DOI: 10.11844/cjcb.2026.07.0012
中国细胞生物学学报 Chinese Journal of Cell Biology 2026, 48(7): 1887–1904 CSTR: 32200.14.cjcb.2026.07.0012

间充质干细胞向角膜细胞方向分化的研究进展

李伶 黄艳 武媛媛 王秋萍 汲婧* 王丽珍* 樊瑜波

(生物力学与力生物学教育部重点实验室, 高端医疗装备与器械创新及转化工业和信息化部重点实验室, 国家医学攻关(医工结合方向)高端医疗装备与器械产教融合创新平台, 北京航空航天大学生物与医学工程学院, 北京 100191)

摘要 角膜作为眼球前部的透明屈光组织, 是视觉形成的关键屏障, 角膜上皮、角膜基质及角膜内皮的结构完整性是维持视觉功能的关键。角膜移植术作为治疗角膜疾病的重要手段, 因供体短缺、免疫排斥等瓶颈问题, 临床推广与应用面临显著挑战。间充质干细胞(mesenchymal stem cells, MSCs)具有自我更新、免疫调节和多向分化特性, 在角膜再生领域展现出独特优势, 为角膜各层组织修复提供了新的治疗策略。该文系统综述MSCs定向分化为角膜上皮细胞、角膜基质细胞及角膜内皮细胞的诱导方法, 为角膜组织工程及再生医学的基础研究与临床转化提供参考。

关键词 间充质干细胞; 角膜; 角膜上皮细胞; 角膜基质细胞; 角膜内皮细胞; 分化

Research Progress on the Differentiation of Mesenchymal Stem Cells Towards Corneal Cells

LI Ling, HUANG Yan, WU Yuanyuan, WANG Qiuping, JI Jing*, WANG Lizhen*, FAN Yubo

(*Key Laboratory of Biomechanics and Mechanobiology*, *Ministry of Education, Key Laboratory of Innovation and Transformation of Advanced Medical Devices*, *Ministry of Industry and Information Technology, National Medical Innovation Platform for Industry-Education Integration in Advanced Medical Devices (Interdiscipline of Medicine and Engineering)*, *School of Biological Science and Medical Engineering*, *Beihang University*, *Beijing 100191*, *China*)

Abstract As the transparent refractive tissue at the front of the eyeball, the cornea serves as a critical barrier for vision. The structural integrity of the corneal epithelium, stroma, and endothelium is essential for maintaining normal visual function. Although corneal transplantation is a vital treatment for corneal diseases, its clinical implementation and application face significant challenges due to donor shortages and immune rejection. MSCs (mesenchymal stem cells), with their self-renewal, immunomodulatory, and multipotent differentiation capabilities, exhibit unique advantages in corneal regeneration and provide novel therapeutic strategies for the repair of corneal tissues across all layers. This review aims to systematically summarize the induction methods for the directional differentiation of MSCs into corneal epithelial cells, corneal keratocytes cells, and corneal endothelial cells, providing references for fundamental research and clinical translation in corneal tissue engineering and regenerative medicine.

Keywords mesenchymal stem cells; cornea; corneal epithelial cells; corneal keratocytes; corneal endothelial cells; differentiation

收稿日期: 2026-02-11 接受日期: 2026-06-25
北京市自然基金重点研究专题(批准号: Z240017)资助的课题
*通信作者。Tel: 010-82315554, E-mail: jingji09714@buaa.edu.cn; lizhenwang@buaa.edu.cn
Received: February 11, 2026 Accepted: June 25, 2026
This work was supported by the Beijing Municipal Natural Science Foundation (Grant No.Z240017)
*Corresponding authors. Tel: +86-10-82315554, E-mail: jingji09714@buaa.edu.cn; lizhenwang@buaa.edu.cn

角膜作为眼球前部的透明屈光组织，是视觉形成的关键屏障，其上皮层、基质层与内皮层构成的三维结构及各层细胞的特异性功能，共同维持着角膜的透明性、屈光性和屏障完整性。上皮层细胞紧密排列抵御外界侵袭，基质层胶原有序排列保障光学特性，内皮层细胞通过主动转运维持角膜水合状态，任何一层细胞的功能异常或数量减少都可能导致角膜混浊甚至失明[1]。目前，角膜移植术仍是治疗终末期角膜疾病的主要手段，但该技术面临着供体角膜严重短缺、患者术后免疫排斥反应高发、复杂角膜损伤修复效果不佳等诸多局限[2]。

干细胞再生疗法的兴起为突破角膜移植术的瓶颈提供了新方向，其通过定向诱导干细胞分化为功能性角膜细胞，实现受损角膜组织的生理性修复，尤其适用于供体不足或多层面损伤的病例[3-4]。在众多干细胞类型中，间充质干细胞(mesenchymal stem cells, MSCs)凭借其独特优势，成为角膜再生领域中颇具应用前景的种子细胞之一[5]。MSCs是具有自我更新能力、免疫调节特性和分化为不同细胞谱系潜能的成体干细胞，可以从多种人体组织(如骨髓、脂肪、脐带、牙髓等)中获得[5-6]。近年来，许多研究证实通过精准调控诱导条件可以实现MSCs向角膜各层细胞的定向分化，其分化效率与功能成熟度不断提升，为构建工程化角膜组织及开发细胞替代疗法奠定了基础[5,7]。本文系统总结了促进MSCs向角膜上皮细胞、角膜基质细胞及角膜内皮细胞方向分化的多种诱导方法，重点分析了不同策略的技术特点、优势局限及应用前景，为推动MSCs在角膜再生治疗中的临床转化提供了参考。

1 MSCs向角膜上皮细胞方向分化

体外诱导MSCs向角膜上皮细胞方向分化的方法主要分为四类：共培养诱导、生物化学因子诱导、生物材料微环境调控以及基因修饰。各类方法的相关研究汇总详见表1。

1.1 共培养诱导

共培养诱导是将两种或两种以上的细胞共同培养于同一环境中的方法，该方法可模拟体内细胞相互作用的生理微环境。共培养可以分为直接共培养和间接共培养，其中间接共培养更便于区分不同的细胞。间接共培养又可以分为Transwell共培养法和条件培养基共培养法。

Transwell共培养法是利用Transwell小室通过膜分隔将不同细胞分开培养，避免不同细胞之间的直接接触，但允许可溶性分子(如生长因子、细胞因子)自由扩散，实现细胞分子信号互通，可以促进MSCs定向分化。如Tsai等[8]在Transwell系统中共培养人脱落乳牙牙髓干细胞(stem cells from human exfoliated deciduous teeth, SHED)与角膜上皮细胞，检测到上皮谱系标记物细胞角蛋白3(cytokeratin 3, CK3)和CK19的表达水平明显增加，说明SHED具有向角膜上皮样细胞转分化的潜力。因此，脱落乳牙可作为获取SHED的理想组织来源，分离得到的SHED具备良好的眼表损伤再生修复潜力。

条件培养基共培养法是利用角膜上皮细胞、角膜缘上皮干细胞(limbal epithelial stem cells, LESCs)等的条件培养基，为MSCs提供可溶性诱导信号，是间接共培养中比较简单的方法。Soleimanifar等[9]认为用角膜上皮细胞培养基，可以促进结膜来源的MSCs(conjunctiva-derived mesenchymal stem cells, CJ-MSCs)分化为角膜上皮细胞，角膜上皮细胞培养基与补充激素上皮培养基(supplemented hormonal epidermal medium, SHEM)联合诱导，可以为干细胞分化为角膜上皮细胞提供更有利的微环境。Venugopal等[10]认为兔角膜缘组织块条件培养基可以诱导兔脂肪组织来源的间充质干细胞(adipose tissue derived-mesenchymal stem cells, AT-MSCs)表达CK3和CK12等角膜上皮标志物的mRNA和蛋白，以热敏聚合物*N*-异丙基丙烯酰胺-co-甲基丙烯酸缩水甘油酯(*N*-isopropylacrylamide-co-glycidyl methacrylate, NGMA)包被培养基底，诱导细胞融合形成细胞片。移植入兔角膜损伤模型，移植后的角膜清晰且光滑。Viveiros等[11]的研究表明，采用亲水化表面改性培养瓶培养角膜上皮外植块，可收获高产量的角膜上皮条件培养基，该培养基能够有效诱导骨髓来源间充质干细胞(bone marrow derived-mesenchymal stem cells, BM-MSCs)向角膜上皮细胞分化。

1.2 生物化学因子诱导

利用特定生长因子、细胞因子或小分子化合物，模拟胚胎发育中的信号调控通路，可以诱导MSCs向角膜上皮细胞谱系定向分化[12]。常用的诱导试剂有骨形成蛋白4(bone morphogenetic protein 4, BMP4)、表皮生长因子(epidermal growth factor, EGF)、胰岛素、全反式维甲酸(all-*trans* retinoic acid, ATRA),

表1 间充质干细胞向角膜上皮细胞分化的方法

Table 1 Methods for differentiating mesenchymal stem cells into corneal epithelial cells

分化方法 Method	MSCs来源 MSCs source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
Co-culture	Human SHED	Co-culture human SHED with corneal epithelial cells in Transwell system; DMEM/F12 (1:1), 5% FBS, 10 ng/mL EGF, 0.5% DMSO	*In vitro*: 7, 14, 21 d	Upregulated expression of CK3 and CK19	Tsai et al/2015[8]
	Human CJ-MSCs	Corneal epithelial cell medium; SHEM: DMEM/F12, 5 ng/mL EGF, 5 μg/mL insulin, 5 μg/mL transferrin, 5 ng/mL sodium selenite, 0.5 μg/mL hydrocortisone, 30 ng/mL cholera toxin A, 0.5% DMSO, 50 μg/mL gentamicin, 1.25 μg/mL amphotericin B, 5% FBS	*In vitro*: 24 d	Expression of CK3, CK12, CK14, CK15, ABCG2, δNp63	Soleimanifar et al/2018[9]
	Rabbit AT-MDSCs	Corneal limbal tissue conditioned medium mixed with low-glucose DMEM at volume ratio 3:1	*In vitro*: 14 d; *In vivo*: 1 month	Expression of CK3, CK12, p63, ABCG2, Pax6; cell sheet transplantation alleviates rabbit corneal injury with clear and smooth cornea	Venugopal et al/2020[10]
	Human BM-MSCs	Corneal epithelial cell medium; DMEM/F12, 10% FBS, 40% corneal epithelial conditioned medium, 5 mL/L TC minimal eagle vitamins, 0.01 U/mL insulin, 15 μg/mL glutathione, 100 IU/mL penicillin, 40 μg/mL gentamicin, 2 μg/mL amphotericin B	*In vitro*: 21 d	Expression of CK3, CK12	Viveiros et al /2024[11]
Biochemical factors	Human BM-MSCs	Induction medium: DMEM, 25 ng/mL BMP4, 10 ng/mL EGF, 1 μmol/L ATRA; differentiation medium: DMEM:F12 (3:1), 5% FCS, 10 ng/mL EGF, 5 μg/mL insulin, 500 ng/mL hydrocortisone, 2 nmol/L triiodothyronine, adenine, 1% penicillin-streptomycin	*In vitro*: 13d	Upregulated mRNA and protein of CK3, CK8, CK12; increased transepithelial electrical resistance	Katikireddy et al/2014[13]
	Human BM-MSCs	DMEM:F12 (3:1), 10% FBS, 10 ng/mL EGF, 5 μg/mL insulin, 0.4 g/L hydrocortisone, 10^{-10} mol/L cholera toxin, 2×10^{-9} mol/L triiodothyronine, 0.18 mmol/L adenine, 2 mmol/L *L*-glutamine	*In vitro*: 10-14 d; *In vivo*: 8 weeks	Expression of CK3, p63, ITGB1; amniotic membrane-engineered grafts reduce corneal neovascularization and reconstruct epithelium in LSCD rat models	Rohaina et al/2014[14]
	Human AT-SCs	MesenPRO RS™, 3 μmol/L CHIR99021, 1 μmol/L E-616452, 500 nmol/L A-83-01, 500 μmol/L VPA, 5 μmol/L tranylcyprmine, 10 μmol/L ATRA	*In vitro*: 3 d	Upregulated E-cadherin, δNp63, ZO-1, occludin, EpCAM, CK19	Setiawan et al/2017[16]
	Human BM-MSCs and AT-MSCs	SHEM: DMEM:F12 (2:1), 2% FBS, 10 ng/mL EGF, 5 μg/mL insulin, 0.4 μg/mL hydrocortisone, 0.5% DMSO, 2 nmol/L triiodothyronine, 0.18 mmol/L adenine, 2 mmol/L *L*-glutamine, 1% antibiotics	*In vitro*: 7 d	Elevated CK12 and ITGB1 expression; enhanced epithelial barrier resistance and wound healing capacity	Nieto-Nicolau et al/2020[15]

