科学通报
评 述
《中国科学》杂志社
SCIENCE CHINA PRESS

间充质干细胞重建眼表及其免疫调节机制研究进展

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2025-04-22 收稿, 2025-06-16 修回, 2025-07-08 接受, 2025-07-08 网络版发表
国家重点研发计划(2022YFD1302201)、国家自然科学基金(32372970)和国家自然科学基金青年科学基金(32101150)资助

摘要 当前针对角膜缘干细胞缺陷症(limbal stem cell deficiency, LSCD)的临床治疗主要依赖异体移植, 但供体不足与术后排异导致众多患者面临治疗困境. 间充质干细胞(mesenchymal stem cells, MSCs)作为一类具有自我更新及多向分化潜能的成体干细胞, 广泛分布于牙髓、脂肪、脐带、骨髓、毛囊等组织中. 因其自体可获取性、可分化为角膜上皮样细胞或通过外泌体介导基质重建及免疫调节特性, 在供体稀缺、免疫排斥风险高的角膜病治疗中展现出极大的应用价值, 尤其为解决双侧LSCD患者供体匮乏与免疫排斥难题提供重要策略. 本文综合评述脐带、牙髓、脂肪、骨髓、毛囊及诱导多能干细胞(induced pluripotent stem cells, iPSCs)来源间充质干细胞在重建角膜领域的研究状况, 分析比较不同间充质干细胞治疗角膜损伤的优劣, 详述其通过细胞接触、分泌可溶性因子以及胞外囊泡(extracellular vesicles, EVs)递送活性分子的方式调控调节性T细胞(regulatory T cells, Tregs)扩增, 深入剖析MSCs重建眼表免疫机制. 此外, 也讨论了在临床转化过程中, 基于MSCs治疗角膜疾病面临的问题、潜在解决方案以及未来发展的广阔前景, 并提出利用3D打印/工程化技术联合MSCs治疗LSCD新方案, 旨在突破MSCs疗法的现有局限性.

关键词 间充质干细胞, 免疫调节, 角膜缘干细胞缺乏症, 眼表重建

角膜作为眼表的一层透明无血管组织, 在眼球成像系统中发挥着至关重要的屈光作用[1]. 由于其直接暴露于外界环境且结构相对脆弱, 容易受到物理、化学以及遗传因素的损害[2]. 任何导致角膜透明度、平滑度、形状或厚度异常的改变, 均会对机体视觉功能产生不利影响. 当前, 圆锥角膜(keratoconus, KC)[3]、角膜内皮衰竭(corneal endothelial failure, CEF)[4]、角膜缘干细胞缺乏症(limbal stem cell deficiency, LSCD)[5]等眼表疾病, 已成为全球眼科医生面临的重大临床挑战. 在上述病理状态下, 角膜缘的角膜上皮干细胞遭受破坏, 导致邻近的结膜上皮细胞侵入并覆盖角膜表面, 进而引发新生血管化、慢性炎症、纤维组织侵入和基质疤痕形成, 最终损害眼表结构, 降低患者视力. 针对这些眼部重疾, 目前临床主要依赖角膜移植来重建眼表[3], 但供体短缺与术后排异严重制约其疗效; 另外, 手术创伤导致的角膜神经丛损伤、泪膜稳定性下降及继发性青光眼等并发症进一步加重治疗负担[6,7]. 因此, 急需探索一种更高效、更安全的治疗方法, 以改善患者预后.

在过去30年, 针对角膜疾病问题的研究主要集中

引用格式: 张沛, 陈茜茜, 蒲玉歌, 等. 间充质干细胞重建眼表及其免疫调节机制研究进展. 科学通报
Zhang P, Chen Q, Pu Y, et al. Advancements in ocular surface reconstruction by mesenchymal stem cells and immunomodulatory mechanism (in Chinese). Chin Sci Bull, doi: 10.1360/CSB-2025-0573

在角膜再生的探索上; 近年来, 伴随干细胞技术的不断发展, 间充质干细胞为角膜病的精准诊疗提供了多方面的创新思路. 间充质干细胞(mesenchymal stem cells, MSCs)来源广泛, 具备多向分化潜能及免疫调节能力, 可通过体外定向诱导分化[8]或者特定分子干预的转分化技术[9]分化为角膜内皮细胞(corneal endothelial cells, CEnCs)、角膜上皮细胞(corneal epithelial cells, CECs)以及角膜基质细胞(corneal keratocyte cells, CKCs), 参与受损角膜的修复; 通过分泌TSG-6、HGF、IL-10等细胞因子抑制炎性介质表达、促进抗炎巨噬细胞极化及调节T细胞活性, 有效减轻慢性炎症, 改善角膜的免疫微环境[10~12]. 另外, 表皮干细胞(epidermal stem cells, EpiSCs)同MSCs一样具有自我更新和多向分化潜能, 是修复角膜的理想细胞来源之一[13], 二者联合治疗的协同作用能够显著加速角膜组织的再生[14], 预测MSCs与EpiSCs的共处理将是未来角膜再生的重要途径. 本综述介绍了与眼表重建相关的最新技术、重要进展和未来挑战, 详细阐述了6种治疗眼角膜损伤的间充质干细胞类型及其免疫调控机制, 并对未来MSCs在角膜修复领域所面临的挑战和发展进行了讨论, 以期为LSCD的眼表重建提供临床和实践参考.

1 角膜缘干细胞的定位与相关疾病

角膜缘干细胞(limbal stem cell, LSCs)位于角膜缘基底区域, 是维持CECs持续更新的关键成体干细胞[15]. 在前期的工作中, 本课题组对LSCs开展了大量的实验研究, 发现其特异性表达p63、ABCG2等角膜缘干细胞标志物及K3/K12和Cx43等角膜上皮标志物, 这些表达为LSCs生物学特征的识别和研究提供了重要分子标记基础[16]. 基底层的LSCs分化为短暂扩增细胞, 随后向角膜中央迁移并分化为终末上皮细胞, 而周边LSCs则向角膜缘迁移以补充干细胞池; 此过程确保新老细胞及时更替, 维持角膜透明性与屏障功能[17].

LSCD是一种以角膜上皮结膜化为特征的眼表疾病, 其发生是由于LSCs数量减少或功能下降, 导致CECs稳态失衡[18]. LSCD发病机理分为先天性和后天获得性两种; 其中, 先天性因素包括先天性无虹膜、角膜缘发育不良、多发性内分泌缺陷等[19]; 后天因素多由于化学损伤、眼表瘢痕化天疱疮(ocular cicatricial pemphigoid, OCP)、史蒂文斯-约翰逊综合征(Stevens-Johnson syndrome, SJS)、中毒性表皮坏死松解症(toxic epidermal necrolysis, TEN)等引发的慢性炎症和纤维化微环境, 造成LSCs凋亡、角膜新生血管化及持续性上皮缺损[20~22].

相关研究表明, 体外扩增培养的LSCs可重建LSCD眼表[23]. 由于无需使用免疫抑制药物, 自体LSCs移植成为治疗LSCD的首选方法; 但双侧病变的患者(如SJS、OCP等)因缺乏健康供体无法适用, 异体移植虽可暂时重建眼表, 但其仍面临免疫排斥反应和产生并发症等风险[24]. 因此, 针对无健康LSCs可供移植的患者, 利用非角膜来源的MSCs重建LSCD眼表具有巨大潜能. 其治疗策略主要包括2类(图1): 一是基于MSCs直接移植的细胞疗法, 发挥多向分化能力促进角膜再生; 二是无细胞疗法, 利用MSCs分泌的胞 外囊泡(extracellular vesicles, EVs)、细胞因子等生物活性物质实现免疫调节和眼表修复[25].

