1/10(keratin 1/10)、桥粒芯蛋白1(desmoglein 1, DSG1)的表达量增加, 进而抑制皮肤炎症恢复适当的皮肤屏障功能, 抑制T细胞介导的免疫应答促进伤口愈合[73]。然而, 关于iPSCs-exos治疗仍然存

在不确定因素，例如其分化潜力受到供体细胞来源的影响，导致iPSCs-exos生成的最佳细胞类型仍不确定，无法充分发挥iPSCs-exos的治疗潜力，所以iPSCs-exos的最佳治疗潜力有待发掘。

2.4.5 胎儿真皮间充质干细胞来源的外泌体(fetal dermal-MSCs-derived exosomes, FD-MSCs-exos) 胎儿真皮间充质干细胞(fetal dermal-MSCs, FD-MSCs)来源于意外流产胎儿的真皮，具有免疫原性低、体外扩增容易、增殖能力和分化能力强、无瘢痕组织修复潜力、高分裂率和较低免疫排斥反应等优点[34]。FD-MSCs-exos通过Notch信号通路激活HDF运动和分泌能力[74]，通过分泌可溶性物质抑制瘢痕疙瘩成纤维细胞(keloid fibroblast, KFs)的生物活性，下调抗凋亡蛋白B淋巴细胞瘤-2(b-cell lymphoma-2, BCL-2)的表达以及上调促凋亡蛋白BCL2相关X蛋白(bcl2-Associated X Protein, BAX)的表达，从而促进KFs的凋亡，进而抑制重塑阶段瘢痕的增生，得到更好的创面愈合效果[71]。

2.4.6 羊水干细胞来源外泌体(amniotic fluid stem cells-derived exosomes, AFSCs-exos) 在成人疾病或新生儿严重疾病的临床前研究证实了AFSCs-exos的再生作用。AFSCs-exos可以影响创面的细胞生长、免疫调节、凋亡、抗炎、抗氧化、血管生成，以及瘢痕形成。特别在抗瘢痕方面，过表达miRNA-146a-5p的AFSCs-exos通过靶向下调趋化因子受体4(c-x-c motif chemokine receptor 4, CXCR4)以及基质细胞衍生因子-1(stromal cell-derived factor-1, SDF1)的表达，促进ECM重塑以及无瘢痕伤口愈合[75]。而AFSCs-exos调节免疫应答的相关组分TGF-β1/2、肝细胞生长因子(hepatocyte growth factor, HGF)、五聚环蛋白(pentraxin)，除了可以抑制纤维化的发生改善瘢痕之外[76]，还能调节炎症阶段的巨噬细胞极化，发挥抗炎作用，证明了AFSCs-exos作为新型治疗方式的可行性。

3 总结和展望

慢性难愈合创面是目前临床面临的严峻挑战之一，常用的治疗方式已经不能满足当前患者的治疗期待，所以，无细胞治疗的Exos逐渐显示出巨大的治疗潜力。Exos含有各种生物活性蛋白质、核酸、miRNA，其中与生物材料的协同治疗模式是目前的研究热点。除了单个Exos应用于创面治疗外，不同Exos能针对伤口愈合的某一个环节发挥最佳的治疗效果，且多个Exos可以在创面愈合的不同阶段发挥联合治疗作用。这提示我们在未来的研究中，可以根据创面的情况采用多种Exos的联合应用来制定具有个性化的最佳治疗方案。

除了所提到的Exos的治疗优势之外，Exos研究也存在许多需要解决的问题，例如：(1) Exos中的非编码RNA、蛋白质、脂质等物质的含量以及最佳应用条件都尚未确定；(2) Exos不仅需要考虑其来源细胞的数量以及质量，还有成本问题，如果没有解决成本问题，Exos依旧不能取代现有的治疗手段；(3) 在Exos作用过程中，如何避免外部因素影响Exos的结构、功能及治疗效果；除此之外，供体自身的健康状况、遗传、性别和年龄等因素，也可能会影响Exos的治疗效果，甚至改变期望的治疗功效；(4) 需要有针对于Exos实验的开发策略，例如局部、全身、注射等各方面的测试实验，确定Exos是否有剂量依赖性，还需要更多不同来源的Exos的短期和长期的安全性评估，来保证其在临床使用的安全性；(5) Exos目前依然处于起步阶段，从临床前研究到临床研究的转化还有很长的一段距离，例如大规模Exos的制备、提取、分离以及长期储存的问题没有统一的标准，而且不同Exos具有异质性，其提取以及鉴定也需要针对性的策略标准。

因此，用于治疗不同慢性创面安全、有效、高效率、易生产的Exos及相关产品还需要去探索开发，需要更多的研究来确定不同创面所适用的最佳Exos类型，最终建立起从Exos来源的干细胞到临床治疗的标准化流程，实现Exos在临床促进创面愈合的广阔应用前景。

参考文献 (References)

[1] DISSEMOND J, ROMANELLI M. Inflammatory skin diseases and wounds [J]. Br J Dermatol, 2022, 187(2): 167-77.