续表1

分化方法 Method	MSCs来源 MSCs source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
Biochemical factors	Human AT-DSCs	MET medium M1: MesenPRO RS™, 3 μmol/L CHIR99021, 1 μmol/L E-616452, 500 nmol/L A-83-01, 500 μmol/L VPA, 5 μmol/L tranylcypromine, 10 μmol/L ATRA, 2% serum, triple antibiotics; MET medium M2: 300 nmol/L CHIR99021, 50 μmol/L VPA; M2/CNT-50 medium (1:1 mixture); pure CNT-50 medium	*In vitro*: M1: 5 d; M2: 7 d; M2/CNT-50: 3 d; CNT-50: 10 d; *in vivo*: 4 weeks	Expression of E-cadherin, δNp63, ZO-1, CK5, CK19; grafts mitigate corneal edema and opacity in alkali-burn LSCD rats	Bandeira et al/2020[19]
	Human WJ-MSCs	Step 1: 10 μmol/L SB505124, 25 ng/mL BMP4, 10 ng/mL EGF, 10 μmol/L ATRA; Step 2: SHEM: DMEM:F12 (2:1), 5% FBS, 10 ng/mL EGF, 1% ITS, 0.5 μg/mL hydrocortisone, 0.05% DMSO, 200 nmol/L adenine, 100 U/mL penicillin, 100 μg/mL streptomycin	Step 1: 3 d; step 2: 6 d	Expression of CK12, CK3, CK15, p63, Pax6	Nguyen et al/2022[17]
	Human AT-DSCs	Step 1: 10 μmol/L SB-505124, 10 μmol/L IWP-2, 50 ng/mL FGF-2, DMEM, 15% FBS, 862 mg/L glutamine, 110 mg/L pyruvate, 1% penicillin/streptomycin; step 2: modified SHEM: DMEM:F12 (2:1), 2% FBS, 5 μg/mL insulin, 10 ng/mL EGF, 4 nmol/L PEDF, 20 ng/mL KGF, 0.4 μg/mL hydrocortisone, 0.5% DMSO, 0.18 mmol/L adenine, 2 nmol/L triiodothyronine	Step 1: 7 d; step 2: 10 d	Expression of αp63, BMI-1	Cadenas-Martin et al/2023[18]
Biological materials	Human CJ-MSCs	Nanofiber scaffold, DMEM/F12, 5% FBS, 1% penicillin-streptomycin, 500 ng/mL hydrocortisone, 5 μg/mL insulin, 2 nmol/L triiodothyronine, adenine, 10 ng/mL EGF	*In vitro*: 12 d	Upregulated CK3, CK8, CK12	Soleimanifar et al/2017[22]
	Human WJ-MSCs	PRGF-enriched collagen/silk fibroin scaffold; DMEM/F12, 5% FBS, 2 mmol/L *L*-glutamine, 1% penicillin-streptomycin (100 μg/mL)	*In vitro*: 9 d, 17 d	Elevated CK3 and CK12 expression	Pourjabbar et al/2024[23]
Gene transfection	Rat AT-MSCs	*Pax6* gene transfected into rat AT-MSCs; DMEM/F12, 10% FBS	*In vitro*: 21 d; *in vivo*: 7 d	Expression of CK3, CK12, E-cadherin; Pax6-modified cells reconstruct ocular surface in rabbit LSCD models	Sun et al/2018[26]
	Mouse BM-MSCs	Pax6-overexpressed mouse BM-MSCs for limbal stem cell differentiation; DMEM/F12, 10% FBS, 500 μL alanyl-glutamine, 500 μL non-essential amino acids, 500 μL penicillin-streptomycin, 10^{-10} mol/L cholera toxin, 0.5 mg/L hydrocortisone, 5 mg/L insulin, 2 mg/L triiodothyronine, 50 μg/L EGF, 5 μg/mL NGF, 10 μg/mL TGF	*In vitro*: 5 d, 10 d	Upregulated p63, CK14	Wu et al/2022[27]
	Mouse BM-MSCs	*Pax6*-transfected mouse BM-MSCs; DMEM:F12, 1/100 penicillin-streptomycin, 20% FBS, 5 mg/mL insulin, 0.4 mg/mL hydrocortisone, 10^{-10} mol/L cholera toxin, 2×10^{-10} mol/L triiodothyronine, 50 ng/mL EGF, 10 ng/mL TGF, 5 ng/mL NGF	*In vitro*: 10 d; *in vivo*: 42 d	Expression of CK14, p63, CK12; Pax6-BM-MSCs form stratified corneal epithelium and repair chemically burned mouse corneas	Gao et al/2023[28]

以及信号通路抑制剂包括糖原合酶激酶3(glycogen synthase kinase 3, GSK3)抑制剂和转化生长因子-β(transforming growth factor-β, TGF-β)受体激酶抑制剂等, 这些分子可以单独或联合发挥诱导MSCs向角膜上皮细胞方向分化的作用。

首先, BMP4、EGF、胰岛素和ATRA常被用来诱导MSCs向角膜上皮细胞方向分化, 上调角膜上皮细胞特异性标志物的表达。如Katikireddy等[13]从人BM-MSCs中筛选出SSEA4$^+$细胞群, 先用含有BMP4、EGF和ATRA的诱导培养基, 然后用含有5%胎牛血清、EGF、胰岛素、氢化可的松、三碘甲状腺原氨酸和腺嘌呤等的分化培养基进行培养, 检测到角膜上皮特异性标志物CK3和CK12的mRNA与蛋白表达水平显著升高。EGF与胰岛素的联合作用对于诱导MSCs向角膜上皮细胞方向分化比较重要, 如Rohaina等[14]用含有10%胎牛血清、EGF、胰岛素、氢化可的松、霍乱毒素、三碘甲状腺原氨酸和腺嘌呤等的培养基诱导人BM-MSCs, 体外检测可见诱导细胞显著表达CK3等角膜上皮特异性标志物。将分化后的细胞与羊膜复合构建组织工程角膜, 并将其移植至LESCs缺陷大鼠模型后, 该移植物可显著提高大鼠角膜透明度、抑制新生血管增殖, 并重建完整角膜上皮层结构。又如Nieto-Nicolau等[15]用含有2%胎牛血清、EGF、胰岛素、氢化可的松、二甲基亚砜、三碘甲状腺原氨酸和腺嘌呤的SHEM分别诱导人BM-MSCs和AT-MSCs, 培养7 d后, BM-MSCs组的CK12和整合素β1(integrin subunit beta 1, ITGB1)的mRNA和蛋白表达水平均显著上调, 取该分化细胞进行跨上皮电阻检测与创面愈合实验, 可见诱导后的细胞的跨上皮电阻提升、细胞创面愈合能力增强, 证实BM-MSCs具备向角膜上皮细胞分化的潜能。但是如果单独使用ATRA, BM-MSCs非特异性细胞角蛋白pan-CK和CK19表达水平显著增加, 而特异性蛋白表达水平增加并不明显, 说明单独使用ATRA可能并不能诱导BM-MSCs分化为功能性角膜上皮细胞, 但仍然具有促进角膜组织再生的能力[15]。

近年来, 靶向特异性信号通路的小分子抑制剂已广泛应用于干细胞定向诱导体系, 可有效提升上皮分化效率。Setiawan等[16]联合多种通路抑制剂[诱导试剂包含GSK3抑制剂CHIR99021、TGF-β1受体激酶抑制剂RepSox(E-616452)、TGF-β1型受体抑制剂A-83-01、组蛋白去乙酰化酶抑制剂丙戊酸(valproic acid, VPA)、单胺氧化酶抑制剂反苯环丙胺及ATRA]干预人AT-MSCs, 持续处理细胞72 h。分子检测结果显示: 介导上皮–间充质转化、维持细胞间质成纤维样表型的间充质特征基因*SNAI2*、*ZEB1*、*VIM*和*ITGA5*表达显著下调; 上皮标志物*E-cadherin*、*δNp63*、*ZO-1*、*occludin*、*EpCAM*和*CK19*表达明显上调。全基因组表达谱分析筛选得到540个上调基因、483个下调基因, 上调基因富集于细胞周期调控、细胞骨架重塑、上皮发育等通路, 下调基因多富集于间质表型、纤维化及胞外基质重构相关通路。同时细胞形态由细长梭形转变为多边形上皮样结构, 细胞迁移能力显著降低, 透射电镜可观察到紧密连接、桥粒样复合物。上述分子与细胞形态变化共同证实, 该小分子抑制剂组合可抑制细胞原有间质表型, 高效诱导上皮–间充质转化, 使细胞获得稳定角膜上皮样特征。Nguyen等[17]先用TGF-β抑制剂SB505124、BMP4、EGF和ATRA的组合处理人脐带华顿氏胶来源的MSCs(Wharton's jelly mesenchymal stem cells, WJ-MSCs) 3 d, 然后用含有5%胎牛血清、EGF、胰岛素–转铁蛋白–亚硒酸钠(insulin-transferrin-sodium selenite, ITS)、氢化可的松、二甲基亚砜和腺嘌呤等的SHEM培养6 d, 即可诱导生成表达角膜上皮细胞特异性标志物CK12的角膜上皮样细胞。其中, ATRA是通过抑制β-连环蛋白由细胞质向细胞核的转位, 从而实现对Wnt信号通路的阻断。Cadenas-Martin等[18]先用含有Wnt抑制剂IWP-2(inhibitor of wnt production 2)、TGF-β抑制剂SB-505124及成纤维细胞生长因子2(fibroblast growth factor 2, FGF-2)的培养基处理人AT-MSCs 7 d, 然后在添加色素上皮衍生因子(pigment epithelium-derived factor, PEDF)和角质细胞生长因子(keratinocyte growth factor, KGF)的改良SHEM中培养10 d, 可以诱导细胞分化为LESCs干细胞。Bandeira等[19]在Setiawan等[16]研究的基础上开展优化实验, 先联合使用GSK3抑制剂、TGF-β抑制剂、VPA与ATRA处理人AT-MSCs, 驱动细胞发生上皮–间充质转化并生成上皮祖细胞; 再采用上皮定向诱导体系持续培养, 使祖细胞进一步分化为成熟上皮样细胞。利用该细胞构建的组织工程角膜移植物可有效修复碱烧伤大鼠受损角膜, 显著缓解角膜水肿并减轻角膜混浊。

