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生物材料在角膜组织工程中的应用及研究进展

潘嘉莉 石晓硕 杨燕宁

武汉大学人民医院眼科中心 湖北 武汉 430060

摘要 角膜移植是治疗角膜疾病的重要手段,但由于全球角膜供体短缺和移植排斥的限制,其临床应用仍面临巨大挑战。随着人口老龄化、角膜疾病高发以及移植需求的增加,寻找替代方案已成为研究重点。近年来,生物工程角膜作为一种潜在解决方案备受关注,旨在通过优化材料促进角膜再生。理想的生物材料应具备良好的机械强度、生物相容性和透明性,并可能具有可降解性和临床可行性。本文综述了角膜组织工程中支架型生物材料的设计策略与应用进展,探讨了细胞与材料的相互作用,以期为角膜组织再生及临床转化提供参考。

关键词 生物材料;组织工程;角膜盲;角膜移植

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Application and research progress of biomaterials in corneal tissue engineering

PAN Jiali, SHI Xiaoshuo, YANG Yanning

Eye Center, Remin Hospital of Wuhan University, Wuhan 430060, Hubei, China

Abstract

Corneal transplantation is a critical treatment for corneal diseases; however, its clinical application faces significant challenges due to the global shortage of donor corneas and the risk of transplant rejection. With the increasing prevalence of corneal diseases, a growing aging population, and rising transplantation demands, finding alternative solutions has become a key research focus. In recent years, bioengineered corneas have emerged as a promising solution. They are designed with optimized materials to promote corneal regeneration. Ideal biomaterials should possess good mechanical strength, biocompatibility, and optical transparency, as well as potential degradability and clinical feasibility. This article reviews the design strategies and application progress of scaffold-type biomaterials in corneal tissue engineering, and discusses the interactions between cells and materials, to provide insights for corneal regeneration and clinical translation.

Key Words Biomaterials; Tissue Engineering; Corneal Blindness; Corneal Transplantation

角膜是眼球前部的重要透明组织,具备无血管特征,并且拥有丰富的神经分布。其主要功能包括光的传输与折射,同时还承担保护眼球免受机械损伤、紫外线辐射及病原感染的关键作用^[1]。角膜移植是治疗角膜损伤和疾病的重要手段,然而,全球

角膜供体短缺已成为移植手术的主要限制因素^[2]。世界卫生组织(World Health Organization, WHO) 2010 年调查^[3]显示,全球有 3 900 万人失明,其中 12%(约 490 万人)为双侧角膜盲,约 2 300 万人为单侧角膜盲。尽管同种异体角膜移植是目前治疗角

作者简介:潘嘉莉,女,2000—,医学硕士生,主要从事眼表及角膜疾病研究,E-mail: 2018305232047@whu.edu.cn。

通信作者:杨燕宁,E-mail: ophyyn@163.com。

膜疾病的最佳选择^[4-5],但供体短缺和移植排斥反应仍是面临的重大挑战。角膜因其独特的免疫特性,使得排斥反应相较于其他实质器官移植有所减少,但长期随访研究^[6]表明,角膜移植仍存在一定的排斥风险。随着生物工程技术的不断发展,开发生物工程化角膜有望解决供体短缺和移植排斥等问题,推动角膜移植领域的进一步进展。

1 角膜组织工程

角膜组织工程(Corneal Tissue Engineering)作为再生医学的前沿领域,通过整合生物材料和细胞技术开发人工角膜替代物,以应对传统移植中的供体短缺和免疫排斥问题。构建具有生物相容性、光学透明度和力学稳定性的角膜替代材料是该领域的核心研究方向。通过精确模拟角膜的分层组织结构,特别是细胞外基质(extracellular matrix, ECM)与细胞间的动态相互作用,采用材料特性优化、细胞类型筛选和先进制造工艺协同的策略,力求全面恢复角膜的光学性能和生理功能。目前,基于天然材料、合成高分子及其复合体系的支架构建技术已取得重要突破,结合3D生物打印等新兴制造方法,在结构仿生和功能重建方面展现出显著优势。然而,内皮细胞的长期存活维持和神经再生等关键问题仍是临床转化面临的主要挑战。这些技术难题的突破将为角膜疾病的治疗带来革命性的解决方案。