2 治疗角膜疾病的间充质干细胞

近年来, MSCs治疗角膜疾病的研究取得显著进展, 其中主要包括脐带间充质干细胞(umbilical cord mesenchymal stem cells, UC-MSCs)、牙髓干细胞(dental pulp stem cells, DPSCs)、脂肪来源干细胞(adipose-derived mesenchymal stem cells, ADSCs)、骨髓间充质干细胞(bone marrow mesenchymal stem cells, BM-MSCs)、毛囊间充质干细胞(hair follicle-derived mesenchymal stem cells, HF-MSCs)和诱导多能干细胞来源间充质干细胞(induced pluripotent stem cell-derived mesenchymal stem cells, iMSCs)等非角膜来源干细胞(表1). 其中BM-MSCs因兼具高效分化、免疫调节以及分泌活性等优势, 在角膜治疗中应用最为广泛.

2.1 脐带间充质干细胞(UC-MSCs)

UC-MSCs来源广泛, 可从脐带血、脐带组织、羊膜中分离获取, 其取材具有非侵入等安全特性[26]. 该细胞群体具备优良的增殖能力, 能迅速完成对受损组织的高效补充; 同时兼具显著的免疫调节功能, 能有效抑制炎症反应和免疫排斥现象[31]. 当前的研究重点在于探讨UC-MSCs在角膜修复领域的多样化应用, 并逐步发展出从细胞治疗到组织工程的全面技术体系. Ye等人[38]通过定向诱导UC-MSCs分化为CEnCs, 并验证其在兔角膜内皮功能障碍模型中的组织再生效果, 为细胞替代疗法提供直接证据.

Li等人[39]进一步探索UC-MSCs的跨胚层分化潜力, 将其诱导为软骨细胞并负载于3D生物打印的聚乙

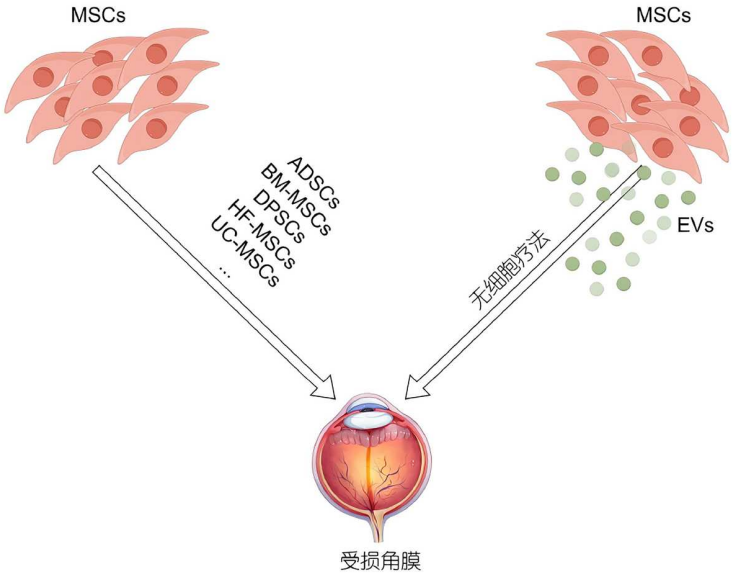

图 1 MSCs及其分泌物恢复角膜功能(本图由Figdraw绘制)
Figure 1 MSCs and their secretions in restoring corneal function (By Figdraw)

表 1 用于角膜损伤修复的MSCs类型及特性[a)]
Table 1 Types and characteristics of MSCs for corneal injury repair

细胞类型	来源	特点	优势	劣势	参考文献
脐带间充质干细胞	脐带组织	从新生儿脐带胶质层分离的多能干细胞	在角膜损伤修复过程中, 表现出良好的抗炎活性、低免疫原性以及高分化潜能	存在供体来源受限问题, 其传代体系的稳定性仍面临技术瓶颈	[26,27]
牙髓干细胞	牙髓组织	存在于牙髓基质中的成体多能干细胞, 具有跨胚层分化潜能	相对容易获取且创伤小, 免疫原性低, 与CECs均起源于胚胎外胚层	定向分化为CECs效率仍待优化, 其长期疗效需通过临床试验与延长随访周期予以验证	[28,29]
脂肪间充质干细胞	脂肪组织	存在于脂肪基质中的成体多能干细胞, 具备自我更新能力及跨胚层分化潜能	增殖能力较强, 微创获取(吸脂术废弃物利用)且具低免疫原性	细胞纯化困难, 且其增殖和分化能力有限	[30,31]
骨髓间充质干细胞	骨髓组织	存在于哺乳动物骨髓基质中的一类具有多向分化潜能的细胞亚群	来源广泛, 兼具抑制炎症与促进组织修复功能	在采样过程中存在损伤风险, 且其传代和分化性能受限	[32,33]
毛囊间充质干细胞	毛囊组织	存在于毛囊隆突区的一类成体多能干细胞亚群	源于患者自身毛囊, 无免疫原性; 眼睑皮肤毛囊储量充足	向角膜细胞分化效率低下, 增殖能力有限, 分化细胞体内存活时间短, 且具潜在致瘤风险	[34,35]
诱导多能干细胞来源间充质干细胞	诱导多能干细胞	由诱导多能干细胞定向分化获得的一类兼具自体安全性、无限增殖潜能与多向分化能力的间充质干细胞亚群	无限增殖能力, 克服了成体MSCs的衰老限制; 源自患者体细胞重编程, 避免免疫排斥; 基因表达更接近胚胎干细胞, 向角膜细胞分化效率高于成体MSCs	诱导多能干细胞(induced pluripotent stem cells, iPSCs)重编程技术存在一定的肿瘤形成风险, 分化为角膜细胞一致性不足, 制备成本高昂, 泪液成分、角膜拓扑结构可能干扰iMSCs的定植与分化	[36,37]

二醇丙烯酸酯支架，增加角膜修复体的生物相容性和角膜的厚度，为结构性缺损的修复提供新方案. Aghamollaei等人[40]则利用UC-MSCs亚群华氏胶源性间充质干细胞与去细胞化角膜基质片结合，展现低免疫原性与细胞外基质完整性，推动无细胞化组织工程角膜向临床转化. 上述三个团队分别从细胞功能重建、生物材料复合与技术路径升级三个维度，系统解决角膜修复中细胞来源稀缺、机械支撑不足及免疫排斥风险等难题，凸显UC-MSCs在眼科再生医学中多重价值的转化潜能.

2.2 牙髓干细胞(DPSCs)

DPSCs起源于神经嵴，与CECs及CEnCs在胚胎发育上具有同源性[28]. DPSCs的获取过程相对简便, 可在拔牙或者牙髓的治疗过程中获得[29]. 在羊膜存在的培养条件下, 实验选取*KRT43*、*KRT12*、*KRT14*、*PAX6*及*GAPDH*等眼部功能相关基因，通过RT-qPCR、免疫组化和Western blot技术，评估DPSCs在角膜修复中的应用潜力. 结果显示DPSCs在基因转录水平和蛋白表达量方面均与LSCs无显著差异. 除分子特征外，在上述培养条件下DPSCs也表现出与LSCs类似的形态特征, 这些结果证明DPSCs具备向LSCs分化的潜能[41]，为其作为角膜修复的替代细胞来源提供重要依据. 为了促进DPSCs向角膜细胞的分化，传统实验方法通常是将DPSCs培养在分化培养基中，通过直接诱导途径使其变为角膜细胞, 如Luzuriaga等人[42]证实, DPSCs在体内外均可被诱导为CECs，但所得细胞存在形态不完整、功能不完善及分化效率低等局限性. 针对此，Bosch等人[43]在利用患者自体DPSCs生成CEnCs治疗LSCD的研究中，开发出一种名为“两步分化法”的细胞分化方式(图2)，即先将DPSCs诱导为神经嵴干细胞(neural crest stem cells, NCSCs), 然后再将NCSCs定向诱导为角膜细胞. 与直接分化法相比, 该法所得角膜细胞的标记基因表达水平明显升高，并且其形貌在光学显微镜下呈现出典型的多边形；“两步分化法”为开发基于DPSCs的角膜细胞再生方案提供了新思路.