1.3 生物材料微环境调控

三维支架系统包括软支架材料、硬支架材料、

复合型支架材料以及无支架三维培养系统[20]。三维支架能为细胞共培养提供仿生基质支撑，此前研究已证实其在人工角膜模型构建中的作用[21]。Soleimanifar等[22]发现使用纳米纤维支架能促进CJ-MSCs的*CK3*基因表达水平显著升高，证实纳米纤维支架和生化因子联合使用可以有效促进MSCs向角膜上皮细胞方向分化。Pourjabbar等[23]开发了一种富含富血小板生长因子的胶原蛋白丝纤维素支架，用来支持WJ-MSCs的增殖，以及其向角膜上皮细胞方向分化，结果观察到CK3和CK12的表达水平显著增加。三维支架介导的干细胞分化技术日趋成熟，与化学诱导手段结合可显著提升分化效率，相关方案已在角膜上皮组织工程研究中得到广泛应用[24]。

1.4 基因修饰

配对盒基因6(paired box gene 6, Pax6)属于Pax转录因子家族，是调控眼部发育的关键转录因子，对维持角膜正常发育稳态至关重要[25]。LESCs不断更新角膜上皮细胞，而*Pax6*作为核心调控基因，可通过外源转染诱导MSCs向角膜上皮细胞、角膜缘干细胞定向分化。Sun等[26]将Pax6转染至大鼠AT-MSCs中，诱导21 d后观察到角膜上皮细胞特异性标志物CK3、CK12和E-cadherin的表达水平增加，将该分化细胞移植至兔角膜损伤模型，细胞可定植生长并重建受损角膜上皮屏障。吴霜等[27]针对小鼠BM-MSCs的研究证实，Pax6过表达联合生化因子共诱导时，细胞向LESCs定向分化的速率与诱导比例均显著提升；该诱导体系还可促进干细胞增殖、抑制细胞凋亡。Gao等[28]进一步发现过表达Pax6能够显著增强BM-MSCs的集落形成能力，且该类干细胞可有效修复化学烧伤造成的小鼠角膜损伤。

在调控MSCs向角膜上皮细胞定向分化的分子网络中，Wnt/β-catenin、TGF-β/Smad、MAPK/ERK及PI3K-Akt通路均发挥核心调控作用。ATRA、Wnt抑制剂与TGF-β抑制剂可协同启动细胞上皮–间充质转化进程，而转录因子Pax6是介导干细胞向上皮谱系定向分化的核心调控分子。目前诱导干细胞分化为角膜上皮细胞的主流策略可分为四类：共培养诱导、生物化学因子诱导、生物材料微环境调控以及基因修饰。其中共培养体系高度模拟眼表生理微环境，诱导效果稳定、上皮表型维持稳定，但体系成分复杂，批次差异大，难以标准化，不利于临床转化；生化小分子组合诱导的可操作性强、条件可控，便于实验重复与机制研究，但单一细胞因子诱导特异性有限，往往需要多因子联用；三维生物材料可优化细胞黏附与形态，改善二维培养的细胞去分化问题；基因转染(Pax6等)可显著提升分化纯度与定向效率，但基因编辑存在生物安全隐患，暂不适合临床应用，而且诱导后上皮细胞屏障功能验证不足，功能成熟度仍有局限。总之，MSCs向角膜上皮细胞分化的研究可以突破角膜上皮种子细胞来源限制，初步重建上皮屏障，并通过旁分泌发挥协同修复作用。但该领域仍存在很多问题，如分化细胞表型纯度与稳定性不足、缺乏成熟仿生诱导体系、移植后长期存活及功能整合效果欠佳，这些问题值得未来进一步深入研究。

2 MSCs向角膜基质细胞方向分化

体外诱导MSCs向角膜基质细胞方向分化的主要方法分为四类：共培养诱导、生物化学因子诱导、生物材料微环境调控、物理调控。各类方法的相关研究汇总详见表2。

2.1 共培养诱导

利用Transwell装置，共培养干细胞和角膜基质细胞，可以诱导干细胞向角膜基质细胞方向分化。如，Zhang等[29]将人AT-MSCs与角膜基质细胞通过Transwell装置进行共培养，观察到角膜基质细胞特异性标志物角膜蛋白多糖(keratocan)和醛脱氢酶3家族成员A1(aldehyde dehydrogenase 3 family, member A1, ALDH3A1)基因和蛋白表达水平升高。角膜基质细胞条件培养基也可以诱导MSCs向角膜基质细胞方向分化。Park等[30]利用角膜基质细胞条件培养基诱导人BM-MSCs，诱导后细胞可表达*keratocan*、*ALDH3A1*与基膜聚糖(*lumican*)，同时α-平滑肌肌动蛋白(α-smooth muscle actin, *α-SMA*)的表达被显著抑制。为明确该条件培养基中发挥诱导作用的核心活性物质，该团队后续实验进一步说明在角膜基质细胞条件培养基中，胰岛素样生长因子结合蛋白2(insulin-like growth factor binding protein 2, *IGFBP2*)是关键活性成分，添加500 ng/mL的IGFBP2时，可以明显促进人BM-MSCs表达*IGFBP2*、*keratocan*和*ALDH1A1*，并下调*α-SMA*水平[31]。Ghiasi等[32]利用角膜基质细胞条件培养基诱导人AT-MSCs向角膜基质细胞方向分化，负载细胞的小切口微透镜在兔角膜基质中具有良好的生物安全性和组织相容性，细胞可长期存活

表2 间充质干细胞向角膜基质细胞分化的方法

Table 2 Methods for differentiating mesenchymal stem cells into corneal stromal cells

分化方法 Method	MSCs来源 Source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
Co-culture	Human BM-MSCs	Seed cells in α-MEM for 24 h, then culture in α-MEM supplemented with 10% keratocyte conditioned medium, gradually increased to 40%; DMEM/F12, 10% FBS	*In vitro*: 15 d	Upregulated *keratocan*, *lumican*, *ALDH1A1*	Park et al/2012[30]
	Human AT-MSCs	DMEM, 10 ng/mL bFGF, 0.1 mmol/L A2-P, 1% heparin-stripped, platelet-poor horse serum, 100 U/mL penicillin, 100 μg/mL streptomycin	*In vitro*: 16 d	Elevated *keratocan* and *ALDH3A1*	Zhang et al/2013[29]
	Human AT-MSCs	DMEM/F12 supplemented with 10% keratocyte conditioned medium, gradually raised to 40%; DMEM/F12, 5% FBS, 1% amniotic matrix extract, 1% A2-P, 1% ITS, 1% non-essential amino acids	*In vitro*: 7, 14, 21 d; *in vivo*: 3 months	Expression of *keratocan*, *lumican*, *ALDH3A1*, *CD34*; cell-laden microlenses exhibit good biocompatibility, long-term cell survival and stable keratocyte phenotype without fibrosis or rejection in rabbit stroma	Ghiasi et al/2023[32]
Biochemical factors	Human AT-MSCs	MEM, 5 ng/mL bFGF, 20% FBS, 1% *L*-glutamine, 1% penicillin-streptomycin	*In vitro*: 14 d; *in vivo*: 10 weeks	Expression of keratocan, *ALDH3A1*, *COLI*; combined with hyaluronic acid ECM scaffold, cells differentiate into functional keratocytes in rabbit cornea	Espandar et al/2012[33]
	Human CSSCs	DMEM, 1 mmol/L A2-P, 2 mmol/L *L*-alanyl-*L*-glutamine, 50 μg/mL gentamicin, 100 μg/mL penicillin; group 1: 10 ng/mL FGF-2; group 2: 0.1 ng/mL TGF-β3; group 3: 10 ng/mL FGF-2+0.1 ng/mL TGF-β3; cultured on polyurea fibrous substrates	*In vitro*: 9 weeks	Upregulated *ALDH3A1*, *keratocan*, *CHST6*, *B3GNT7*; suppressed fibrotic α-SMA phenotype	Wu et al/2013[36]
	Human LSSCs	DMEM, 10 ng/mL bFGF, 0.1 ng/mL TGF-β3, 1 mmol/L A2-P; cultured on aligned nanofiber substrates	*In vitro*: 7 d, 30 d; *in vivo*: 2, 4 weeks	Expression of *ALDH3A1*, *AQP1*, *keratocan*, *PTGDS*; fiber gel grafts inhibit corneal scarring and form lamellar stroma with native-like collagen arrangement in mouse injury models	Basu et al/2014[37]
	Human DPCs	DMEM, 10 ng/mL bFGF, 0.1 ng/mL TGF-β3, 1 mmol/L A2-P; seeded on 700 nm-diameter PCL nanofibers	*In vitro*: 21 d; *in vivo*: 1, 5 weeks	Expression of *ALDH3A1*, *AQP1*, *CHST6*, *keratocan*, *PTGDS*, *B3GNT7*; *in vitro* differentiated cells secrete human type I collagen and keratocan after intrastromal injection in mice without opacity or immune rejection	Syed-Picard et al/2015[38]
	Human AT-MSCs	10 ng/mL bFGF, 0.1 mmol/L A2-P, 2 mmol/L glutamine, gradient ATRA (0.1, 1, 10 μmol/L)	*In vitro*: 7 d, 14 d	Upregulated *ALDH3A1*, *keratocan*, *lumican*, *decorin*, *COLI*	Lynchl et al/2017[42]
	Human TMSCs	10 ng/mL KGF/EGF, recombinant human keratocyte growth factor, 10 ng/mL EGF, 1% horse serum, 10 000 U/mL streptomycin/penicillin	*In vitro*: 14 d	Elevated *keratocan*, *ALDH3A1*, *PAX6*, *ABCG2*; reduced *SOX2* and *Notch*	Park et al/2017[44]