[2] WU J, CHEN L H, SUN S Y, et al. Mesenchymal stem cell-derived exosomes: the dawn of diabetic wound healing [J]. World J Diabetes, 2022, 13(12): 1066-95.

[3] HAN G, CEILLEY R. Chronic wound healing: a review of current management and treatments [J]. Adv Ther, 2017, 34(3): 599-610.

[4] PÄTH G, PERAKAKIS N, MANTZOROS C S, et al. Stem cells in the treatment of diabetes mellitus-focus on mesenchymal stem cells [J]. Metabolism, 2019, 90: 1-15.

[5] MAZINI L, ROCHETTE L, ADMOU B, et al. Hopes and limits of adipose-derived stem cells (ADSCs) and mesenchymal stem

cells (MSCs) in wound healing [J]. Int J Mol Sci, 2020, 21(4): 1306.
[6] HADE M D, SUIRE C N, MOSSELL J, et al. Extracellular vesicles: emerging frontiers in wound healing [J]. Med Res Rev, 2022, 42(6): 2102-25.
[7] XIE M, WU D, LI G, et al. Exosomes targeted towards applications in regenerative medicine [J]. Nano Select, 2021, 2(5): 880-908.
[8] PSARAKI A, NTARI L, KARAKOSTAS C, et al. Extracellular vesicles derived from mesenchymal stem/stromal cells: the regenerative impact in liver diseases [J]. Hepatology, 2022, 75(6): 1590-603.
[9] NALLAKUMARASAMY A, JEYARAMAN M, MAFFULLI N, et al. Mesenchymal stromal cell-derived extracellular vesicles in wound healing [J]. Life, 2022, 12(11): 1733.
[10] SHIN K O, HA D H, KIM J O, et al. Exosomes from human adipose tissue-derived mesenchymal stem cells promote epidermal barrier repair by inducing *de novo* synthesis of ceramides in atopic dermatitis [J]. Cells, 2020, 9(3): 680.
[11] TUTUIANU R, ROSCA A M, IACOMI D M, et al. Human mesenchymal stromal cell-derived exosomes promote *in vitro* wound healing by modulating the biological properties of skin keratinocytes and fibroblasts and stimulating angiogenesis [J]. Int J Mol Sci, 2021, 22(12): 6239.
[12] ZHOU C, ZHANG B, YANG Y, et al. Stem cell-derived exosomes: emerging therapeutic opportunities for wound healing [J]. Stem Cell Res Ther, 2023, 14(1): 107.
[13] SHI J, MA X, SU Y, et al. MiR-31 mediates inflammatory signaling to promote re-epithelialization during skin wound healing [J]. J Invest Dermatol, 2018, 138(10): 2253-63.
[14] GOLCHIN A, HOSSEINZADEH S, JOUYBAR A, et al. Wound healing improvement by curcumin-loaded electrospun nanofibers and BFP-MSCs as a bioactive dressing [J]. Poly Adv Technol, 2020, 31(7): 18885.
[15] BROUGHTON G 2ND, JANIS J E, ATTINGER C E. The basic science of wound healing [J]. Plast Reconstr Surg, 2006, 117(7 Suppl): 12S-34S.
[16] SPAKOVA T, JANOCKOVA J, ROSOCHA J. Characterization and therapeutic use of extracellular vesicles derived from platelets [J]. Int J Mol Sci, 2021, 22(18): 9701.
[17] LAFFONT B, CORDUAN A, PLÉ H, et al. Activated platelets can deliver mRNA regulatory Ago2•microRNA complexes to endothelial cells via microparticles [J]. Blood, 2013, 122(2): 253-61.
[18] BAO H, CHEN YX, HUANG K, et al. Platelet-derived microparticles promote endothelial cell proliferation in hypertension via miR-142-3p [J]. FASEB J, 2018, 32(7): 3912-23.
[19] GIDLÖF O, VAN DER BRUG M, OHMAN J, et al. Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression [J]. Blood, 2013, 121(19): 3908-S26.
[20] GASPAR R S, FERREIRA P M, MITCHELL J L, et al. Platelet-derived extracellular vesicles express NADPH oxidase-1 (Nox-1), generate superoxide and modulate platelet function [J]. Free Radic Biol Med, 2021, 165: 395-400.
[21] FRENCH S L, BUTOV K R, ALLAEYS I, et al. Platelet-derived extracellular vesicles infiltrate and modify the bone marrow during inflammation [J]. Blood Adv, 2020, 4(13): 3011-23.