2.3 脂肪来源的间充质干细胞(ADSCs)

本课题组从鸡和兔体内分离并培养ADSCs，通过相关实验证实ADSCs与DPSCs一样，具备多向分化能力[44]. 在特定的诱导条件下, ADSCs和DPSCs均能分化为CECs、CEnCs和CKCs[31]. 但ADSCs的获取方式比DPSCs更加便捷，可以通过微创的脂肪抽吸手术获得, 对机体的生理状况影响较小. 另外, ADSCs能够通过旁分泌途径分泌血小板衍生生长因子(platelet-derived growth factor, PDGF)、血管内皮生长因子(vascular endothelial growth factor, VEGF)等多种生长因子, 它们有助于角膜的再生、伤口的愈合, 并能减轻炎症反应. 更为关键的是，ADSCs产生的旁分泌细胞外囊泡可用于无细胞治疗策略，从而降低了使用活细胞引起的免疫排斥和肿瘤激活等风险[45].

基于ADSCs的上述优势，多个研究团队通过不断优化实验逐步完善了LSCD治疗方案的可行性和高效性. Bandeira等人[46]提出一种体外分化方法，利用小分子抑制剂诱导ADSCs分化为上皮祖细胞，使其与纤维

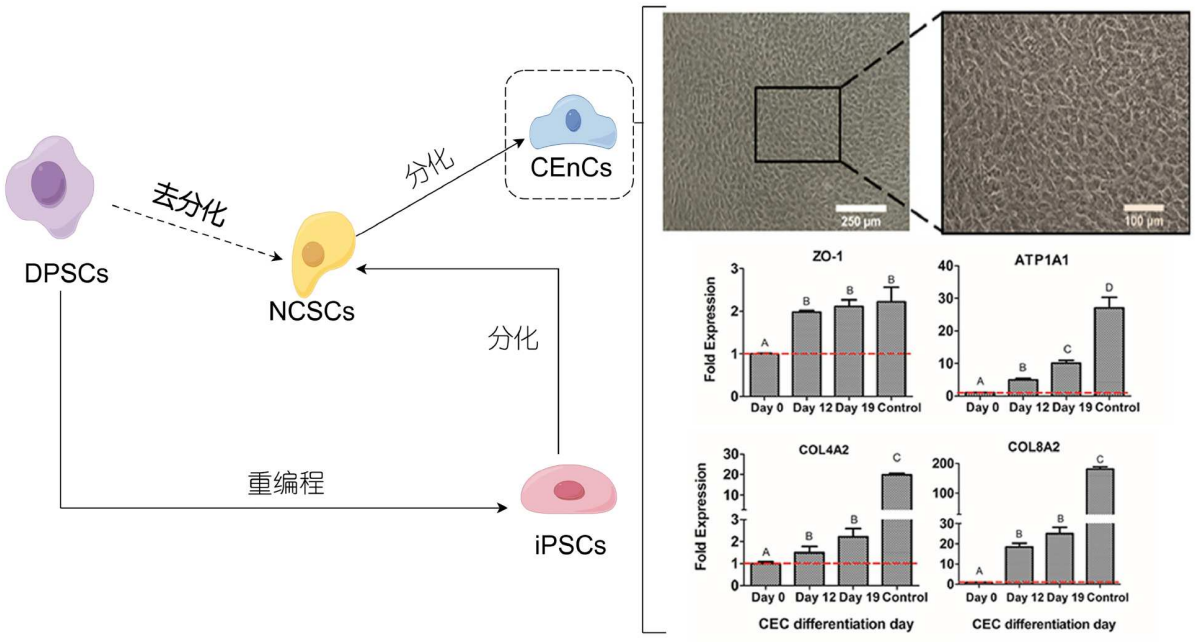

图 2 自体DPSCs两步分化途径生成CEnCs(本图由Figdraw绘制)
Figure 2 Two-step pathway of autologous DPSCs into CEnCs (by Figdraw)

蛋白凝胶结合成组织工程化上皮片，移植至碱烧伤大鼠模型中发现其显著促进角膜上皮再生并抑制炎症反应；该研究强调了体外定向分化和生物支架在功能化细胞构建中的重要性. Galindo等人[47]则聚焦于ADSCs的体内免疫调节作用，以羊膜为支架将ADSCs移植至部分/完全LSCD模型中，发现其能迁移至炎症区域，减少该区域炎症反应，抑制角膜新生血管形成，同时恢复CK3、E-cadherin等角膜上皮标志物和CK15、p63等角膜缘干细胞标记物的表达，这些结果为ADSCs的旁分泌效应提供依据. Park等人[30]另辟蹊径，利用ADSCs的条件培养基(conditioned medium, CM)局部滴注化学烧伤性角膜(图3)，发现CM通过上调rhEGF、HGF等修复因子和抑制IL-1β、VEGF等炎症介质来加速上皮愈合，证明ADSCs的疗效不仅依赖细胞移植，其分泌物亦具有独立修复潜能. 以上研究从直接细胞移植到无细胞疗法、从体外分化到体内调控，共同验证ADSCs治疗效果，逐步丰富LSCD治疗方案，为临床转化提供多样化参考.

2.4 骨髓间充质干细胞(BM-MSCs)

近年来，BM-MSCs因其多向分化潜能与免疫调节特性，逐步成为眼表修复的研究热点[48]. BM-MSCs用于角膜修复已在材料优化、机制解析及临床转化等方面取得了显著进展(表2)[49~55]. 与ADSCs一样，BM-MSCs利用细胞治疗手段来修复眼表不仅涉及细胞分化替代，还依赖于旁分泌作用，即通过分泌TGF-β、PGE2等因子减少T细胞增殖与DC细胞活化，进而改善角膜炎症微环境. 在兔角膜基质缺损模型中，自体BM-MSCs通过PKH26标记证实其局部移植可促进角膜基质增厚与上皮重建，证明BM-MSCs是分化为LSCs的理想来源之一[56].

在大鼠角膜碱烧伤模型中发现，结膜下注射自体BM-MSCs可显著减少$CD68^+$巨噬细胞浸润，下调TNF-α、IL-2等促炎因子及VEGF的表达，进而抑制新生血管形成并加速角膜上皮再生[57]. 虽然该研究确认BM-MSCs可通过免疫调节(如抑制T细胞活化和DC细胞成熟)重塑局部微环境，但未观察到其分化为CECs的直接证据. 兔BM-MSCs在纤维蛋白胶支架与角膜基质细胞共培养条件下，可表达角膜基质特异性蛋白Keratocan，参与角膜基质形成，证实BM-MSCs具有跨胚层分化为角膜细胞的潜力[58]. 此外，BM-MSCs的分化能力与实验条件相关，当BM-MSCs处于角膜损伤部位中细胞因子梯度的特定微环境时，可以定向分化为CECs，直接参与组织再生[59]. 以上基于细胞治疗手段的研究从炎症抑制、组织再生等角度展现出BM-MSCs在眼表修复中具有较强的免疫调节和分化替代能力.