续表2

分化方法 Method	MSCs来源 Source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
	Human CSSCs	DMEM/F12, 50 μg/mLA2-P, 10 μg/mL human insulin, 5.5 μg/mL human transferrin, 6.7 ng/mL sodium selenite, 1% nonessential amino acids, antibiotics,10 ng/mL FGF-2, 0.1 ng/mL TGF-β3, 10 μmol/L ATRA	*In vitro*: 21 d	Expression of *ALDH3A1*, *CD34*, *keratocan*, *COL1A1*, *lumican*	Sidney and Hopkinson/2018[43]
	Human PDLSCs	Step 1: suspension spheroid culture in DMEM/F12, 1% B27, 1% N2, 0.5 mmol/L β-mercaptoethanol, 15% chick embryo extract, 5% FBS; Step 2: culture on human amniotic matrix and porcine corneal matrix with DMEM/F12, 20 ng/mL bFGF, 0.1 ng/mL TGF-β3, 1 mmol/L A2-P, MEM amino acids, MEM non-essential amino acids, ITS	Step 1: 14 d; step 2: 15 d	Upregulated *ALDH3A1*, *keratocan*, *lumican*, *CHST6*, *B3GNT7*, *COL8A2*	Yam et al/ 2018[40]
	Human CSSCs; Human AT-MSCs; Human BM-MSCs; Human UC-MSCs	DMEM, 10 ng/mL FGF-2, 1 ng/mL TGF-β3, 1 mmol/L A2-P	*In vitro*: 7 d	Expression of *keratocan*, *lumican*, *CHST6*, *PTGDS*, *ALDH3A1*, *PAX6*	Dos et al/2019[39]
	Canine limbal CSSCs	DMEM, 10 ng/mL bFGF, 0.1 mmol/L A2-P, 50 μg/mL gentamicin, 1% penicillin/streptomycin, 1% glutamine	*In vitro*: 21 d	Elevated *keratocan*, *lumican*, *ALDH1A3*	Kafarnik et al/2020[34]
	Rat LSSCs	5% GelMA hydrogel infused into PCL-PEG microfiber scaffolds to form fiber-reinforced 3D hydrogel; DMEM/F12, 10 ng/mL bFGF, 1 mmol/L ascorbic acid, 1% ITS-X, 1× glutamine	*In vitro*: 7 d, 2 weeks, 4 weeks; *in vivo*: 3 months	Expression of *ALDH3A1*, *keratocan*, *AQP1*; rat intrastromal grafts show high corneal transparency, negligible neovascularization, well-integrated regenerated stroma with uniform type VI collagen secretion	Kong et al/2020[35]
	Human BM-MSCs	DMEM/F12, 1% MEM vitamins, 1% MEM non-essential amino acids, 20 ng/mL bFGF, 0.1 ng/mL TGF-β3, 1 mmol/L A2-P, 1% ITS, 1% penicillin/streptomycin; 3D bioprinting: BM-MSCs encapsulated in composite hydrogel with 0.5% agarose and 0.2% type I collagen	*In vitro*: 14 d, 21 d	Upregulated *lumican*, *keratocan*, *ALDH1A1*, *ALDH3A1*, *COLI*	Taoum et al/2025[41]
Biological materials	Human TMSCs	Pre-differentiation medium: DMEM,10% FBS, 1% penicillin/streptomycin, and 10 ng/mL KGF/EGF; long-term 3D culture medium for cells encapsulated in co-dECM bioink: DMEM, 10% FBS, and 1% penicillin/streptomycin	*In vitro*: 14 d; *in vivo*: 56 d (mouse), 28 d (rabbit)	Expression of *keratocan* and *ALDH3A1*; mild inflammation in mouse subcutaneous grafts; stable graft morphology without obvious immune rejection in rabbit cornea, maintaining keratocyte phenotype	Kim et al/2019[45]

续表2

分化方法 Method	MSCs来源 Source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
	Human PMSCs	Bioink: 3% sodium alginate, 6 mg/mL dCECM, 10% type B gelatin at volume ratio 2:1:1; differentiation medium: DMEM/F12, 5% NuSera, 1× ITS, 0.5 mmol/L *L*-ascorbic acid, 1× MEM vitamins, 1× penicillin/streptomycin/amphotericin B, 250 μg/mL dCECM	*In vitro*: 28 d	Elevated *keratocan* and *ALDH3A1* expression	Marin-Tapia et al/2025[46]
Physical regulation	Human PDLSCs	3% equiaxial dome-shaped mechanical strain via Flexcell tension system; DMEM, 10 ng/mL bFGF, 0.1 ng/mL TGF-β3, 1 mmol/L A2-P; four groups: strain alone, chemical induction alone, combined treatment, sequential treatment	*In vitro*: 3 d, 7 d; cell sheet construction: 12 d	Upregulated *ALDH3A1*, *CD34*, *keratocan*, *lumican*, *COLI*, *COLV*	Chen et al/2018[47]
	Human dental follicle stem cells	Supercritical CO_2 decellularized porcine corneal ECM, α-MEM, 10% FBS	*In vitro*: 21 d	Expression of *keratocan*, *ATP1A1*, *N-cadherin*	Lin et al/2025[49]

并维持角膜基质细胞功能, 无纤维化或排斥风险。

2.2 生物化学因子诱导

FGF-2[亦称碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)和抗坏血酸-2-磷酸盐(ascorbate-2-phosphate, A2-P)]、TGF、ATRA、KGF、EGF等常被用来诱导干细胞向角膜基质细胞方向分化。有些研究单独或联合使用生物化学因子, 也有的研究把生物化学因子和生物材料结合起来, 生物材料可模拟角膜基质胶原纤维有序排列的特征, 共同诱导MSCs分化为功能性的角膜细胞。

在诱导干细胞向角膜基质细胞方向分化的生物化学因子中, bFGF是被使用较多的生物化学因子。如Espandar等[33]将经5 ng/mL bFGF诱导的人AT-MSCs复合透明质酸衍生胞外基质支架移植至兔角膜基质, 细胞可在体内存活长达10周, 并分化为功能性角膜基质细胞。Kafarnik等[34]研究证实, 犬角膜基质边缘分离的角膜基质干细胞(corneal stromal stem cells, CSSCs)具备典型间充质干细胞特征; 采用10 ng/mL bFGF联合0.1 mmol/L A2-P处理该细胞后, 细胞呈特征性星状形态, 角膜基质特异性标志物*keratocan*、*lumican*、*ALDH1A3*表达显著上调, 同时*Pax6*、*N-cadherin*与*α-SMA*表达水平降低。Kong等[35]的研究表明, 10 ng/mL bFGF、1%胰岛素–转铁蛋白–硒–乙醇胺添加剂(insulin-transferrin-selenium-ethanolamine additive, ITS-X)和1 mmol/L抗坏血酸的组合能够促进大鼠角膜源基质干细胞(limbal stromal stem cells, LSSCs)表达基质细胞特异性的ALDH3A1、keratocan、水通道蛋白1(aquaporin-1, AQP1)基因和蛋白质。他们制备了网格状的聚己内酯–聚乙二醇微纤维支架, 并将明胶甲基丙烯酸酯(gelatin methacrylate, GelMA)水凝胶注入支架中, 通过调整纤维间距获得能模拟基质结构且具有与天然角膜最相似特性的最优结构。无论是在体外还是体内, 生物化学因子与生物材料的协同作用均能够维持角质细胞的表型, 并诱导与天然基质组织相似的正交排列的细胞外基质分泌。

另外, 由bFGF、TGF-β3和A2-P构成的诱导方案, 常用于诱导MSCs向角膜基质细胞分化。如Wu等[36]设置三组处理干预人CSSCs, 持续培养9周, 分别采用10 ng/mL FGF-2、0.1 ng/mL TGF-β3单独诱导, 或两种因子联用并添加1.0 mmol/L A2-P。检测结果显示, 角膜基质细胞标志物ALDH3A1、keratocan、碳水化合物硫酸转移酶6(carbohydrate sulfotransferase 6, CHST6)、UDP-葡萄糖醛酸: 半乳糖-β-1,3-*N*-乙酰葡糖胺转移酶7(UDP-GlcNAc:Gal-β-1,3-*N*-acetylglucosaminyltransferase 7, B3GnT7)表达显著上调, 而纤维连接蛋白额外域A(fibronectin extra domain-A, Fn-EDA)与α-SMA的表达被抑制。将上述诱导后的各组细胞接种至聚酯脲醛纤维基质培养后可见: FGF-2组胶原纤维沿单一方向排布, TGF-β3

组形成垂直于纤维走向的胶原层, FGF-2与TGF-β3联合诱导组可形成多层板状结构, 内部胶原纤维呈正交排列, 该结构模式与人角膜基质组织相似。Basu等[37]采用10 ng/mL FGF-2、0.1 ng/mL TGF-β3联合1.0 mmol/L A2-P诱导人LSSCs, 并将细胞接种于定向排布的纳米纤维基底上进行培养。诱导后的细胞可分化为角膜基质细胞, 稳定表达特征标志物ALDH3A1、AQP1、keratocan与前列腺素D合酶(prostaglandin D2 synthase, PTGDS), 还可构建富含胶原蛋白、硫酸角质素蛋白聚糖的厚层板状基质样组织。将该基质样组织移植至小鼠角膜损伤区域, 能够有效抑制瘢痕生成; 新生修复组织呈层状结构, 胶原排布模式与天然角膜基质高度一致。同样, Syed-Picard等[38]采用10 ng/mL bFGF、0.1 ng/mL TGF-β3联合1 mmol/L A2-P诱导人牙髓干细胞(dental pulp stem cells, DPCs), 并将细胞接种于聚己内酯纳米纤维基底。诱导细胞可在基因与蛋白层面表达角膜基质细胞特异性分子; 将细胞注射至小鼠角膜基质后, 能够分泌含人I型胶原蛋白与keratocan的胞外基质, 既不会破坏角膜透明度, 也无明显免疫排斥现象。在此基础上, Dos等[39]对比多种来源间充质干细胞的角膜基质分化潜能: 使用10 ng/mL bFGF、1 ng/mL TGF-β3及1 mmol/L A2-P分别处理人AT-MSCs、BM-MSCs、UC-MSCs与CSSCs, 各类干细胞均可向角膜基质细胞分化, 表现为角膜基质特征标志物表达上调; 其中CSSCs的定向分化潜能最优。

尽管bFGF、TGF-β3与A2-P的组合常应用于诱导MSCs向角膜基质细胞分化, 但不同研究采用的作用浓度存在一定差异。如Yam等[40]先悬浮培养人牙周膜干细胞(periodontal ligament-derived stem cells, PDLSCs)制备细胞球, 再将细胞接种于人羊膜基质、猪角膜基质, 使用20 ng/mL bFGF、0.1 ng/mL TGF-β3与1 mmol/L A2-P开展诱导; 检测结果表明, *ALDH3A1*、*keratocan*、*lumican*、*CHST6*、*B3GNT7*、VIII型胶原α2链(collagen type VIII alpha 2, *COL8A2*)等角膜基质细胞相关基因表达均显著上调。Taoum等[41]将人BM-MSCs包埋于含0.5%琼脂糖和0.2% I型胶原蛋白的复合水凝胶中, 使用20 ng/mL bFGF、0.1 ng/mL TGF-β3和1 mmol/L A2-P诱导培养人BM-MSCs。结果显示*lumican*、*keratocan*、*ALDH1A1*、*ALDH3A1*和I型胶原蛋白等角膜基质细胞相关的标志物表达显著上调, 原因是, 三维培养比二维细胞培养系统更能模拟角膜基质, 为干细胞向角膜基质细胞的分化提供了更优越的环境。

此外, ATRA也可以促进干细胞向角膜基质细胞方向分化。如Lynch等[42]在使用10 ng/mL bFGF和0.1 mmol/L A2-P的基础上, 分别添加0.1、1、10 μmol/L的ATRA, 结果显示ATRA上调了角质细胞特异性标志物的表达, 提高了I型胶原蛋白的含量。另外, Sidney和Hopkinson[43]使用无血清培养基并补充10 ng/mL FGF-2、0.1 ng/mL TGF-β3和10 μmol/L ATRA, 能够使间充质化的细胞再次回到角膜基质细胞的表型, 但是单独使用ATRA并不足以刺激细胞外基质蛋白的产生。