2 用于角膜组织工程的生物材料

用于角膜替代物构建的生物材料需同时满足结构仿生和功能仿生的双重需求,既要精确模拟天然角膜的层级化组织结构,又要复现其独特的光学特性和生理功能。这类支架材料必须具备与角膜组织相匹配的机械强度、良好的生物相容性、优异的光学透明性、可控的生物降解特性以及临床可操作性等重要特征。根据材料来源和基本特性的差异,用于角膜组织工程的生物材料主要可分为天然高分子和合成高分子两大体系。天然高分子材料因其固有的生物活性和细胞识别位点,能够为角膜细胞提供更接近生理状态的微环境;而合成高分子材料则通过灵活的分子设计和可调控的合成工艺,实现材料理化性能的精确定制。下文将系统讨论天然衍生材料和合成高分子材料的基本特性、改性策略及其在角膜组织工程中的具体应用,以期

膜替代物的研发提供理论基础(图1)。

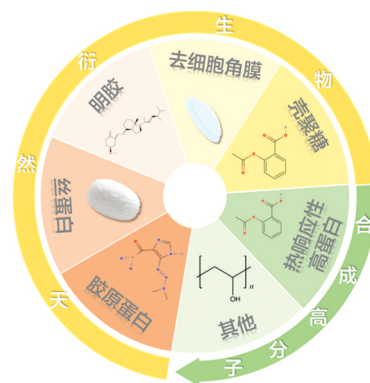


图1 角膜组织工程生物材料的概念示意图

2.1 天然衍生材料

2.1.1 胶原蛋白 胶原蛋白是大多数组织^[7]细胞外基质的主要成分,其三肽序列精氨酸-甘氨酸-天冬氨酸(Arginine-Glycine-Aspartic Acid, RGD)可被整合素识别,在细胞黏附、迁移和增殖中发挥重要作用^[8]。I型胶原因易获取且成本较低,被广泛用于组织工程,而III型胶原在机械性能和光学特性方面更优^[9]。作为角膜基质的主要成分,胶原在构建仿生角膜基质方面至关重要^[10-11],可支持角膜细胞及神经再生^[8, 12-14],但因机械强度和弹性较低,限制其直接应用^[15-16]。交联技术因此被广泛应用,以提升其机械性能和化学稳定性,同时保留生物学优势。

不同交联方法对胶原膜的力学及光学性能影响显著。例如,1-乙基-3-(3-二甲胺丙基)碳二亚胺[1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, EDC]交联可增强抗拉强度,而紫外线诱导的葡萄糖交联可改善弹性和强度,使其接近天然角膜^[17]。交联胶原水凝胶既可用于构建角膜细胞生长的膜状结构^[18],也可作为三维支架封装基质细胞,以模拟全层角膜组织^[10-11],但机械性能仍有局限^[19]。多种交联方法(如脱水热交联、EDC及戊二醛交联)已被证实可支持人角膜上皮细胞(human corneal epithelial cells, HCECs)、人角膜成纤维细胞(human corneal fibroblasts, HCFs)及兔角膜上皮细胞(rabbit corneal epithelial cells, rCECs)的增殖和分化^[8, 18, 20-22]。

为改善胶原的力学性能及生物稳定性,研究者开发了多种复合材料。例如, I型猪源胶原与2-甲基丙烯酰氧乙基磷酸胆碱(2-methacryloyloxyethyl phosphorylcholine, MPC)形成的互穿网络水凝胶,兼具高透明度、机械强度和抗酶解性,可在猪角膜

模型中促进角膜细胞及神经再生^[23-24]。胶原-壳聚糖复合膜在猪角膜移植实验^[15, 25]中,能在 12 个月内促进角膜上皮、基质及神经的再生。DUAN 等人^[26]发现,结合特定细胞黏附肽修饰的聚合物可促进角膜上皮细胞的黏附及增殖,同时促进神经轴突的生长。