由于干细胞体内移植后自身活力下降且易被机体免疫排异，越来越多的研究关注来源于MSCs的具有性

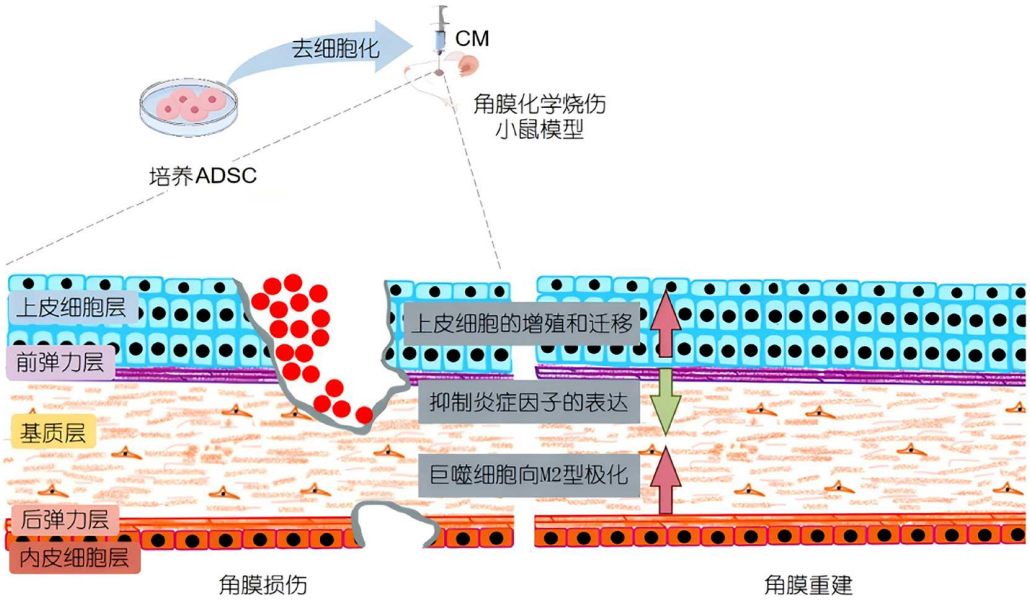

图 3 ADSCs的条件培养基治疗角膜上皮损伤(本图由Figdraw绘制)
Figure 3 ADSC-CM treatment for corneal epithelial injury (By Figdraw)

表 2 基于BM-MSCs的角膜修复实验研究对比[a)]

Table 2 A comparative study on the use of BM-MSCs in corneal repair

疗法	应用方式	研究目标	模型	主要结果	参考文献
细胞疗法	支架负载	优化支架材料	体外BM-MSCs与甲基丙烯酸酰化明胶, 水凝胶共培养	30% 水凝胶组, Keratocan表达增加显著	[49]
		构建组织工程角膜(图4)	兔角膜碱烧伤模型	角膜透明度和血管化程度显著降低, 角膜厚度接近正常角膜	[50]
		证实MSCs通过免疫调节修复角膜	大鼠角膜化学, 烧伤模型	视力恢复与炎症抑制相关(非分化依赖)	[51]
	基质注射	抑制炎症、血管生成	兔角膜碱烧伤模型	血管面积减少, IL-1β/TLR4表达下降	[52]
无细胞疗法	3D培养	3D微环境优化, 分泌组活性	聚己内酯-明胶电纺纤维支架共培养MSC, 兔角膜器官模型	CFCs划痕闭合率提高, α-SMA表达减少	[53]
	凝胶递送	可重复凝胶系统, 提升分泌组疗效	碱烧伤模型	瘢痕减少, CD44介导HA/MSC-S协同效应	[54]
	体外干预	外泌体治疗内皮功能障碍	受损hCECs模型	hCECs增殖增加, 迁移率提高	[55]

a) HA: 透明质酸(hyaluronic acid); hCECs: 人角膜内皮细胞(human corneal endothelial cells); MSC-S: 间充质干细胞分泌组(mesenchymal stem cell secretome); CFCs: 角膜成纤维细胞(corneal fibroblast cells)

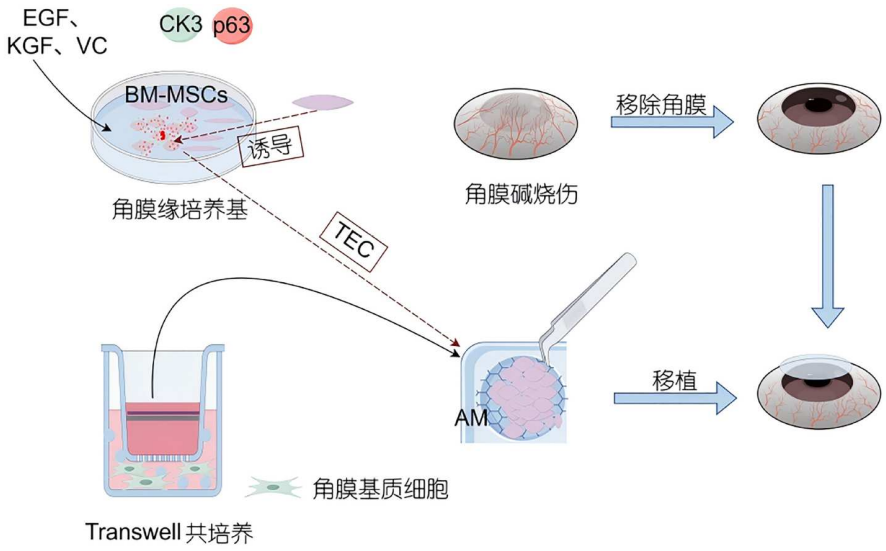

图 4 构建BM-MSCs源性TEC修复碱烧伤角膜. AM: 羊膜; TEC: 组织工程角膜(本图由Figdraw绘制)

Figure 4 Construction of BM-MSCs-derived TEC for repairing alkali-burned cornea. AM: Amniotic membrane; TEC: tissue-engineered cornea (By Figdraw)

能稳定、容易保存、无免疫原性等优势的EVs, 能以无细胞治疗的方式来弥补MSCs移植带来的不足[60]. BMSC-EVs携带microRNA、蛋白质等活性成分, 可模拟母细胞的修复功能, 同时规避免疫排斥风险[61]. Li等人[62]探讨BMSC外泌体(exosomes, Exos)对糖尿病视网膜病变小鼠炎症的调节作用, 研究发现BMSC-EVs中*miR-125a-5p*能抑制高葡萄糖诱导的内质网应激, 通过提升CECs活力、增殖和迁移能力来改善DK. Saccu等人[63]研究表明, BMSC-EVs在体外能促进hCECs的伤口闭合, 在体内能调节组织中IL-6炎症因子分泌水平、血管生成程序以及诱导炎症细胞死亡, 恢复血-视网膜屏障功能, 加速角膜损伤恢复(图5).

2.5 其他间充质干细胞

自体HF-MSCs凭借其来源丰富、易扩增特性成为角膜再生理想细胞源之一. HF-MSCs经无3T3饲养层的角膜缘成纤维细胞条件培养基诱导转分化为CK12/*pax6*$^{+}$角膜样细胞, 直接在纤连蛋白凝胶形成多层角膜样上皮片层, 其形态标记物表达均与原代组织吻合, 移植至LSCD小鼠后成功重建透明角膜[34]; Olszewski等