[22] ZHAO G, LIU F, LIU Z, et al. MSC-derived exosomes attenuate cell death through suppressing AIF nucleus translocation and enhance cutaneous wound healing [J]. Stem Cell Res Ther, 2020, 11(1): 174.
[23] NIU S H, LI B, GU H C, et al. Knowledge mapping of extracellular vesicles in wound healing: a bibliometric analysis (2002-2022) [J]. Int Wound J, 2023, 20(8): 3221-40.
[24] HU Y, QU H, HE J, et al. Human placental mesenchymal stem cell derived exosomes exhibit anti-inflammatory effects via TLR4-mediated NF-κB/MAPK and PI3K signaling pathways [J]. Pharmazie, 2022, 77(3): 112-7.
[25] ZHANG Y, SHI L, LI X, et al. Placental stem cells-derived exosomes stimulate cutaneous wound regeneration via engrailed-1 inhibition [J]. Front Bioeng Biotechnol, 2022, 10: 1044773.
[26] ZHOU W, SILVA M, FENG C, et al. Exosomes derived from human placental mesenchymal stem cells enhanced the recovery of spinal cord injury by activating endogenous neurogenesis [J]. Stem Cell Res Ther, 2021, 12(1): 174.
[27] LI X, XIE X, LIAN W, et al. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model [J]. Exp Mol Med, 2018, 50(4): 1-14.
[28] WANG J, WU H, PENG Y, et al. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways [J]. J Nanobiotechnology, 2021, 19(1): 202.
[29] SHI R, JIN Y, HU W, et al. Exosomes derived from mmu_circ_0000250-modified adipose-derived mesenchymal stem cells promote wound healing in diabetic mice by inducing miR-128-3p/SIRT1-mediated autophagy [J]. Am J Physiol Cell Physiol, 2020, 318(5): C848-C56.
[30] SHI R, JIN Y, ZHAO S, et al. Hypoxic ADSC-derived exosomes enhance wound healing in diabetic mice via delivery of circ-Snhg11 and induction of M2-like macrophage polarization [J]. Biomed Pharmacother, 2022, 153: 113463.
[31] HSU H H, WANG A Y L, LOH C Y Y, et al. Therapeutic potential of exosomes derived from diabetic adipose stem cells in cutaneous wound healing of db/db mice [J]. Pharmaceutics, 2022, 14(6): 1206.
[32] ZHANG Y, BAI X, SHEN K, et al. Exosomes derived from adipose mesenchymal stem cells promote diabetic chronic wound healing through SIRT3/SOD2 [J]. Cells, 2022, 11(16): 2568.
[33] WU S C, KUO P J, RAU C S, et al. Increased angiogenesis by exosomes secreted by adipose-derived stem cells upon lipopolysaccharide stimulation [J]. Int J Mol Sci, 2021, 22(16): 8877.
[34] WANG X, JIAO Y, PAN Y, et al. Fetal dermal mesenchymal stem cell-derived exosomes accelerate cutaneous wound healing by activating notch signaling [J]. Stem Cells Int, 2019, 2019: 2402916.
[35] HU L, WANG J, ZHOU X, et al. Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts [J]. Sci Rep, 2020, 10(1): 6693.
[36] ZHOU Y, ZHAO B, ZHANG X L, et al. Combined topical and systemic administration with human adipose-derived mesenchymal stem cells (hADSC) and hADSC-derived exosomes mark-

edly promoted cutaneous wound healing and regeneration [J]. Stem Cell Res Ther, 2021, 12(1): 257.

[37] PAULI A, RINN J L, SCHIER A F. Non-coding RNAs as regulators of embryogenesis [J]. Nat Rev Genet, 2011, 12(2): 136-49.

[38] ZHONG X Q, WANG D, CHEN S, et al. Umbilical cord blood-derived exosomes from healthy term pregnancies protect against hyperoxia-induced lung injury in mice [J]. Clin Transl Sci, 2023, 16(6): 966-77.

[39] SONG Y, WANG B, ZHU X, et al. Human umbilical cord blood-derived MSCs exosome attenuate myocardial injury by inhibiting ferroptosis in acute myocardial infarction mice [J]. Cell Biol Toxicol, 2021, 37(1): 51-64.