当前胶原材料多呈片状结构,研究者尝试多种策略优化角膜仿生支架。ZHANG 等人^[17]通过在硬性隐形眼镜模具中干燥交联胶原,成功构建出具有天然角膜形态的胶原膜。为提高操作性,KONG 团队^[27]采用电纺聚(乳酸-共-乙醇酸)[Poly(lactic-co-glycolic acid), PLGA]与胶原复合形成增强型“夹层”结构,但该复合材料的透光率不足。此外,胶原玻璃化凝胶(collagen vitrigel, CV)通过调控交联度,在透明度和机械强度间取得平衡,已被证实可促进上皮层形成,并降低炎症反应^[10, 28-29]。在仿生结构构建方面,GRIFFITH 等人^[30]采用交联胶原-硫酸软骨素(chondroitin sulfate, CS)支架,并在其上接种角膜上皮、基质及内皮细胞,以构建多层角膜基质。GERMAIN 等人^[31]将原代 HCECs 与 HCFs 共培养,在交联胶原凝胶内培养 3 d,可形成 4~5 层角膜上皮和基底膜成分。另一种方法是利用冷冻干燥及交联技术,在多孔胶原支架内植入角膜成纤维细胞,以促进其迁移及基质分泌,但透明性仍需优化^[32]。然而,该方法生成的支架仍未能完全重现天然角膜的透明性。BUILLES 等人^[33]通过加入蛋白聚糖改善透明度,尽管支架在兔角膜模型中表现出较好的修复能力,但存活率仍有待提升。

2.1.2 丝蛋白 丝素蛋白(silk fibroin, SF)来源于家蚕(bombyx mori)茧壳,因其优异的生物相容性、可控降解性及机械性能,在组织工程和再生医学中备受关注。其前体溶液通常经溴化锂溶解、透析后获得。SF 的高透明性使其成为角膜工程的潜在材料,可支持角膜上皮细胞的培养,并在渗透性方面表现良好^[34-35],然而,由于缺乏天然 ECM 蛋白,SF 膜在细胞黏附方面略逊于羊膜^[36]。为改善其生物学性能,可偶联 RGD 修饰的胶原,以增强人角膜缘上皮细胞(human corneal limbal epithelial cells, HCLEs)、HCFs 和人角膜内皮细胞(human corneal endothelial cells, HCENs)的黏附性与增殖能力^[37-39],或采用多聚赖氨酸进行表面修饰^[40]。

多孔 SF 膜可支持角膜上皮细胞与基质细胞的双层共培养,并促进人角膜基质干细胞(human corneal stromal stem cells, HCSSCs)的 ECM 分泌^[41]。

掺入芦荟凝胶(aloe vera gel, AV)可提高兔角膜内皮细胞(rabbit corneal endothelial cells, rCENs)的存活率和形态保持能力^[42]。此外,PEG 诱导可形成高孔隙率结构,以调控降解速率,但可能降低力学性能,并影响角膜缘上皮细胞(human limbal epithelial cells, HLEC)的增殖与层化^[43-44]。研究^[45]表明,植入兔角膜基质的 RGD 修饰 SF 膜可在半年内保持透明性和结构稳定性,无免疫排斥或新生血管生成。通过增加孔隙^[41]或引入丙酰胺^[46],可进一步调控降解速率。

除 SF 外,家蚕丝的另一种蛋白丝胶蛋白(silk sericin, SS)因潜在的致敏性长期被忽视,但研究^[47]表明其具备良好生物相容性,可增强 HCLEs 细胞黏附,具备角膜上皮再生潜力。近期,APPLEGATE 等人^[48]开发了一种基于黄素单核苷酸的光交联技术,可将丝素溶液转化为高弹性、透明的水凝胶,且所使用的材料均为生物友好型,为角膜组织工程提供了新思路。