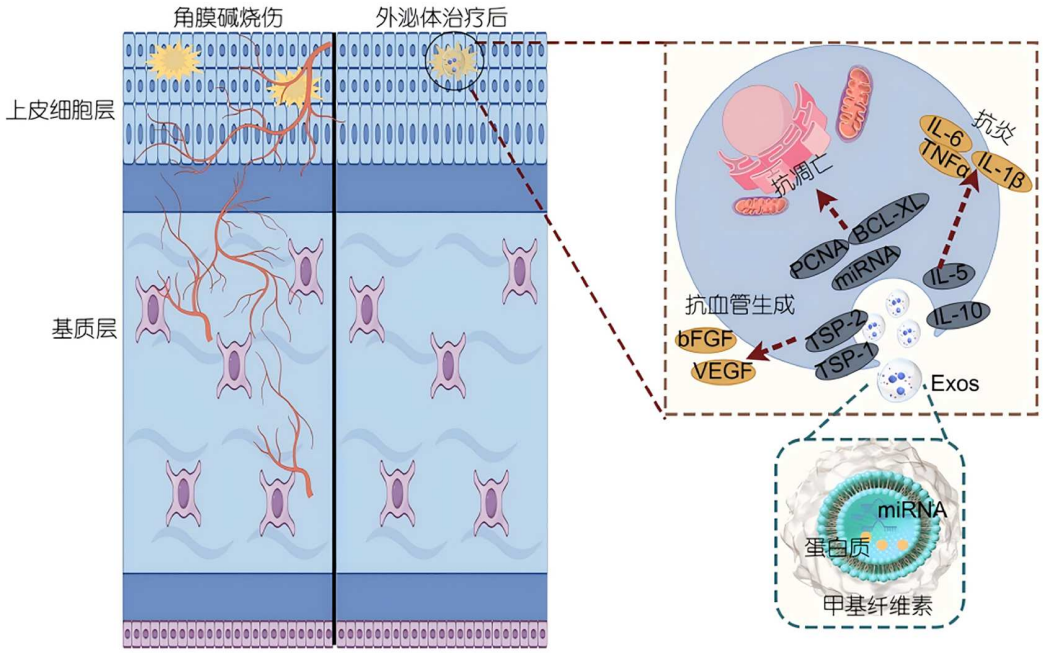

图 5 MSC-EVs修复角膜伤口(本图由Figdraw绘制)
Figure 5 MSC-EVs repair corneal wound (By Figdraw)

人[35]证实，眼睑活检来源的HF-MSCs具有跨胚层分化潜能，尽管高龄患者干细胞活力轻微下降，但自发形成的球状细胞可识别德斯梅特膜仿生拓扑结构，分化为功能性角膜内皮样细胞，为全年龄段患者提供自体内皮替代方案.

HF-MSCs可通过转分化上皮修复与跨胚层内皮再生作用，为角膜盲治疗开辟新路径，而iMSCs凭借其强增殖力与低异质性也已成为再生医学新前沿. RNA测序显示iMSCs高表达*Hox*家族基因，且具更强免疫抑制功能，iMSCs通过线粒体捐赠显著提高*Ndufs4*基因敲除小鼠的RGC存活率与视网膜功能[36]；Tang等人[37]研究发现，热敏壳聚糖水凝胶缓释的iMSC-EVs可下调胶原基因与TRAM2蛋白表达，同步促进上皮/基质修复并抑制瘢痕形成，促进角膜再生. 尽管iMSCs外泌体递送与组织靶向修复展现出巨大潜力，其仍存在重编程致瘤风险与产品标准化挑战.

3 MSCs的免疫调节机制

受损组织的修复与重建涉及炎症反应、组织新生以及结构重建等一系列病理学阶段. 在修复过程中，干细胞移植被视为一种潜在的治疗策略，然而，移植细胞在宿主免疫系统的免疫清除下难以长期存活、无法发挥功能，成为干细胞应用的主要障碍之一[64]. 大量研究表明，MSCs的免疫调节特性对于促进其在体内分化和参与眼表修复尤为关键[65]. 这些调节特性通过细胞间接触、可溶性因子分泌、Tregs调控以及EVs传递活性分子等多种机制，共同抑制免疫反应的过度激活，调节角膜组织的微环境，最终有助于炎症部位的稳定和修复(图6).

3.1 基于细胞间接触的免疫调节

3.1.1 CD80 靶点结合介导

CD80以二聚体形式存在于MSCs细胞膜上，可与其他细胞的CD28、CTLA-4和PD-L1等靶点相结合[66~68]. 结合的靶点及所处的免疫环境不同，CD80发挥的功能不同；如与T细胞表面CD28结合，可促进T细胞活化和增殖，进而引发炎症反应[69]；与Tregs表面CTLA-4结合，则抑制炎症反应[70]. Mittal等人[71]证实，当MSC通过表面分子CD80直接与Tregs互作时，会促进Tregs在移植物部位的浸润，增强其对Th1细胞的抑制功能，延长角膜移植物存活时间.

3.1.2 非靶点直接接触

Coulson-Thomas等人[72]发现，当MSCs暴露于炎症细胞时，可合成富含硫酸软骨素蛋白聚糖-多能蛋白聚

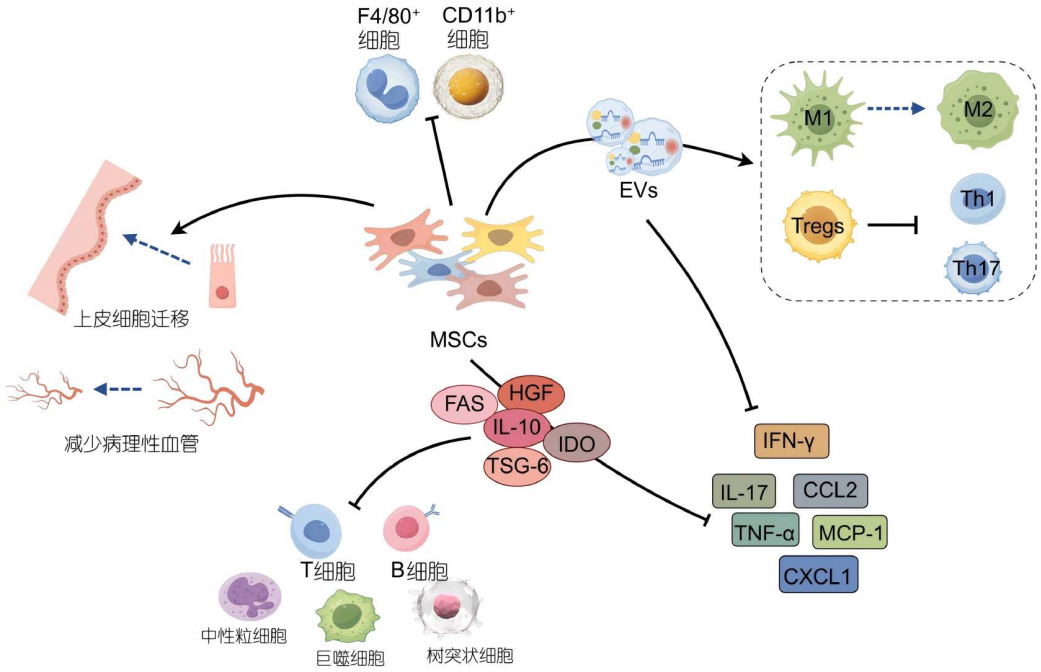

图 6 间充质干细胞免疫调节机制. M1: M1型巨噬细胞; M2: M2型巨噬细胞; Th1: 辅助性T细胞1型; Th17: 辅助性T细胞17型; Tregs: 调节性T细胞(本图由Figdraw绘制)

Figure 6 Immunomodulatory mechanism of MSCs. M1: M1 macrophages; M2: M2 macrophages; Th1: T helper 1 cell; Th17: T helper 17 cell; Tregs: regulatory T cells (By Figdraw)

糖的细胞外糖萼, 该结构通过与重链修饰的HA基质实现稳定组装, 为Tregs细胞提供黏附和增殖的支架, 改善角膜免疫微环境. Kang等人[31]通过直接共培养与Transwell分隔培养对比发现, 当MSCs与外周血单核细胞直接接触时, 显著抑制外周血单核细胞增殖, 而通过微孔隔断的非接触条件下抑制作用完全丧失, 表明细胞接触在MSCs的抑制作用中发挥至关重要的作用; 同时验证了MSCs的局部使用对于角膜移植存活率的提高比全身给药更有效.