还有研究者使用KGF和EGF作为小分子诱导剂, 结合生物墨水提供的物理线索联合诱导MSCs向角膜基质细胞分化。如Park等[44]利用10 ng/mL KGF/EGF诱导人鼻甲来源间充质干细胞(turbinate-derived mesenchymal stem cells, TMSCs), 细胞表达角膜基质细胞标志物*keratocan*和*ALDH3A1*, 而干细胞标志物*SOX2*和*Notch*的表达水平明显下降。

2.3 生物材料微环境调控

为角膜组织工程开发特制的生物墨水对于构建角膜的天然结构和功能至关重要。Kim等[45]发现牛角膜来源的脱细胞外基质(cornea-derived decellularized extracellular matrix, Co-dECM)在胶原蛋白和糖胺聚糖的定量测量结果方面与天然角膜相似, 并且具有适宜的透明度有利于视力恢复, 还含有多种生长因子, 包括FGF、TGF和胰岛素样生长因子。将10 ng/mL KGF/EGF预处理的人TMSCs培养在Co-dECM胶体中, 检测到角膜基质层的代表性标志物keratocan和ALDH3A1的表达, 将负载干细胞的Co-dECM复合体植入小鼠和兔子体内, 生物相容性良好, 并有助于维持角膜基质细胞的特性。Marin-Tapia等[46]以海藻酸钠、脱细胞角膜细胞外基质(decellularized corneal extracellular matrix, dCECM)与B型明胶为原料制备生物墨水, 系统评价不同配比墨水的黏度、剪切稀化及黏弹性特征。结果显示, 配比为3%海藻酸钠、6 mg/mL dCECM、10% B型明胶(体积比2:1:1)的3G10生物墨水流变特性与打印效果最优, 成型稳定、具备角膜生理曲率的支架结构, 拥有高形状保真度、接近天然角膜的透光率, 杨氏模量也处于角膜生理区间。将人胎盘源间充质干细胞(placenta-derived mesenchymal stem cells, PMSCs)封装于该墨水内培养, 细胞呈伸长形态, 角膜基质细

胞特异性标志物*keratocan*、*ALDH3A1*表达显著上调。

2.4 物理调控

角膜基质承受的穹顶形应变是重要生理特征之一, 体外给干细胞加载力学刺激也可以促进其向角膜基质方向分化。如Chen等[47]利用Flexcell张力系统给人PDLSCs加载3%静态穹顶形机械应变, 结果表明角膜基质细胞相关基因包括*ALDH3A1*、*CD34*、*keratocan*、*lumican*、I型胶原蛋白和V型胶原蛋白的表达水平明显上调, 力学刺激能够促进人PDLSCs向角膜基质细胞方向分化, 而且力学因素与生物化学因子的协同作用显著。Zhang等[48]进一步证明3%的应变可以通过上调*ALDH3A1*的表达来影响角膜基质细胞增殖和迁移, 这些发现为进一步研究角膜应变及其与角膜损伤修复之间的关系提供了有价值的见解。天然角膜脱细胞基质可通过物理结构引导MSCs定向分化。近期研究表明, 将牙囊干细胞接种于经超临界二氧化碳脱细胞处理的猪角膜基质支架培养后, 细胞可表达角膜基质特征标志物, 提示该细胞具备向角膜基质样细胞定向分化的潜能[49]。

对于诱导MSCs向角膜基质细胞方向的分化, FGF/MAPK、TGF-β/Smad、Wnt信号通路可能起了重要作用, 力学信号可以通过细胞骨架–核机械传导促进*ALDH3A1*等关键基因的表达。基质细胞条件培养基和复合生化因子诱导(联用bFGF、TGF-β3和抗坏血酸)是目前主要诱导MSCs向角膜基质细胞方向分化的方法, 三维仿生支架和力学物理刺激是近年来的补充优化手段。其中条件培养基组分不明确, 标准化生产难度大; 生化组合诱导体系成熟, 可稳定表达Keratocan、ALDH3A1等角膜基质特征标志物; 脱细胞角膜基质等仿生材料可模拟天然角膜胶原微环境, 维持基质细胞静息表型; 适度机械力学刺激可协同生化信号, 提升分化效率与基质胶原有序分泌能力, 但长期体外培养易出现细胞纤维化转型, 破坏角膜基质透明特性。总之, MSCs向角膜基质层分化的研究可以解决角膜基质修复的力学支撑难题、抑制角膜基质瘢痕形成、构建三维组织工程支架。目前存在的主要问题是分化细胞的基质分泌功能与排列有序性不足、诱导分化的调控机制尚不明确、三维支架与细胞的适配性仍有待优化等。

3 MSCs向角膜内皮细胞方向分化

体外诱导MSCs向角膜内皮细胞方向分化的方法主要分为三类: 共培养诱导、生物化学因子诱导、以及物理调控。各类方法的相关研究汇总详见表3。

3.1 共培养诱导法

共培养干细胞和角膜内皮细胞, 可以诱导干细胞向角膜内皮细胞方向分化。Dai等[50]建立多模式诱导体系处理人AT-MSCs: 一方面采用重组细胞穿透蛋白、小分子重编程试剂联合模拟微重力生物反应器完成细胞非遗传直接重编程; 另一方面分别开展两组共培养实验, 一是与角膜内皮细胞直接接触共培养, 二是借助Transwell小室与角膜基质细胞间接共培养。经上述处理后的AT-MSCs由纺锤形转变为典型多边形, 同时表达*CD31*、*AQP1*、*ZO-1*等角膜内皮特征标志物。

条件培养基可以诱导MSCs向角膜内皮细胞方向分化。Joyce等[51]使用了三种不同的培养基, 包括MSCs基础培养基、用于培养晶状体上皮细胞的基质培养基和晶状体上皮细胞条件培养基, 诱导脐带血来源间充质干细胞(umbilical cord blood-derived mesenchymal stem cells, UCB-MSCs)向角膜内皮细胞方向分化。体外结果显示, 在晶状体上皮细胞基础培养基与条件培养基中培养的UCB-MSCs、ZO-1、N-cadherin均可定位于细胞边缘; 体内移植实验证实, UCB-MSCs能够归巢至角膜内皮损伤区域并呈现角膜内皮样表型, 其中以晶状体上皮细胞条件培养基诱导的细胞内皮特征最为典型。此外, Bosch等[52]利用第三磨牙来源牙髓干细胞构建角膜内皮细胞单层。该团队先通过悬浮培养体系诱导牙髓干细胞向神经嵴干细胞转化, 为后续分化创造适宜微环境; 再将所得细胞置于角膜内皮细胞条件培养基中继续培养, 结果显示牙髓干细胞可分化为多边形内皮样细胞, ZO-1、钠钾ATP酶α1亚基(Na^+/K^+-ATPase α1 subunit, ATP1A1)、IV型胶原α2链(collagen type IV alpha 2, COL4A2)、COL8A2等内皮特征标志物基因和蛋白表达水平显著上调。除外源干细胞诱导方案外, 眼内固有间充质细胞同样可调控角膜内皮修复。近期研究证实, 后角膜缘间充质基质细胞可依靠旁分泌效应, 显著提升过渡区角膜内皮祖细胞的增殖、迁移、创面修复、集落形成能力并维持细胞干性; 其制备的条件培养基亦能够有效促进角膜内皮再生[53]。

表3 间充质干细胞向角膜内皮细胞分化的方法

Table 3 Methods for differentiating mesenchymal stem cells into corneal endothelial cells

分化方法 Method	MSCs来源 Source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
Co-culture	Human AT-MSCs	Direct co-culture with corneal endothelial cells; DMEM/F12, 10% FBS, 0.1 mmol/L non-essential amino acids, 1 mmol/L sodium pyruvate, 2 mmol/L *L*-glutamine, 100 μmol/L β-mercaptoethanol, 5 ng/mL bFGF, 1% penicillin-streptomycin	*In vitro*: 38 d	Expression of *Nanog*, *CD31*, *AQP1*, *ZO-1*	Dai et al/2014[50]
	Human UCB-MSCs	MSC basal medium: DMEM, 10% FBS, 1% penicillin-streptomycin; LECBM: ATCC EMEM, 20% FBS, 1% penicillin-streptomycin; LECCM: SV40-transformed human lens epithelial cell supernatant diluted 1:1 with LECBM	*In vitro*: 21 d; *in vivo*: 14 d	Expression of *NCAM1*, *TFAP2β*, *CDK15*, *MEIS1*, *COL8A1*; ZO-1 and N-cadherin localized at cell borders; cells home to damaged endothelial regions with endothelial-like phenotype, especially in LECCM	Joyce et al/2012[51]
	Human dental pulp stem cells from third molars	Suspension culture for neural crest progenitor generation, followed by corneal endothelial conditioned medium culture; corneal endothelial conditioned medium: high-glucose DMEM, 5% FBS, 1% *L*-alanyl-*L*-glutamine, 2 ng/mL bFGF, 0.1 mmol/L 2-mercaptoethanol, 10 ng/mL heregulin β, 10 ng/mL activin A, 200 ng/mL IGF-I	*In vitro*: 40 d	Expression of ZO-1, ATP1A1, COL4A2, COL8A2	Bosch et al/2021[52]
Biochemical factors	Human UC-MSCs	MEM, 0.5 mmol/L BIO, 10 μmol/L Y-27632, 1% ITS-G, 4 nmol/L triiodothyronine, 0.5 mg/mL hydrocortisone, 50 mg/mL ascorbic acid, 1 mmol/L $CaCl_2$, 1 mmol/L sodium pyruvate, MEM amino acids, MEM essential vitamin mixture	*In vitro*: 9, 11 d; *in vivo*: 8 d	Expression of CDH2, Pitx2, COL4A2, SLC4A4, Car2, COL8A2 and ATP1A1; tissue-engineered endothelium maintains corneal thickness and transparency in bullous keratopathy rabbit models	Yamashita et al/2018[55]
	Human AT-MSCs	Neural differentiation medium: DMEM, 1% FBS, 100 ng/mL bFGF, 10 μmol/L forskolin; Ali et al[58], protocol Wagoner et al[59], protocol	*In vitro*: 30-40 d	Expression of S100, ATP1A1, ZO-1, AQP1	Marta et al/2021[57]
	Human UC-MSCs	Corneal endothelial differentiation medium: human endothelial-SFM, 0.1% PVA, 0.5 mmol/L BIO, 10 μmol/L Y-27632, 1% ITS-G, 0.02 mg/mL A2-P, 1 μmol/L SB431542, 2.5 μmol/L H-1152, 0.2 mg/mL $CaCl_2$	*In vitro*: 16 d; *in vivo*: 21 d	Expression of ATP1A1, AQP1, COL8A1, COL8A2, ZO-1, N-cadherin, NCAM and CD166; intracameral injection alleviates corneal opacity and neovascularization in endothelial dysfunction rabbit models compared with blank and undifferentiated MSCs groups	Ye et al/2022[56]
Physical regulation	Human foreskin-derived MSCs	DMEM, 4.5 g/L glucose, pyruvate-free glutamine, 1% FBS, 1% penicillin/streptomycin	*In vitro*: 24 d	Expression of *ZO-1*, *ATP1A1*, *Pitx2*, *COL8*	Gutermuth et al/2019[61]