相比胶原,SF 制备与改性更便捷,并可通过表面图案化和孔结构调控细胞行为。例如,沟槽深度和宽度可影响角膜基质细胞的排列,层叠多孔 SF 膜或海绵结构可构建三维角膜模型^[49]。然而,SF 支架层数远低于天然角膜(约 200 层,总厚度 500 μm),仍需优化以更好模拟角膜基质结构。此外,尽管化学修饰可提升 SF 生物相容性,但角膜组织富含多种功能性蛋白,仍需探索更精准的表面改性策略。目前研究主要集中在 SF 表面结构与孔隙率对细胞行为的影响,其在体内的长期生物相容性仍有待深入研究。

2.1.3 明胶 明胶作为胶原蛋白的部分水解产物,被广泛应用于角膜组织工程支架的制备。然而,纯明胶材料存在热不稳定性问题,在生理条件下会快速降解,限制了其单独作为支架材料的应用^[50]。为改善这一缺陷,研究者通常采用以下两种主要交联策略:一是脱水热处理,这种方法不仅能显著提高材料的机械性能,还能增强其光学透明性及白蛋白渗透性;二是化学交联,通过与戊二醛、EDC/N-羟基琥珀酰亚胺(N-Hydroxysuccinimide, NHS)等交联剂反应形成稳定的三维网络结构。研究表明,经过适当交联处理的明胶支架,其理化性质更接近天然角膜组织,在透明度、力学性能和降解速率等方面均优于未交联的胶原膜,展现出更好的临床应用潜力。此外,在明胶膜上培养的原代 HCENs 可表达 ZO^{-1} 、 $\text{Na}^{+}/\text{K}^{+}$ -ATP 酶及 N-钙黏蛋

白,并形成完整的内皮单层^[51]。

MIMURA等人^[52]发现戊二醛交联的明胶支架可促进兔角膜成纤维细胞(rabbit corneal keratocytes, rCKs)及其前体细胞的黏附与ECM沉积,并可用于兔角膜基质袋移植。采用EDC/NHS交联并结合冷冻干燥,可调控明胶膜的孔隙率、杨氏模量和溶胀比,其力学特性可通过调整明胶浓度及交联参数优化^[53]。LAI^[54]发现添加CS可促进rCKs生长,但会降低杨氏模量及抗蛋白酶降解能力。此外,NIU等^[55]通过在EDC交联过程中加入肝素,制备了透明明胶支架,增强碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)的吸附与释放能力,同时提高柔韧性,支持HCENs生长并维持其正常内皮功能。兔角膜移植实验表明,该支架可与基质逐步融合。

LAI等^[56]开发了一种基于透明质酸功能化的明胶微球可作为三维培养系统,支持rCKs扩增,并在兔眼实验中展现良好耐受性。近年来,半合成明胶甲基丙烯酸酯化物(gelatin methacryloyl, GelMA)因其优异的生物相容性和可调控的物理特性受到关注。RIZWAN等人^[57]通过在紫外线交联前,将GelMA前聚物溶液置于4℃条件下孵育1 h,可获得力学性能更优的GelMA+水凝胶。其均匀且较小的孔径分布有助于HCENs的黏附与存活,并在兔角膜移植实验中表现出较高的细胞存活率。明胶及其衍生物在角膜组织工程中具有重要应用价值。通过优化交联策略及复合材料的选择,可进一步提升其力学性能与生物相容性,以更好地支持角膜细胞生长及功能重建。

2.1.4 去细胞角膜 去细胞角膜(decellularized cornea, DC)作为目前临床应用最成熟的生物工程角膜材料,已在全球范围内实现了从实验室研究到产业化应用的跨越。这类材料因其与天然角膜高度相似的机械性能和光学特性^[58]以及较低的免疫原性^[59],成为解决角膜供体短缺问题的有效方案。然而,由于人类DC的适用组织来源有限,大多数研究采用猪角膜。研究^[60-61]表明,DC能支持角膜细胞体外生长,并促进动物模型中的组织再生。目前全球已有多款DC产品获批并投入临床使用,包括中国的艾欣瞳®及优得清®、欧盟的Acornea®、美国的BioCornea®及韩国的KeraNatural®等。截至2023年,全球累计完成超4万例DC移植,5年存活率为52%~83%。