3.2 基于可溶性细胞因子进行免疫调节

3.2.1 TSG-6

TSG-6是MSCs的重要抗炎产物之一[73], 其表达不仅受TLR/NF-κB信号通路调控, 还与MSCs的储存条件相关. 在高度炎症环境下, MSCs基于TLR/NF-κB信号通路上调TSG-6表达, 诱导巨噬细胞从促炎M1表型向抗炎M2表型极化, 并显著降低TNF-α与IL-6水平[74]. 在低温保存条件下, MSCs可以提高TSG-6表达水平, 显著减少角膜创面$CD45^+$免疫细胞浸润[75]. Oh等人[76]的研究证实, 在角膜遭受化学和机械损伤后, 注射重组人TSG-6能够同步抑制包括IL-6、IL-1β在内的促炎因子以及CXCL1/CCL2等趋化因子的表达; 该研究还发现, 重组人TSG-6通过降低MMP-9的活性, 有效延缓了角膜基质的降解过程. Figueroa-Haro等人[77]利用转基因技术改造MSCs, 使其过度表达TSG-6, 在角膜炎症环境下增强MSCs的迁移和再生能力. Ko等人[78]利用TSG-6成功地将肺部的单核细胞/巨噬细胞转化为高水平表达MHC II类、$B220^+CD11b^+$的免疫抑制性细胞, 这些细胞能够抑制T细胞的增殖以及Th1/Th17细胞的分化, 从而在小鼠体内诱导免疫耐受; TSG-6的利用增强了小鼠对角膜移植排斥反应和自身免疫炎症的抵抗能力.

3.2.2 HGF

角膜损伤引发的炎症微环境可显著上调MSCs的HGF分泌水平[79]. HGF作为角膜修复的关键因子, 不仅能够通过激活c-Met受体直接促进CECs的迁移和增殖[80], 还能通过免疫调节作用来抑制急性和慢性炎症反应. Mittal等人[81]揭示, MSCs以HGF依赖性方式促进角膜移植物存活, 通过分泌HGF阻断引流淋巴结中树突状细胞(dendritic cells, DCs)的活化, 减少抗原向

T细胞的递呈；同时HGF还可抑制Th1型细胞分化，促进Tregs扩增(图7). Astaridewi等人[82]利用白藜芦醇预处理MSCs，提高其HGF分泌量，下调IL-2、IFN-γ等Th1型细胞因子，抑制$CD4^+$T细胞浸润；同时减少VEGF分泌，改善炎症微环境，提升眼表病变的治疗效果.

3.2.3 IL-10

IL-10是一种高效的免疫调节因子，可通过抑制促炎因子释放和调控免疫细胞表型，提高自身免疫耐受性以及减少炎症期间的组织损伤[83]. Lu等人[84]发现，过表达IL-10的MSCs (IL-10-BM-MSCs)，能显著提高$CD68^+$巨噬细胞中*lncRNA 003946*的表达，抑制巨噬细胞MHC-II分子及CD80/CD86共刺激因子的表达，减少角膜基质内$CD4^+$T细胞及$CD68^+$巨噬细胞浸润；另外还发现，IL-10-BM-MSCs诱导眼表引流淋巴结中$CD4Foxp3^+$Tregs的显著增加，表明IL-10可能直接参与并放大MSCs介导的免疫调节. 同样，Abughanam等人[85]也发现，利用MSCs能有效增加$Foxp3^+$Treg细胞的数量，并诱导其分泌更多的IL-10，直接抑制TNF-α的产生，重建外周耐受性，抑制自身免疫攻击.

3.2.4 TGF-β

TGF-β对MSCs的免疫调节功能有着显著的影响. TGF-β能够抑制NF-κB和MAPK信号通路磷酸化，降低IL-6、TNF-α等促炎因子表达，进一步减少中性粒细胞与单核细胞浸润[86]. Lynch等人[87]发现，TGF-β预处理的MSCs能基于Smad2/3途径高效诱导Tregs的扩增，并上调Tregs中CD73和PD-L1等免疫检查点的表达量，增强其对效应T细胞的抑制能力，提高小鼠角膜移植物的生存率. Lohan等人[88]研究发现，异体MSCs治疗可增加受体引流淋巴结Tregs数量，该效应与TGF-β介导的Foxp3表达水平升高密切相关. 此外，具有免疫抑制作用TGF-β1一旦过度表达，能通过下调*miR-29*表达来促进胶原基因表达，导致角膜瘢痕的形成[89]；反之，利用TGF-β3则能抑制纤维化过程[90]. 因此，在角膜损伤修复过程中，TGF-β1和TGF-β3的平衡对于控制炎症反应和组织纤维化至关重要.

3.3 基于调控Tregs细胞发挥免疫调节

3.3.1 Tregs细胞

Tregs在诱导和维持眼部免疫耐受中发挥重要作

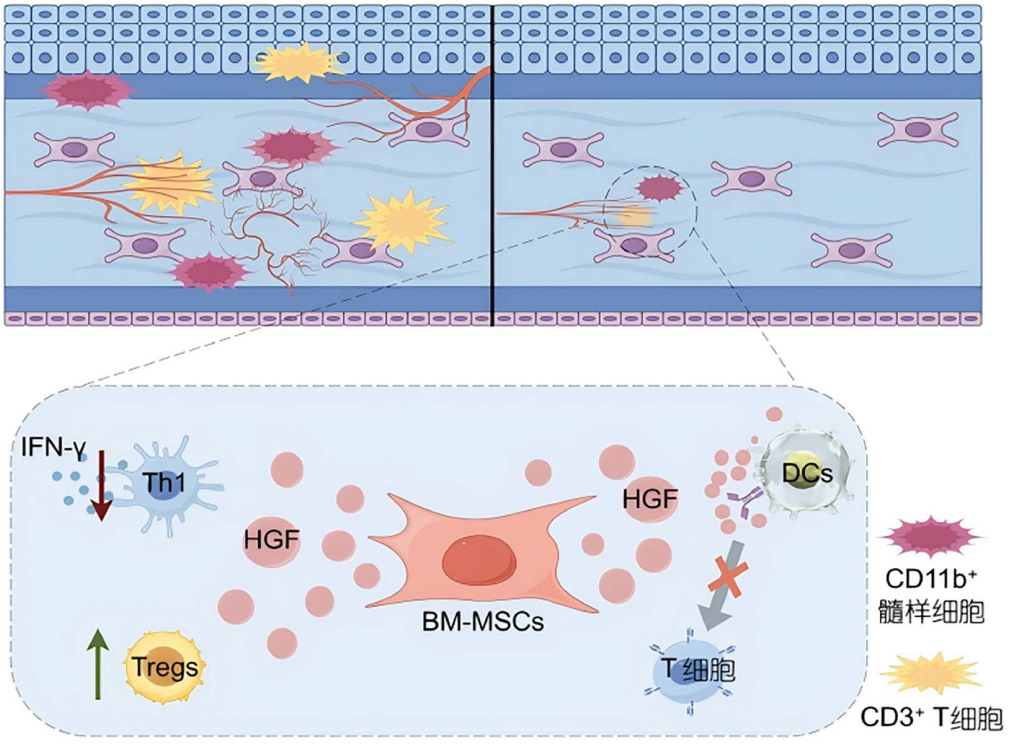

图 7 BM-MSCs分泌HGF介导角膜移植物免疫耐受的微环境重塑(本图由Figdraw绘制)
Figure 7 BM-MSCs secrete HGF to promote immune tolerance and microenvironment remodeling in corneal grafts (By Figdraw)

用. MSCs可以促进Tregs的分化[91], Tregs反过来可放大MSCs的免疫效应[92]. Piekarska等人[93]研究发现, Tregs与MSCs接触后, 上调MSCs表面CD39/CD73腺苷酸酶的表达, 这两种酶通过“ATP-腺苷”代谢轴将胞外ATP水解为免疫抑制性腺苷, 抑制Th1/Th17细胞的增殖. Lu等人[94]发现, MSCs通过CCL22/CCR4轴募集Foxp3$^+$/CD4$^+$Tregs至移植部位, 显著减少CD4$^+$ T细胞及CD68$^+$巨噬细胞浸润, 从而延长移植角膜的存活时间. Bian等人[95]发现, 清除Foxp3$^+$ Tregs会导致角膜移植物排斥指数升高, CD4$^+$ T细胞浸润增加, 导致移植物存活率降低. 因此, 在使用MSCs治疗角膜疾病时, 炎症的改善与否可能与Tregs细胞的免疫调控直接相关.