续表3

分化方法 Method	MSCs来源 Source	培养基 Culture media	分化周期 Duration	检测结果 Results	作者/发表日期 Author/year
	Human WJ-MSCs	Endothelial medium 2: DMEM, 20% FBS, 1% penicillin/streptomycin, 0.1% CFA; serum-free human endothelial medium: DMEM, 20 ng/mL bFGF, 10 ng/mL EGF, 1% penicillin/streptomycin	*In vitro*: 16 d; *in vivo*: 5 d	Expression of ZO-1, ATP1A1, Pitx2, COL8 electrophysiological functional verification and transparent cornea observed in rabbit models	Feiertag et al/2020[62]
	Human eyelid epidermal hair follicle neural crest stem cells	Serum-free endothelial medium, 20 ng/mL bFGF, 20 ng/mL EGF, 1% penicillin/streptomycin, 0.1% ciprofloxacin	*In vitro*: 24 d	Expression of Nanog, Sox-2, Pitx2, ATP1A1, CoL8A1, ZO-1, Oct-4, Pax6, SSEA4, Tra1-60, CD34, CD49f	Olszewski et al/2022[63]

3.2 生物化学因子诱导

角膜来源前体(cornea-derived precursors, COPs)是从成年角膜基质分离的干细胞, 表现出多能神经嵴来源干细胞的特征。Hatou等[54]以小鼠和人COPs为种子细胞, 在添加ATRA与GSK-3β抑制剂(可激活Wnt/β-catenin信号通路)的培养基中, 构建出具备生理功能的组织工程角膜内皮。ATRA和GSK-3β抑制剂均上调了*Pitx2*的表达, 这是一种参与眼前节发育的同源盒基因, GSK-3β抑制剂还提高了ATP1A1表达水平。把小鼠和人的组织工程角膜内皮移植到兔子角膜中, 可以保持角膜的透明性和厚度。一些研究旨在用小分子生物化学因子诱导MSCs向角膜内皮细胞方向分化。Yamashita等[55]发现, 在用含GSK-3β抑制剂BIO培养基诱导分化后, 人脐带中的间充质干细胞(umbilical cord mesenchymal stem cells, UC-MSCs)形成了多边形的类角膜内皮细胞结构, 将组织工程角膜内皮移植到水疱性角膜病兔子模型中, 可以维持角膜厚度和透明度。Ye等[56]联合GSK-3β抑制剂BIO、ROCK抑制剂Y-27632及H-1152诱导人UC-MSCs获得角膜内皮样细胞。将分化细胞移植入角膜内皮损伤兔模型后, 人线粒体DNA示踪实验证明移植细胞可稳定定植; 相较于空白对照组与单纯干细胞注射组, 移植该分化细胞可显著减轻角膜内皮损伤兔模型的角膜混浊程度, 并抑制新生血管增生。Marta等[57]采用不同前人的方法诱导人AT-MSCs向角膜内皮细胞方向分化, Ali等[58]的方案主要是先利用Noggin(BMP抑制剂)和SB431542(TGF-β抑制剂)获得神经嵴细胞, 然后诱导神经嵴细胞向角膜内皮细胞方向分化; 而Wagoner等[59]的方案主要是先利用CHIR99021(GSK3抑制剂)和SB431542(TGF-β抑制剂)获得神经嵴细胞, 然后诱导神经嵴细胞向角膜内皮细胞方向分化, 两种方案在信号作用、成分和时序等有所差异。结果显示, Ali等[58]的方案在诱导效率、时间成本、经济性方面更具优势; Wagoner等[59]的方案在角膜内皮标志性基因与功能蛋白表达方面更优。

3.3 物理调控

角膜内皮细胞在体内生理状态下处于复杂的机械微环境中, 而体外实验证实, 机械因素会影响角膜内皮细胞的行为和功能。例如, Muhammad等[60]证明了微型和纳米拓扑可以促进供体来源的原发性人角膜内皮细胞的增殖并维持功能。然而, 关于利用机械力影响干细胞向角膜内皮细胞分化的研究却比较少。一些研究报告指出, 底物地形和基质刚度可以影响干细胞向角膜内皮细胞方向的分化。例如, Gutermuth等[61]研究了Descemet膜样微地形图(Descemet's membrane-like microtopography, DLT)诱导人MSCs向类角膜内皮细胞分化过程中的机械转导作用。经DLT诱导后的干细胞可呈现典型多边形形态, 该体系不仅上调*ZO-1*、*ATP1A1*、*Pitx2*、*COL8*等角膜内皮特征基因的转录水平, 还能促进上述对应蛋白的表达。此外, Feiertag等[62]将人WJ-MSCs接种于复合DLT微形貌的胶原蛋白基底上培养, 诱导后的细胞呈角膜内皮典型六边形形态, ZO-1、ATP1A1、Pitx2、COL8等角膜内皮相关基因与蛋白表达水平均显著上调; 该细胞单层可形成40 mV跨膜电位差, 移植后能够维持角膜透明状态。另外, Olszewski等[63]将老年眼睑皮肤来源毛囊干细胞接种于带DLT印记的膜载体、采用无血清体系培养, 研究证实无需额外添加生化诱导因子, 即可诱导干细胞分化为角膜内皮样细胞; 该类细胞可在基因与蛋白层面表达ZO-1、ATP1A1、

Pitx2、COL8等角膜内皮特征标志物。因此, 从眼睑活检表皮提取的毛囊干细胞, 可能是为任何年龄段患者提供自身角膜内皮替代的有前景细胞来源。

对于诱导MSCs向角膜内皮细胞方向的分化, Wnt/β-catenin、ROCK、TGF-β信号通路可能是比较重要的, 底物拓扑与刚度可通过机械信号增强ZO-1、ATP1A1表达, 提升泵功能。诱导MSCs向角膜内皮细胞方向分化的主要方法是生化诱导, 或者结合角膜内皮细胞共培养, 还可以依托基质刚度、微拓扑结构等物理微环境调控实现形态与功能优化。其中小分子生化诱导体系成分明确、易质控, 是比较贴近临床转化的诱导模式, 但不同研究建立的小分子诱导方案尚无统一最优标准; 物理微环境调控可诱导细胞形成典型六角形态, 强化细胞间连接与屏障功能。总之, MSCs向角膜内皮层分化的研究可以解决角膜内皮供体缺乏问题、重建角膜内皮的水液转运功能、降低免疫排斥风险, 现阶段该方向的发展仍存在多重瓶颈: 现有诱导策略种类有限、分化效率不佳, 分化细胞难以完全成熟, 分化分子机制尚未被阐明, 体内移植后功能持久性较差; 此外, 角膜内皮特异性标志物的研究进展远滞后于角膜上皮与角膜基质细胞, 仍有待系统深入挖掘。

4 总结和展望

综上所述, MSCs可以向角膜上皮细胞、角膜基质细胞及角膜内皮细胞方向分化, 诱导生成的角膜细胞在移植应用中有望改善患者视力, 为角膜疾病治疗提供新路径。目前体外诱导MSCs向各类角膜细胞方向分化的方法主要包括: 共培养诱导、生物化学因子诱导、生物材料微环境调控、物理调控和基因修饰, 上述所有诱导策略汇总见图1。其中, MSCs向角膜上皮细胞及角膜基质细胞方向分化的研究较为充分, 而MSCs向角膜内皮细胞方向分化的诱导策略较为单一, 相关研究报道相对较少。从现有研究来看, 共培养体系诱导效果稳定, 可有效诱导MSCs表达角膜谱系特征标志物, 但体系构成较为复杂, 实验批次差异明显, 难以实现标准化制备, 后续可朝着无饲养层、成分明确的诱导体系优化完善。单纯生化因子诱导操作简便、条件易调控, 适合开展机制探究, 但单一因子诱导特异性有限, 实际研究多采用多因子诱导, 仍需进一步优化配比浓度与作用时间。依托生物材料诱导能够仿生模拟角膜天然微环境, 利于维持细胞表型稳态并促进基质胶原有序排布, 但材料制备工艺、生物相容性及体内降解适配性仍有提升空间。基因修饰手段可明显提升分化靶向性与诱导效率, 却存在潜在生物安全风险, 临床转化应用受到限制, 更建议优先开发安全的非基因干预方案。物理微环境通过力学刺激、表面拓扑结构、基质刚度等条件调控细胞命运, 具备安全无创、无外源因子干扰的优势, 但其内在调控机制和最优作用参数, 仍有待后续深入挖掘。单一诱导方式各有优势与不足, 将不同策略合理联用、取长补短, 是后续优化MSCs角膜定向分化体系的重要方向, 也可为后续标准化诱导方案构建及临床转化研究提供参考思路。

在分子调控层面, MSCs向角膜三层细胞的定向分化, 依赖多条信号通路协同参与。MSCs向上皮细胞分化主要受Wnt/β-catenin、TGF-β/Smad、MAPK/ERK、PI3K-Akt通路调控, ATRA联合Wnt及TGF-β抑制剂可启动上皮–间充质转化, 再辅以EGF、BMP4能够推动上皮谱系定向分化; 向基质细胞分化主要受FGF/MAPK、TGF-β/Smad、Wnt通路调控, bFGF协同TGF-β3、A2-P可明显上调角膜基质特征标志物表达; 向内皮样细胞分化主要受Wnt/β-catenin、ROCK、TGF-β通路调控, GSK-3β、ROCK及TGF-β相关小分子抑制剂可调控神经嵴谱系走向, 助力内皮样细胞功能成熟。ATRA是角膜三系分化中应用较为广泛的诱导因子, 最终分化走向受因子配伍、作用时序、基质刚度、表面拓扑及力学信号等多维度条件共同调控, 不同参数组合可分别偏向诱导上皮、基质或内皮谱系形成, 而各类信号通路与调控因子之间的交叉互作机制, 仍需要继续深入研究。

在临床转化方面, MSCs凭借来源广泛、易获取、多向分化潜能突出及良好的免疫调节能力, 已在角膜上皮、基质及内皮再生研究中展现出重要应用价值, 是突破传统角膜移植供体短缺、免疫排斥等临床瓶颈的理想种子细胞。研究表明, MSCs可通过自体或异体方式用于角膜损伤修复, 其中自体/同基因来源干细胞整体治疗效果更具优势[64-65]。同时已有临床试验证实, 异体MSCs应用于角膜治疗时无明显免疫排斥反应, 安全性良好, 不存在明显致瘤性与异位增殖隐患, 体现出较低的免疫原性, 也具备可靠的异体移植应用潜力[3]。尽管当前研究已在诱