技术方面,DC的制备工艺已形成了相对成熟

的体系。传统的DC方法包括使用不同类型的洗涤剂[如Triton X-100、十二烷基磺酸钠(Sodium Dodecyl Sulfate, SDS)、3-[(3-胆酰胺丙基)二甲基氨基]-1-丙磺酸(3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate, CHAPS)]^[61-62]、冷冻-解冻法以及低高渗溶液冲击等,其中0.5% SDS被证实能在有效去除细胞成分的同时较好地保留ECM结构^[62-63]。SHAFIQ等^[64]发现氯化钠与核酸酶合用可完全去除细胞成分,支持成纤维细胞和上皮细胞生长,并保留上皮基底膜的完整性。近年来,基于物理方法的去细胞技术得到了发展。HASHIMOTO等人^[65]提出的高静压法无需洗涤剂,能消除细菌和病毒,同时保留胶原质。然而,由于需要昂贵的设备,没有被广泛使用。LIANG等^[66]通过使用超临界二氧化碳(supercritical carbon dioxide, SC-CO₂)大幅缩短了处理时间,还可避免化学试剂的残留风险。另一种方法是结合胰磷脂酶A2和SDS,能够保留糖胺聚糖和角膜基质的结构并去除大部分异种细胞^[67],并保持与天然角膜相似的光学与机械特性,支持上皮、基质细胞的完整生长,并在再生过程中缓慢降解,与周围基质融合^[68-69]。不过需要注意的是,当前DC产品主要适用于板层移植,要实现全层角膜的完美替代仍需克服内皮细胞再生等关键技术难题。通过将人类胚胎干细胞(human embryonic stem cells, hESCs)衍生的角膜缘上皮细胞样细胞(limbal epithelial cell-like cells, LEC-like)和角膜内皮细胞样细胞(corneal endothelial cell-like cells, CEC-like)在猪源DC支架两侧进行三维共培养,成功构建出具有完整分层结构的全层人工角膜替代物。该替代物不仅厚度和力学性能与天然兔角膜高度匹配,更在移植后8周即实现透明度恢复^[70],这表明DC是一种极具前景的全层角膜生物工程材料。

2.1.5 壳聚糖 壳聚糖来源于甲壳类生物的外骨骼或真菌细胞壁,是一种天然多糖,通过乙酰化甲壳素合成,在角膜生物工程中常用作羊膜的替代材料,用于眼表重建。研究^[71]显示,初代牛角膜上皮细胞(bovine corneal epithelial cells, BCECs)在纯壳聚糖膜上培养时,增殖速度较快,附着能力优于羊膜,并能维持BCECs表型。经表面修饰的壳聚糖能有效支持角膜上皮细胞的生长,而未修饰基材则无法实现这一点^[72]。

为促进角膜内皮再生,WANG等^[73]将聚己内酯掺入壳聚糖膜中,从而调控其降解速率,制备适

用于内皮再生的支架。在这种复合膜上培养的 BCECs 表现出正常形态并形成连续单层结构。由于壳聚糖在生理溶剂中的溶解性较差,研究转向其水溶性衍生物——羟乙基壳聚糖(hydroxyethyl chitosan, HECTS)。与天然人类角膜相比,HECTS 具有类似的光学透明度和水分含量,并且更高的葡萄糖渗透性^[74]。由羟丙基壳聚糖、明胶和 CS 制成的膜能促进 HCENs 和 rCECs 的生长,并且在兔模型中表现出良好的耐受性^[75-76]。尽管壳聚糖在角膜生物工程展现出巨大潜力,但其更适合作为复合支架的组成成分使用,而非单独制备纯壳聚糖支架,因其单一材料难以满足角膜再生的复杂需求。

2.2 合成高分子材料

2.2.1 热响应性高分子 刺激响应聚合物,如聚(N-异丙基丙烯酰胺) [Poly(N-isopropylacrylamide), PNIPAM] 及其共聚物,因其热响应的亲水-疏水特性,在角膜生物工程中得到广泛应用。这些聚合物可在无酶解作用下脱离细胞膜,形成单层无载体细胞,为角膜上皮和内皮的构建提供新策略。已有研究利用温度响应性基质生成角膜上皮^[76]和内皮细胞^[77],其中内皮构建更受关注。