3.3.2 Th17 /Tregs的平衡

CD4$^+$ T细胞亚群是角膜疾病发生与发展的病理学基础, 它在角膜组织中含量丰富, 与角膜表面的病原体和外来抗原接触后会被激活[96]. 一旦被激活, CD4$^+$ T细胞亚群可以分化为不同的效应细胞亚群, 其中Th17细胞和Tregs细胞间失衡会引发角膜炎症或免疫抑制等问题[97]. 具体来说, Th17细胞通过分泌IL-17参与抗炎反应, 并抑制Tregs细胞的功能; 而Tregs细胞是抑制自身免疫和炎症反应的重要调节细胞[98]. 因此, MSCs通过抑制Th17细胞的免疫反应并增强Tregs细胞功能, 被认为是促进移植体存活的潜在机制之一. Fernandes-Cunha等人[54]发现, MSC-S上调CECs中CD44受体的表达, 促进其与HA的黏附, 加速上皮再生; 同时, 高表达的CD44激活TGF-β/Smad3通路驱动Tregs生成, 抑制Th1/Th17增殖分化, 共同发挥组织修复和免疫耐受的双重作用(图8). Zhang等人[99]发现, MSCs可基于*miR-1246*/*NFAT5*信号轴调节CD4$^+$ T细胞功能, 降低Th17细胞分化, 同时上调Tregs特异性转录因子Foxp3, 改善Th17/Tregs细胞失衡, 进而抑制炎症反应. Mendiratta等人[97]发现, 经缺氧和凋亡预处理的MSCs能显著提高PGE2和IDO等免疫调节因子的分泌, 这一过程通过磷酸化信号传导与转录因子STAT3激活来抑制Th17细胞的分化; 同时激活STAT5信号通路以促进Tregs的扩增, 增强免疫调节能力; 上述结果表明可基于STAT3/STAT5信号通路调节Th17/Tregs之间的平衡, 维持和重建角膜组织稳态.

3.4 基于EVs介导进行免疫调节

相关研究表明, MSCs的治疗潜力在很大程度上归功于其分泌的EVs, 这些囊泡具有抗炎、抗凋亡以及促进组织再生等功效[100]; EVs是一类由MSCs分泌的膜性结构, 包括外泌体、微囊泡和凋亡小体等, 携带一系列如蛋白质、RNA等被膜脂质双层封装的生物活性分子[101]. Liu等人[102]聚焦移植物抗宿主病相关眼表损伤,

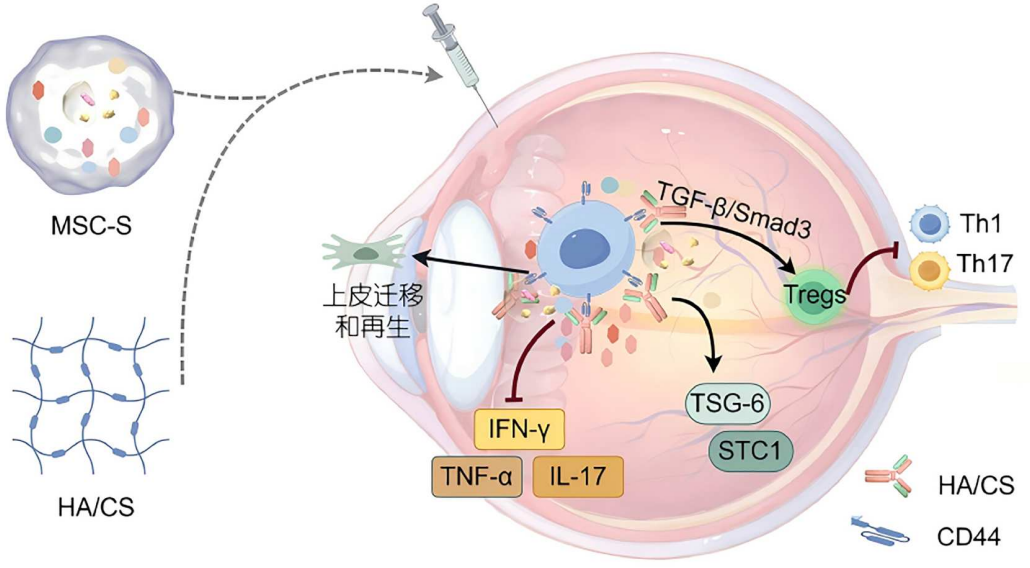

图 8 MSC-S/HA/CS凝胶基于CD44介导的角膜修复-免疫调节协同机制. CS: 硫酸软骨素; HA: 透明质酸; MSC-S: 间充质干细胞分泌组(本图由Figdraw绘制)

Figure 8 CD44-mediated corneal repair-immunomodulation synergy in MSC-S/HA/CS hydrogel. CS: Chondroitin sulfate; HA: hyaluronic acid; MSC-S: mesenchymal stem cell secretome (By Figdraw)

证实MSC-EVs携带的*miR-22*可通过靶向淋巴内皮细胞ICAM-1，阻断T细胞向角膜及结膜的迁移浸润，降低Th1/Th17型炎症因子(如IFN-γ、TNF-α、IL-17)水平. Xu等人[103]开展研究，发现3D培养MSC的外泌体可通过递送*miR-150-5p*靶向抑制*PDCD4*基因表达，驱动巨噬细胞由促炎M1型向抗炎M2型极化，减轻角膜炎症并促进修复.

Ong等人[104]研究发现MSC-exo能上调角膜中$CD163^+$、$CD206^+$ M2巨噬细胞比例，降低IL-1β、IL-8和TNF-α等促炎细胞因子水平，下调与中性粒细胞浸润相关的细胞因子CXCL1、α-MPO的表达，发挥抗炎表型优势. Rautavaara[105]研究发现MSC-EVs可抑制T细胞向Th1和Th17等促炎表型的分化，诱导Tregs产生. 另外，利用干眼症(dry eye disease, DED)模型开展相关实验证实，MSC-EVs可抑制干燥应激诱导的NLRP3炎性小体形成、caspase-1激活和IL-1β表达增加，减少角膜上皮缺损与炎性细胞浸润，减轻DED症状[106]. 综上，MSC-EVs通过递送miRNA等生物活性分子、抑制Th1/Th17分化来重建适应性免疫稳态；下调中性粒细胞趋化因子与促炎因子表达、靶向NLRP3-caspase-1/IL-1β通路抑制固有免疫过度激活. 基于EVs介导进行免疫调节不仅有效缓解角膜上皮缺损和炎性浸润，更为移植物抗宿主病、DED等眼表疾病的治疗提供重建微环境稳态的创新依据.