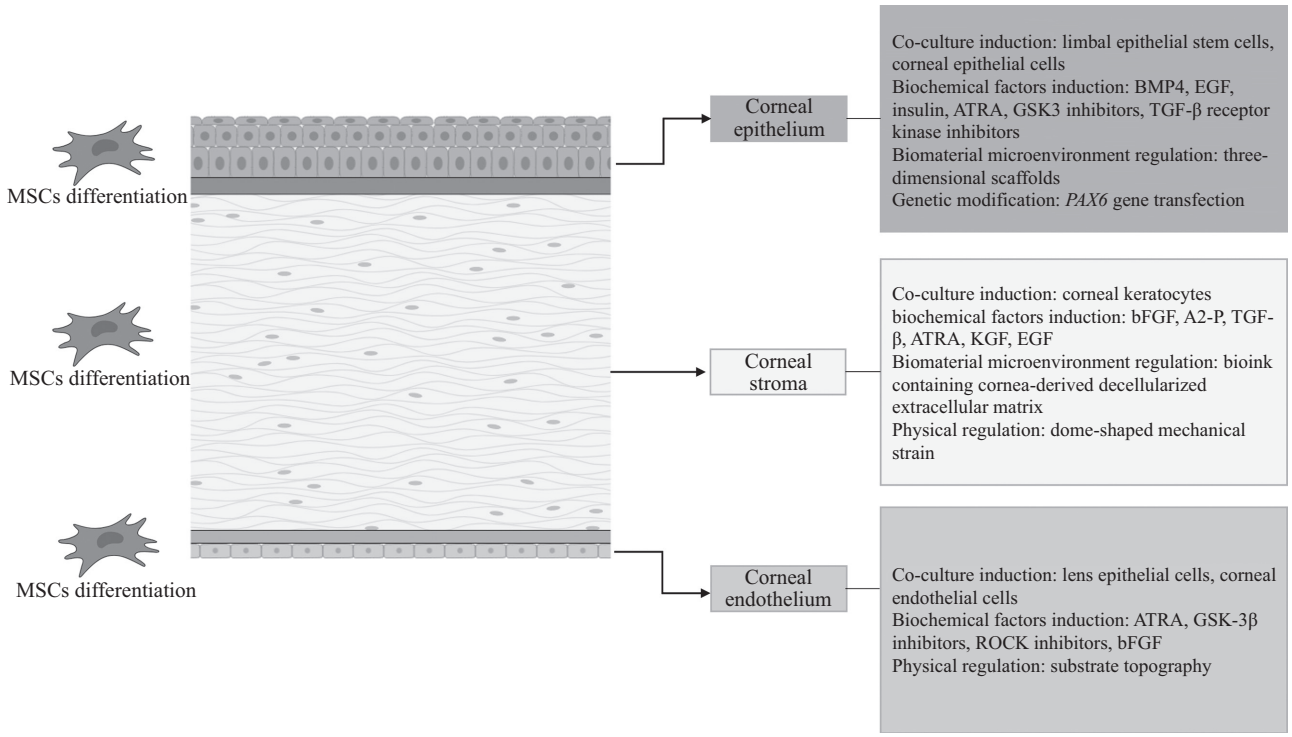

图1 MSCs向角膜上皮、角膜基质和角膜内皮细胞体外分化的方法

Fig.1 The methods for the *in vitro* differentiation of MSCs into corneal epithelial cells, corneal stromal cells, and corneal endothelial cells

导方法上取得丰富积累，涵盖共培养、生物化学因子、生物材料、基因转染及物理调控等多种策略，但不同方法之间的效率、稳定性、标准化程度与临床适用性仍存在明显差异，不同组织来源MSCs的分化效能也具有天然异质性；同时，现有诱导获得的角膜样细胞在基质胶原有序排列、角膜内皮泵功能及上皮屏障功能成熟度方面仍与天然细胞存在差距，行业内尚未形成统一的分化评价标准与质控体系；传统含血清培养体系难以满足GMP临床级制备要求，自体细胞取材创伤、异体细胞潜在免疫排斥、基因修饰与长期小分子干预的远期生物安全性仍需更系统的验证；此外，适配角膜各层结构特点的仿生支架、高效递送方式及体内长期定植与功能维持机制仍需进一步优化。针对上述瓶颈，当前领域仍处于基础研究与临床前探索阶段，尚未形成成熟可标准化的临床转化方案，相关优化策略仍在不断探索与验证中：研究多聚焦于筛选与比较骨髓、脂肪、脐带、牙髓、鼻甲等不同组织来源MSCs向角膜三系细胞分化的潜能差异，以期优选分化效率更高、表型更稳定的种子细胞来源；同时持续优化共培养、生化因子组合、仿生材料及物理调控等单一或联合诱导策略，提升分化细胞的标志物表达与功能成熟度；逐步探索建立统一的分化评价指标与质量控制思路，推动诱导体系向成分明确、可重复方向发展；并围绕低免疫原性、高安全性方案开展临床前探索，以期降低异体移植排斥风险、减少基因修饰依赖。截至目前，经体外定向诱导分化的MSCs来源功能性角膜上皮、基质及内皮细胞，仍处于临床前研究阶段，尚未实现规范化临床转化与应用。相关疗法仍需大量临床前有效性与安全性验证，以及规范化临床试验支撑，距离真正进入临床应用仍有较长的路要走。

干细胞分化不仅受生化因子调控，还受到力学刺激、基质刚度与底物拓扑结构等物理微环境因素的影响，精准调控上述物理信号可为构建高效诱导分化体系提供新思路[66]。以细胞、生物材料支架与生物活性因子联合构建的组织工程化人工角膜，已在角膜修复研究中展现了良好的应用前景，为角膜再生治疗提供了重要基础[67]。此外，角膜类器官技术因仿生还原度更高，现已成为该领域前沿研究热点。该技术可通过分步诱导分化在体外构建同时包含角膜上皮、基质、内皮三类细胞的类器官结构，

类器官可表达各层细胞特征标志物，并组装形成有序排列的胶原纤维，能够高度模拟天然角膜的组织结构与生理功能，为角膜损伤修复、遗传性角膜疾病机制探究、眼科药物筛选以及组织移植治疗提供全新研究载体[68]。未来需进一步优化干细胞诱导策略，明晰分化机制，推动多诱导方法协同应用，并聚焦功能化组织工程角膜的开发，加强基础与临床的衔接，同时关注治疗的长期安全性、成本效益与临床可及性，最终推动角膜损伤修复相关技术真正实现临床转化与应用。

参考文献 (References)

[1] Raut A K, Mohapatra S, Siddiqui I G, et al. The human cornea: unraveling its structural, chemical, and biochemical complexities [J]. Chemistry & Biodiversity, 2025, 22(4): e202402224.

[2] Tay C, Reddy H, Mehta J S, et al. Advances in corneal transplantation [J]. Eye, 2025, 39(13): 2497-2508.

[3] Iliadis I, Pechnikova N A, Poimenidou M, et al. Applications of modern cell therapies: the latest data in ophthalmology [J]. Life, 2025, 15(10): 1610.

[4] 惠延年. 眼科再生医学的进展与前景[J]. 国际眼科杂志, 2025, 25(10): 1547-1550.
Hui Y N. Progress and prospects in regenerative medicine in ophthalmology [J]. International Eye Science, 2025, 25(10): 1547-1550.

[5] de Miguel M P, Cadenas-Martin M, Stokking M, et al. Biomedical application of MSCs in corneal regeneration and repair [J]. International Journal of Molecular Sciences, 2025, 26(2): 695.

[6] 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[J]. 科学通报, 2026, 71(3): 771-787.
Zhang P, Chen Q Q, Pu Y G, et al. Advancements in ocular surface reconstruction by mesenchymal stem cells and immunomodulatory mechanism [J]. Chinese Science Bulletin, 2026, 71(3): 771-787.

[7] Bhujel B, Oh S H, Kim C M, et al. Mesenchymal stem cells and exosomes: a novel therapeutic approach for corneal diseases [J]. International Journal of Molecular Sciences, 2023, 24(13): 10917.

[8] Tsai C L, Chuang P C, Kuo H K, et al. Differentiation of stem cells from human exfoliated deciduous teeth toward a phenotype of corneal epithelium *in vitro* [J]. Cornea, 2015, 34(11): 1471-1477.

[9] Soleimanifar F, Mortazavi Y, Nadri S, et al. Coculture of conjunctiva derived mesenchymal stem cells (CJMSCs) and corneal epithelial cells to reconstruct the corneal epithelium [J]. Biologicals, 2018, 54: 39-43.

[10] Venugopal B, Shenoy S J, Mohan S, et al. Bioengineered corneal epithelial cell sheet from mesenchymal stem cells-A functional alternative to limbal stem cells for ocular surface reconstruction [J]. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2020, 108(3): 1033-1045.

[11] Viveiros M M H, Zucoloto L H, Shiguematsu A I, et al. Comparison of techniques for corneal epithelium cell culture for the collection of conditioned medium [J]. Arquivos Brasileiros De Oftalmologia, 2024, 87(2): e2022.

[12] 牛鹏, 吕洋. 眼表细胞分化的机制及其在疾病治疗中的潜在应用[J]. 眼科新进展, 2025, 45(7): 583-588.
Niu P, Lü Y. The mechanism of ocular surface cell differentiation and its potential application in disease treatment [J]. Recent Advances in Ophthalmology, 2025, 45(7): 583-588.

[13] Katikireddy K R, Dana R, Jurkunas U V, et al. Differentiation potential of limbal fibroblasts and bone marrow mesenchymal stem cells to corneal epithelial cells [J]. Stem Cells, 2014, 32(3): 717-729.

[14] Rohaina C M, Then K Y, Ng A M, et al. Reconstruction of limbal stem cell deficient corneal surface with induced human bone marrow mesenchymal stem cells on amniotic membrane [J]. Translational Research, 2014, 163(3): 200-210.

[15] Nieto-Nicolau N, Martin-Antonio B, Muller-Sanchez C, et al. *In vitro* potential of human mesenchymal stem cells for corneal epithelial regeneration [J]. Regenerative Medicine, 2020, 15(3): 1409-1426.

[16] Setiawan M, Tan X W, Goh T W, et al. Inhibiting glycogen synthase kinase-3 and transforming growth factor-beta signaling to promote epithelial transition of human adipose mesenchymal stem cells [J]. Biochemical and Biophysical Research Communications, 2017, 490(4): 1381-1388.

[17] Nguyen H T, Theerakittayakorn K, Somredngan S, et al. Signaling pathways impact on induction of corneal epithelial-like cells derived from human Wharton's jelly mesenchymal stem cells [J]. International Journal of Molecular Sciences, 2022, 23(6): 3078.

[18] Cadenas-Martin M, Arnalich-Montiel F, de Miguel M P, et al. Derivation of limbal stem cells from human adult mesenchymal stem cells for the treatment of limbal stem cell deficiency [J]. International Journal of Molecular Sciences, 2023, 24(3): 2350.

[19] Bandeira F, Goh T W, Setiawan M, et al. Cellular therapy of corneal epithelial defect by adipose mesenchymal stem cell-derived epithelial progenitors [J]. Stem Cell Research & Therapy, 2020, 11(1): 14.

[20] Orash Mahmoudsalehi A, Soleimani M, Stalin Catzim Rios K, et al. Advanced 3D scaffolds for corneal stroma regeneration: a preclinical progress [J]. Journal of Materials Chemistry B, 2025, 13(21): 5980-6020.

[21] Li J M, Li Q Y, Lu Z X, et al. Injectable microcarriers enhance stem cell delivery and promote corneal regeneration for alkali burn [J]. Chemical Engineering Journal, 2025, 517: 164582.

[22] Soleimanifar F, Mortazavi Y, Nadri S, et al. Conjunctiva derived mesenchymal stem cell (CJMSCs) as a potential platform for differentiation into corneal epithelial cells on bioengineered electrospun scaffolds [J]. Journal of Biomedical Materials Research Part A, 2017, 105(10): 2703-2711.

[23] Pourjabbar B, Shams F, Heidari Keshel S, et al. Proliferation and differentiation of Wharton's jelly-derived mesenchymal stem cells on PRGF-treated hydrogel scaffold [J]. Regenerative Medicine, 2024, 19(11): 549-560.