通过 PNIPAM 接枝修饰培养表面,从兔角膜缘干细胞成功构建多层角膜上皮,并利用聚(氟化乙烯二氟) [Poly(vinylidene fluoride), PVDF] 膜进行自体移植,该细胞层可在不缝合的情况下迅速附着于暴露的角膜基质并重建上皮。此外,PNIPAM 脱落的 HCEN 细胞层在兔模型中恢复了角膜透明度,无炎症反应或排斥现象^[77-78]。体外实验^[77-78]表明,该方法维持 Na^+/K^+ -ATP 酶活性及泵位,且有适度水合作用促进角膜透明度恢复。共聚改性可优化 PNIPAM 的性能,如 N-异丙基丙烯酰胺(N-Isopropylacrylamide, NIPAM)与环氧甲基丙烯酸酯共聚可调控临界温度并引入生物分子整合能力,而 NIPAM 与二乙二醇甲基丙烯酸酯共聚可加速细胞脱落,使临界温度更接近生理范围^[79]。此外,TEICHMANN 等^[80]采用聚(乙烯基甲基醚)开发了具有热响应特性的细胞载体,通过调节层厚、表面刚度和生物活性分子修饰,实现了对 HCEN 细胞黏附与脱落的精准可控调节。

由于热脱落获得的细胞通常较为脆弱,选择合适的输送方法对于移植手术的成功至关重要。因此,研究者探索了不同载体分别用于上皮层(如 PVDF 膜)^[76]和内皮(基质床和明胶)层的移植^[77-78],

以期有效提高该技术临床应用的可行性与手术成功率。

2.2.2 其他合成高分子水凝胶 多种合成材料,如聚乙烯醇 [Poly(vinyl alcohol), PVA]^[81]、聚(2-羟乙基甲基丙烯酸酯) [Poly(2-hydroxyethyl methacrylate), PHEMA]^[82]、聚(乙二醇)二丙烯酸酯 [Poly(ethylene glycol) Diacrylate, PEGDA]^[83]、聚(乳酸-共-乙醇酸)^[84]及聚(乙烯醇)/聚(丙烯酸) [Poly(ethylene glycol)/Poly(acrylic acid), PEG/PAA] 基水凝胶^[85],已作为角膜细胞支架或人工角膜替代物广泛应用于生物工程。尽管这些材料具有优异的渗透性、可调节的力学和光学性能,但若不进行表面修饰,它们普遍缺乏良好的细胞附着功能^[86]。

为了改善细胞附着和生长能力,电纺 PVA、PHEMA 水凝胶和 PEGDA 水凝胶分别通过 I 型胶原、磷酸基团和 RGD 肽进行表面修饰^[81-82, 87]。同样, I 型胶原涂覆的 PLGA 薄膜能够更好地支持 rCEN 细胞^[84]。采用 ZnS/PHEMA/PAA 水凝胶制备的人工角膜表现出较高折射率^[85],而 PEG/PAA 水凝胶则具有较高的葡萄糖渗透性,并能在体内维持光学透明长达 2 周^[88]。新型可降解弹性体聚(甘油琥珀酸酯)被制成薄膜,能够维持 HCECs 的生存,但目前尚未见其力学性能及光学性能的相关研究报告^[89]。

3 小结与展望

综上所述,生物工程化角膜替代物在解决角膜供体短缺方面具有巨大潜力。尽管天然高分子材料如胶原蛋白、丝素和明胶等在生物相容性、透明性和可降解性方面表现优异,但仍面临微结构模拟和神经再生的挑战。合成聚合物虽需表面修饰,但在支持细胞生长和输送方面有重要应用前景。未来的研发应聚焦于多层结构构建、神经恢复和组织再生。随着生物材料、细胞生物学及生物打印等新兴技术的发展,生物工程化角膜替代物有望为角膜移植提供可靠、个性化的解决方案,改善患者生活质量。



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