4 总结与展望

近年来研究表明，直接移植MSCs以及利用其衍生物，能分别基于细胞疗法和无细胞疗法2种策略促进组织再生和发挥免疫调控来改善角膜损伤；其中，脐带、牙髓、脂肪、骨髓、毛囊及iPSCs来源的MSCs均可发挥角膜修复作用，但由于缺乏分离和鉴定MSCs的标准流程，难以对不同类别MSCs的治疗效果进行有效对比分析；并且，不同实验室、不同分离方法及不同培养体系，也会导致MSCs在整体基因表达、表型、增殖速率和分化能力等结果出现不一致性[107].

针对此，未来研究可尝试开发MSCs分离鉴定的标准化体系和通用型MSCs；其中，通用型MSCs能成为同种异体治疗的“现货型”细胞来源，尤其在急诊创伤、器官损伤等时间敏感场景下，具有无需配型等待、单剂成本低于自体疗法等明显优势. 近期，Wang等人[108]针对*B2M*基因的超级增强子进行表观遗传抑制，成功构建新型通用型干细胞GLOBES，其在炎症微环境中将HLA-I表达精准维持在“Goldilocks水平”，同步规避T/NK细胞激活，且无需改变DNA序列，该策略基于免基因编辑为通用型细胞疗法提供一个新视角.

不同来源的MSCs都具有多向分化潜能，能分泌EGF、HGF等细胞因子及MSC-EVs来促进CECs增殖，加速损伤愈合. 同时，它们还能抑制病理性新生血管形成，维持角膜无血管微环境稳态；并通过细胞接触、分泌可溶性因子以及EVs递送活性分子的方式，调控Tregs的扩增机制，调节角膜炎症微环境. 然而，其临床应用仍受限于以下多个问题：(1) 慢性炎症区域的高ROS水平及IL-17过表达会诱导MSCs功能耗竭，降低其旁分泌活性；(2) MSCs来源EVs亚群异质性会导致疗效差；(3) MSCs修复损伤后仍易形成角膜瘢痕，并可能进一步发展为角膜盲；(4) MSCs定向分化为CECs的效率较低，难以满足严重LSCD的修复需求等.

为解决上述应用局限，当前研究聚焦工程化EVs递送系统的开发，通过靶向修饰、加载功能性分子[109](如抗纤维化miRNA、促神经再生蛋白等)，或调整表面电荷(如阳离子化)以增强EVs与CECs结合[110]，提高其在炎症区域的富集效率，延长滞留时间以克服IL-17介导的细胞排异；也有研究从CECs中分离外泌体，基于“DNA拉链”技术开发出一种杂合外泌体囊泡，能有效递送siRNA治疗药物到角膜，显著减少眼表炎症细胞因子的分泌[111]，在提高疗效的同时对使用LSCs下游细胞分泌EVs治疗LSCD具有一定的启发意义. 另外，Huang等人[112]通过建立无血清的定向诱导分化体系，从人羊膜上皮干细胞中诱导分化出角膜基质细胞结合明胶基水凝胶支架来构建仿生角膜基质，能快速恢复兔角膜结构，并通过蛋白聚糖高效重塑组织微环境，减少疤痕形成；Villatoro等人[113]研究发现，犬角膜缘干细胞分泌物对成纤维细胞增殖具有一定抑制作用，也能减少角膜瘢痕的形成.

最后，针对MSCs对角膜上皮的定向分化效率较低问题，本课题组开展相关研究[114,115]证明EpiSCs具有较强的角膜上皮定向分化潜能，但局部免疫失衡会降低其在慢性炎症微环境中的存活率，随时间的推移导致不断失活. 而MSC-EVs基于SDF-1/CXCR4信号轴[116,117]，可增强EpiSCs向角膜损伤区的定向迁移，抑制促炎因子释放、改善局部免疫失衡，并明显促进角膜上皮屏障功能的恢复. 因此，可设计共载MSC-EVs/EpiSCs的3D打印生物工程角膜支架，EVs前处理能抑制局部炎症，为后续EpiSCs的定向分化提供微环境支

持, 加快实现角膜高效修复. 我们相信, 通过不断加强学术界与产业界的紧密合作和持续创新, 更加有望迎来适宜且高效的重建眼表新方案, 进一步推动组织工程角膜技术的临床应用与发展.

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Summary for “间充质干细胞重建眼表及其免疫调节机制研究进展”

Advancements in ocular surface reconstruction by mesenchymal stem cells and immunomodulatory mechanism

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Current clinical treatment for limbal stem cell deficiency (LSCD) relies mainly on allogeneic transplantation, but the lack of donors and postoperative rejection has resulted in many patients facing treatment difficulties. Mesenchymal stem cells (MSCs), a class of adult stem cells with self-renewal and multidirectional differentiation potential, are widely distributed in various tissues, including dental pulp, adipose tissue, umbilical cord, bone marrow, and hair follicles. Due to their autologous availability, the ability to differentiate into corneal epithelial-like cells, and unique properties of mediating matrix reconstruction and immune regulation via exosomes, this approach presents significant potential in treating corneal diseases. These characteristics establish MSCs as a crucial approach to tackling the combined issues of donor scarcity and immune rejection in patients with bilateral LSCD, providing an innovative alternative for the functional and structural restoration of the ocular surface. This review systematically examines the roles of recent advancements in research on MSCs derived from umbilical cord, dental pulp, adipose tissue, bone marrow, hair follicles, and induced pluripotent stem cells (iPSCs) for corneal reconstruction. It analyzes and compares the strengths and weaknesses of different MSCs in treating corneal injuries. Various research groups have systematically addressed challenges such as the scarcity of cell sources, insufficient mechanical support, and the risk of immune rejection through three dimensions: cell function reconstruction, biomaterials combination, and technological pathway upgrades. However, the long-term survival and functioning of transplanted cells in the host’s immune system remain significant obstacles in the application of stem cells. Therefore, the immunomodulatory properties of MSCs are crucial to promote their in vivo differentiation and participation in ocular surface repair. This article focuses on elucidating how MSCs regulate the expansion of Tregs through CD80 target binding and non-target-mediated cell contact, as well as the secretion of soluble factors such as TSG-6, HGF, IL-10, and TGF-β, and the delivery of regulatory molecules like miR-22 and miR-150-5p via extracellular vehicles (EVs). These mechanisms aim to restore the balance between Th17 and Tregs, providing an in-depth analysis of the immune mechanisms involved in MSC-mediated ocular surface reconstruction. This review discusses the challenges encountered in the clinical translation of MSC-based therapies for corneal diseases. These challenges include functional exhaustion resulting from chronic inflammatory microenvironments, variability in efficacy due to source heterogeneity, inadequate differentiation efficiency, and the potential for postoperative scarring. Current research is focusing on innovative solutions based on engineering technologies to mitigate these bottlenecks. The research includes the development of engineered EV delivery systems featuring targeted modifications, the loading of functional molecules, and the adjustment of surface charge to enhance binding to corneal cells and improve enrichment in inflammatory regions; the preparation of microneedle arrays for precise and sustained exosome delivery; and the directed differentiation of corneal stromal cells in combination with gelatin-based hydrogel scaffolds to construct biomimetic corneal stroma and reduce fibrosis. Recent research findings suggest potential future directions for the development of MSCs. Based on this, the article proposes a new treatment plan for LSCD utilizing 3D technology and engineering combined with MSCs, aiming to overcome the current limitations of MSC therapy. This work aims to disseminate recent research on ocular surface reconstruction utilizing MSCs and MSC-EVs, facilitating comprehension for non-professionals and encouraging discussion with leading researchers. Through ongoing innovation and collaboration between academia and industry, we expect the development of more efficient and clinically appropriate MSC-based treatment strategies.

mesenchymal stem cells, immunomodulatory, limbal stem cell deficiency, ocular surface reconstruction

doi: 10.1360/CSB-2025-0573