[40] PANG Y, MA M, WANG D, et al. Embryonic stem cell-derived exosomes attenuate transverse aortic constriction induced heart failure by increasing angiogenesis [J]. Front Cardiovasc Med, 2021, 8: 638771.

[41] SINGLA D K, JOHNSON T A, TAVAKOLI DARGANI Z. Exosome treatment enhances anti-inflammatory M2 macrophages and reduces inflammation-induced pyroptosis in doxorubicin-induced cardiomyopathy [J]. Cells, 2019, 8(10): 1224.

[42] TAVAKOLI DARGANI Z, SINGLA D K. Embryonic stem cell-derived exosomes inhibit doxorubicin-induced TLR4-NLRP3-mediated cell death-pyroptosis [J]. Am J Physiol Heart Circ Physiol, 2019, 317(2): H460-71.

[43] WANG N, LI X, ZHONG Z, et al. 3D hESC exosomes enriched with miR-6766-3p ameliorates liver fibrosis by attenuating activated stellate cells through targeting the TGFβRII-SMADS pathway [J]. J Nanobiotechnol, 2021, 19(1): 437.

[44] JIANG M, JIANG X, LI H, et al. The role of mesenchymal stem cell-derived EVs in diabetic wound healing [J]. Front Immunol, 2023, 14: 1136098.

[45] YAN C, XÜ Y, LIN Z, et al. Human umbilical cord mesenchymal stem cell-derived exosomes accelerate diabetic wound healing via ameliorating oxidative stress and promoting angiogenesis [J]. Front Bioeng Biotechnol, 2022, 10: 829868.

[46] HU Y, RAO S S, WANG Z X, et al. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function [J]. Theranostics. 2018, 8(1): 169-84.

[47] XIU C, ZHENG H, JIANG M, et al. MSCs-derived miR-150-5p-expressing exosomes promote skin wound healing by activating PI3K/AKT pathway through PTEN [J]. Int J Stem Cells, 2022, 15(4): 359-71.

[48] ZHU Z, ZHANG X, HAO H, et al. Exosomes derived from umbilical cord mesenchymal stem cells treat cutaneous nerve damage and promote wound healing [J]. Front Cell Neurosci, 2022, 16: 913009.

[49] CHEN C Y, RAO S S, REN L, et al. Exosomal DMBT1 from human urine-derived stem cells facilitates diabetic wound repair by promoting angiogenesis [J]. Theranostics, 2018, 8(6): 1607-23.

[50] GUO Z, SU W, ZHOU R, et al. Exosomal MATN3 of urine-derived stem cells ameliorates intervertebral disc degeneration by antisenescence effects and promotes NPC proliferation and ECM synthesis by activating TGF-β [J]. Oxid Med Cell Longev, 2021, 2021: 5542241.

[51] WU X, LUO Y, CHEN J, et al. Transplantation of human menstrual blood progenitor cells improves hyperglycemia by promoting endogenous progenitor differentiation in type 1 diabetic mice [J]. Stem Cells Dev, 2014, 23(11): 1245-57.

[52] DALIRFARDOUEI R, JAMIALAHMADI K, JAFARIAN A H, et al. Promising effects of exosomes isolated from menstrual blood-derived mesenchymal stem cell on wound-healing process in diabetic mouse model [J]. J Tissue Eng Regen Med, 2019, 13(4): 555-68.

[53] CHEN L, QU J, MEI Q, et al. Small extracellular vesicles from menstrual blood-derived mesenchymal stem cells (MenSCs) as a novel therapeutic impetus in regenerative medicine [J]. Stem Cell Res Ther, 2021, 12(1): 433.

[54] HE X, DONG Z, CAO Y, et al. MSC-derived exosome promotes M2 polarization and enhances cutaneous wound healing [J]. Stem Cells Int, 2019, 2019: 7132708.

[55] ZHANG L, OUYANG P, HE G, et al. Exosomes from microRNA-126 overexpressing mesenchymal stem cells promote angiogenesis by targeting the PIK3R2-mediated PI3K/Akt signalling pathway [J]. J Cell Mol Med, 2021, 25(4): 2148-62.

[56] SHEN C, TAO C, ZHANG A, et al. Exosomal microRNA-93-3p secreted by bone marrow mesenchymal stem cells downregulates apoptotic peptidase activating factor 1 to promote wound healing [J]. Bioengineered, 2022, 13(1): 27-37.

[57] JONES B A, PEI M. Synovium-derived stem cells: a tissue-specific stem cell for cartilage engineering and regeneration [J]. Tissue Eng Part B Rev, 2012, 18(4): 301-11.