[24] Fresenko E E, Ma J X, Giegengack M, et al. Stem cell-derived corneal epithelium: engineering barrier function for ocular surface repair [J]. International Journal of Molecular Sciences, 2025, 26(15): 7501.

[25] Polisetti N, Schlunck G, Reinhard T, et al. PAX6 expression pat-

terns in the adult human limbal stem cell niche [J]. Cells, 2023, 12(3): 400.

[26] Sun J, Liu W H, Deng F M, et al. Differentiation of rat adipose-derived mesenchymal stem cells into corneal-like epithelial cells driven by PAX6 [J]. Experimental and Therapeutic Medicine, 2018, 15(2): 1424-1432.

[27] 吴霜, 邹星星, 高杰, 等. 转录因子配对盒基因6对骨髓间充干细胞向角膜缘干细胞样细胞分化的影响[J]. 中国组织工程研究, 2022, 26(24): 3858-3864.
Wu S, Zou X X, Gao J, et al. Effect of paired box gene 6 protein on the differentiation of bone marrow mesenchymal stem cells into keratolimbal stem cell-like cells [J]. Journal of Chinese Tissue Engineering Research, 2022, 26(24): 3858-3864.

[28] Gao J, Ding L, Xin Y, et al. Pax6-induced proliferation and differentiation of bone marrow mesenchymal stem cells into limbal epithelial stem cells [J]. Stem Cells and Development, 2023, 32(13/14): 410-421.

[29] Zhang S, Espandar L, Imhof K M, et al. Differentiation of human adipose-derived stem cells along the keratocyte lineage *in vitro* [J]. Journal of Clinical and Experimental Ophthalmology, 2013, 4: 11435.

[30] Park S H, Kim K W, Chun Y S, et al. Human mesenchymal stem cells differentiate into keratocyte-like cells in keratocyte-conditioned medium [J]. Experimental Eye Research, 2012, 101: 16-26.

[31] Park S H, Kim K W, Kim J C, et al. The role of insulin-like growth factor binding protein 2 (IGFBP2) in the regulation of corneal fibroblast differentiation [J]. Investigative Ophthalmology & Visual Science, 2015, 56(12): 7293-7302.

[32] Ghiasi M, Hashemi M, Salimi A, et al. Combination of natural scaffolds and conditional medium to induce the differentiation of adipose-derived mesenchymal stem cells into keratocyte-like cells and its safety evaluation in the animal cornea [J]. Tissue & Cell, 2023, 82: 102117.

[33] Espandar L, Bunnell B, Wang G Y, et al. Adipose-derived stem cells on hyaluronic acid-derived scaffold: a new horizon in bioengineered cornea [J]. Archives of Ophthalmology, 2012, 130(2): 202-208.

[34] Kafarnik C, Mcclellan A, Dziasko M, et al. Canine corneal stromal cells have multipotent mesenchymal stromal cell properties *in vitro* [J]. Stem Cells and Development, 2020, 29(7): 425-439.

[35] Kong B, Chen Y, Liu R, et al. Fiber reinforced GelMA hydrogel to induce the regeneration of corneal stroma [J]. Nature Communications, 2020, 11(1): 1435.

[36] Wu J, Du Y Q, Mann M M, et al. Bioengineering organized, multilamellar human corneal stromal tissue by growth factor supplementation on highly aligned synthetic substrates [J]. Tissue Engineering Part A, 2013, 19(17/18): 2063-2075.

[37] Basu S, Hertsemberg A J, Funderburgh M L, et al. Human limbal biopsy-derived stromal stem cells prevent corneal scarring [J]. Science Translational Medicine, 2014, 6(266): 266ra172.

[38] Syed-Picard F N, Du Y, Lathrop K L, et al. Dental pulp stem cells: a new cellular resource for corneal stromal regeneration [J]. Stem Cells Translational Medicine, 2015, 4(3): 276-285.

[39] Dos Santos A, Balayan A, Funderburgh M L, et al. Differentiation capacity of human mesenchymal stem cells into keratocyte lineage [J]. Investigative Ophthalmology & Visual Science, 2019, 60(8): 3013-3023.

[40] Yam G H, Teo E P, Setiawan M, et al. Postnatal periodontal ligament as a novel adult stem cell source for regenerative corneal cell therapy [J]. Journal of Cellular and Molecular Medicine, 2018, 22(6): 3119-3132.

[41] Taoum A, Friede R, Thaden O, et al. 3D differentiation of bone-marrow derived mesenchymal stromal cells into the keratocyte lineage for corneal bioprinting [J]. Advanced Healthcare Materials, 2025, 14(28): e2405073.

[42] Lynch A P, Ahearne M. Retinoic acid enhances the differentiation of adipose-derived stem cells to keratocytes *in vitro* [J]. Translational Vision Science & Technology, 2017, 6(1): 6.

[43] Sidney L E, Hopkinson A. Corneal keratocyte transition to mesenchymal stem cell phenotype and reversal using serum-free medium supplemented with fibroblast growth factor-2, transforming growth factor-beta3 and retinoic acid [J]. Journal of Tissue Engineering and Regenerative Medicine, 2018, 12(1): e203-e215.

[44] Park M, Kim B, Kim H, et al. Human turbinate-derived mesenchymal stem cells differentiated into keratocyte progenitor cells [J]. Journal of Clinical and Experimental Ophthalmology, 2017, 8: 8.

[45] Kim H, Park M N, Kim J, et al. Characterization of cornea-specific bioink: high transparency, improved *in vivo* safety [J]. Journal of Tissue Engineering, 2019, 10: 2041731418823382.

[46] Marin-Tapia H A, Romero-Salazar L, Mayorga-Rojas M, et al. Scaffold-free extrusion-based 3D bioprinting of cornea constructs using a decellularized corneal extracellular matrix based bioink and human placenta-derived mesenchymal stem cells [J]. Macromolecular Bioscience, 2025, 25(12): e00276.

[47] Chen J, Zhang W, Backman L J, et al. Mechanical stress potentiates the differentiation of periodontal ligament stem cells into keratocytes [J]. British Journal of Ophthalmology, 2018, 102(4): 562-569.

[48] Zhang Q, Zhou X, Zhang W, et al. Corneal strain influences keratocyte proliferation and migration through upregulation of ALDH3A1 expression [J]. The FASEB Journal, 2024, 38(23): e70236.

[49] Lin K M, Chen Y R, Ho M H, et al. The cell-fate decision of dental follicle stem cells (DFSCs) with porcine corneal extracellular matrix (ECM) [J]. Journal of Dental Science, 2025, 20(3): 1605-1614.

[50] Dai Y, Guo Y, Wang C, et al. Non-genetic direct reprogramming and biomimetic platforms in a preliminary study for adipose-derived stem cells into corneal endothelia-like cells [J]. PLoS One, 2014, 9(10): e109856.

[51] Joyce N C, Harris D L, Markov V, et al. Potential of human umbilical cord blood mesenchymal stem cells to heal damaged corneal endothelium [J]. Molecular Vision, 2012, 18: 547-564.

[52] Bosch B M, Salero E, Nunez-Toldra R, et al. Discovering the potential of dental pulp stem cells for corneal endothelial cell production: a proof of concept [J]. Frontiers in Bioengineering and Biotechnology, 2021, 9: 617724.

[53] Xiao Y, Mcghee C N J, Zhang J, et al. Posterior limbal mesenchymal stromal cells promote proliferation and stemness of transition zone cells: a novel insight into corneal endothelial rejuvenation [J]. Investigative Ophthalmology & Visual Science, 2025, 66(1): 44.

[54] Hatou S, Yoshida S, Higa K, et al. Functional corneal endothelium derived from corneal stroma stem cells of neural crest origin by retinoic acid and Wnt/β-catenin signaling [J]. Stem Cells and Development, 2013, 22(5): 828-839.

[55] Yamashita K, Inagaki E, Hatou S, et al. Corneal endothelial regeneration using mesenchymal stem cells derived from human umbilical cord [J]. Stem Cells and Development, 2018, 27(16): 1097-1108.

[56] Ye E A, Chung H S, Park Y, et al. Induction of corneal endothelial-like cells from mesenchymal stem cells of the umbilical cord [J]. International Journal of Molecular Sciencesi, 2022, 23(23): 15408.

[57] Marta C M, Adrian M, Jorge F D, et al. Improvement of an effective protocol for directed differentiation of human adipose tissue-derived adult mesenchymal stem cells to corneal endothelial cells [J]. International Journal of Molecular Sciences, 2021, 22(21): 11982.

[58] Ali M, Khan S Y, Vasanth S, et al. Generation and proteome profiling of PBMC-originated, iPSC-derived corneal endothelial cells [J]. Investigative Ophthalmology & Visual Science, 2018, 59(6): 2437-2444.

[59] Wagoner M D, Bohrer L R, Aldrich B T, et al. Feeder-free differentiation of cells exhibiting characteristics of corneal endothelium from human induced pluripotent stem cells [J]. Biology Open, 2018, 7(5): bio032102.

[60] Muhammad R, Peh G S, Adnan K, et al. Micro- and nano-topography to enhance proliferation and sustain functional markers of donor-derived primary human corneal endothelial cells [J]. Acta Biomaterialia, 2015, 19: 138-148.

[61] Gutermuth A, Maassen J, Harnisch E, et al. Descemet's membrane biomimetic microtopography differentiates human mesenchymal stem cells into corneal endothelial-like cells [J]. Cornea, 2019, 38(1): 110-119.

[62] Feiertag E, Maassen J, Mueller A, et al. From cord to eye: Wharton jelly-derived stem cells differentiate into corneal endothelial-like cells [J]. Cornea, 2020, 39(7): 877-885.

[63] Olszewski C, Maassen J, Guenther R, et al. Mechanotransductive differentiation of hair follicle stem cells derived from aged eyelid skin into corneal endothelial-like cells [J]. Stem Cell Reviews and Reports, 2022, 18(5): 1668-1685.

[64] Norte-Munoz M, Gallego-Ortega A, Lucas-Ruiz F, et al. Immune recognition of syngeneic, allogeneic and xenogeneic stromal cell transplants in healthy retinas [J]. Stem Cell Research & Therapy, 2022, 13(1): 430.

[65] Norte-Munoz M, Lucas-Ruiz F, Gallego-Ortega A, et al. Neuroprotection and axonal regeneration induced by bone marrow mesenchymal stromal cells depend on the type of transplant [J]. Frontiers in Cell and Developmental Biology, 2021, 9: 772223.

[66] 刘晶劼, 王丽强, 黄一飞. 间充质干细胞诱导方法及分化为角膜组织的研究进展[J]. 中华眼科杂志, 2022, 58(6): 461-466.
Liu J J, Wang L Q, Huang Y F, et al. The research progress of mesenchymal stem cells induction and differentiation into corneal tissue [J]. Chinese Journal of Ophthalmology, 2022, 58(6): 461-466.

[67] Alaminos M, Crespo P V, Garcia J M, et al. Bioartificial human corneas generated by tissue engineering. A historical and technical review [J]. Histology and Histopathology, 2026, 41(1): 25-37.

[68] Giannaccare G, Filippo L, Carina S, et al. Current landscape and future prospects of corneal regenerative medicine [J]. Ophthalmology Therapy, 2026, 15(1): 85-128.