[58] LI M, KE Q F, TAO S C, et al. Fabrication of hydroxyapatite/chitosan composite hydrogels loaded with exosomes derived from miR-126-3p overexpressed synovial mesenchymal stem cells for diabetic chronic wound healing [J]. J Mater Chem B, 2016, 4(42): 6830-41.

[59] TAO S C, GUO S C, LI M, et al. Chitosan wound dressings incorporating exosomes derived from microRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full-thickness skin defects in a diabetic rat model [J]. Stem Cells Transl Med, 2017, 6(3): 73647.

[60] ZHAO B, ZHANG Y, HAN S, et al. Exosomes derived from human amniotic epithelial cells accelerate wound healing and inhibit scar formation [J]. J Mol Histol, 2017, 48(2): 121-32.

[61] FU S, ZHANG H, LI X, et al. Exosomes derived from human amniotic mesenchymal stem cells facilitate diabetic wound healing by angiogenesis and enrich multiple lncRNAs [J]. Tissue Eng Regen Med, 2023, 20(2): 295-308.

[62] CHEN W, CHANG S, YANG C, et al. Schwann cell-like cells derived from human amniotic mesenchymal stem cells promote sciatic nerve repair through an exosome-induced SOX2/FN1 pathway *in vitro* [J]. Int J Mol Med, 2022, 49(6): 80.

[63] YANG R, LIU F, WANG J, et al. Epidermal stem cells in wound healing and their clinical applications [J]. Stem Cell Res Ther, 2019, 10(1): 229.

[64] YANG H, XU H, WANG Z, et al. Analysis of miR-203a-3p/SOCS3-mediated induction of M2 macrophage polarization to promote diabetic wound healing based on epidermal stem cell-derived exosomes [J]. Diabetes Res Clin Pract, 2023, 197: 110573.

[65] DUAN M, ZHANG Y, ZHANG H, et al. Epidermal stem cell-derived exosomes promote skin regeneration by downregulating transforming growth factor-β1 in wound healing [J]. Stem Cell Res Ther, 2020, 11(1): 452.

[66] YAN Y, WU R, BO Y, et al. Induced pluripotent stem cells-derived microvesicles accelerate deep second-degree burn wound healing in mice through miR-16-5p-mediated promotion of keratinocytes migration [J]. Theranostics, 2020, 10(22): 9970-83.

[67] BO Y, LIU B, YANG L, et al. Exosomes derived from miR-16-5p-overexpressing keratinocytes attenuates bleomycin-induced skin fibrosis [J]. Biochem Biophys Res Commun, 2021, 561: 113-9.

[68] TENCATE R. Repair and regeneration of oral tissues [M]. Toronto: Mosby, 2003.

[69] KNIGHT R, BOARD-DAVIES E, BROWN H, et al. Oral progenitor cell line-derived small extracellular vesicles as a treatment for preferential wound healing outcome [J]. Stem Cells Transl Med, 2022, 11(8): 861-75.

[70] ZHOU A K, JOU E, LU V, et al. Using pre-clinical studies to explore the potential clinical uses of exosomes secreted from induced pluripotent stem cell-derived mesenchymal stem cells [J]. Tissue Eng Regen Med, 2023, 20(6): 793-809.

[71] KIM S, LEE S K, KIM H, et al. Exosomes secreted from induced pluripotent stem cell-derived mesenchymal stem cells accelerate skin cell proliferation [J]. Int J Mol Sci, 2018, 19(10): 3119.

[72] YOON J, LEE S K, PARK A, et al. Exosome from IFN-γ-primed induced pluripotent stem cell-derived mesenchymal stem cells improved skin inflammation and barrier function [J]. Int J Mol Sci, 2023, 24(14): 11635.

[73] ESTEBAN-VIVES R, ZIEMBICKI J, SUN CHOI M, et al. Isolation and characterization of a human fetal mesenchymal stem cell population: exploring the potential for cell banking in wound healing therapies [J]. Cell Transplant, 2019, 28(11): 1404-19.

[74] JIAO Y, WANG X, ZHANG J, et al. Inhibiting function of human fetal dermal mesenchymal stem cells on bioactivities of keloid fibroblasts [J]. Stem Cell Res Ther, 2017, 8(1): 170.

[75] HA D H, KIM H K, LEE J, et al. Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapeutics and skin regeneration [J]. Cells, 2020, 9(5): 1157.

[76] BERETTI F, ZAVATTI M, CASCIARO F, et al. Amniotic fluid stem cell exosomes: therapeutic perspective [J]. Biofactors, 2018, 44(2): 158-67.