微针递送生物技术药物的研究进展

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摘要: 微针作为一种新型的透皮给药系统, 能够显著改善皮肤的渗透性, 增强药物的透皮递送效果, 在突破皮肤角质层屏障方面显示出独特的优势。这一特性使得微针给药系统在生物技术药物的递送方面展现出巨大的潜力。传统的生物技术药物递送途径主要是注射给药, 这种方式给患者带来疼痛、皮肤红肿等问题, 导致患者依从性较差。此外, 生物技术药物的生产、运输和储备都需要严格的低温条件来保持其活性, 增加成本。相比之下, 微针给药系统具有诸多优势, 为生物技术药物的递送提供了新的途径和解决方案。本文对递送生物技术药物的微针给药系统进行概述, 总结了微针体系在生物技术药物方面的研究进展。

关键词: 微针; 经皮递送; 生物技术药物; 分类; 稳定性

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Progress in delivering biotechnology drugs on microneedles

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Abstract: As a new transdermal drug delivery system, microneedles can significantly improve skin permeability, enhance drug transdermal delivery, and demonstrate unique advantages in breaking stratum corneum barrier of skin. This feature enables microneedles to demonstrate enormous potential in delivering biotechnology drugs. The traditional delivery method for biotechnology drugs is mainly injection, which brings problems such as pain and skin redness to patients, leading to poor patient compliance. In addition, the production, transportation, and storage of biotechnology drugs require strict low-temperature conditions to maintain their activity and increase cost output. Microneedles, by contrast, have many benefits, providing new avenues and solutions for biomolecular delivery. Accordingly, this review introduced the microneedle drug delivery system for delivery biotechnology drugs, and summarized the research progress of microneedle systems in biotechnology drugs.

Key words: microneedle; transdermal delivery; biotechnology drug; classification; stability

皮肤是人体与外界环境之间的屏障, 是人体最大、最复杂的器官, 成人皮肤的面积为1.5～2.0 m^2, 约占成人体重的15%, 并且它的复杂结构及物理化学特性使其能有效抵御微生物、紫外线辐射、化学物质、过敏原的渗透和水分流失等外来因素[1-3]。皮肤由表皮 (130～180 μm)、真皮 (2 000～3 000 μm) 和皮下组织组成, 表皮的最外层是由角质形成细胞组成的角质层 (15～25 μm), 中间真皮层是由纤维结构蛋白组成的, 位于皮下脂肪上, 这些层的厚度差别很大[4,5]。药物被动扩散到皮肤中通常会受到角质层的阻碍, 这个致密层包含死去的角质细胞、细胞间脂质基质和角质桥粒, 从而形成所谓的“砖和砂浆”结构, 其中“砖”象征角质细胞, “砂浆”象征连续的脂质基质[6]。只有中等亲脂性化合物 (分子质量范围为100～500 Da, log$P$为1.0～

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3.0) 才能绕过皮肤富含脂质的结构进入下层皮肤, 由角质细胞和脂质基质之间的共价键形成的紧密连接构成了皮肤的主要保护屏障功能, 也是药物递送的主要限速屏障[7]。

透皮给药是一种无痛的全身给药方法, 一般是将药物制剂应用到完好健康的皮肤上[8]。一般而言, 透皮给药的药物都是低分子量、适度的亲脂性和高效力, 主要通过跨细胞、细胞间和皮肤附属器官 (汗腺、皮脂腺和毛囊) 途径渗透进和穿过皮肤[9]。药物先透过角质层, 然后通过更深的表皮和真皮层, 药物在真皮层不蓄积, 当药物到达真皮层时, 可通过真皮微循环进行全身吸收[10]。与传统的给药方法相比, 透皮给药具有多种优势[11]。它可以为肠胃外途径提供一种非侵入性替代方法, 从而避免针头恐惧症等问题[12]。此外, 药物的药代动力学曲线更均匀, 保持血药水平稳定在治疗的有效范围内, 避免波峰波谷现象, 从而最大限度地降低了毒副作用的风险。由于给药频率的减少, 它可以提高患者的依从性, 也适用于失去知觉或呕吐的患者, 或依赖自我给药的患者[13]。透皮给药避免了肝脏首过代谢, 从而提高了生物利用度[14]。关于使用皮肤作为疫苗接种策略的新位点, 已知该器官在表皮层和真皮层都充满树突状细胞, 这些细胞在免疫反应中发挥核心作用, 使透皮给药成为治疗性蛋白质和多肽的有吸引力的接种途径[15,16]。对于便宜和非侵入性疫苗接种手段, 特别是在发展中国家是非常急需的[17]。

尽管从皮肤出发是一条很好的给药途径, 但很少有治疗活性分子能自然穿透皮肤的角质层。因此, 许多研究使用化学和物理增强技术, 例如离子电渗疗法、超声电渗疗法、电穿孔、微晶换肤术、激光消融和化学渗透增强剂来克服角质层屏障, 进而增加皮肤渗透性[18]。虽然这些方法可以增加药物输送到皮肤中, 但它们通常会引起皮肤刺激或涉及需要能源的大型设备, 这限制了它们在医学中的应用。因此, 尽管有些药物是局部给药或作为透皮贴剂给药, 但大多数都是通过皮下注射来向皮肤输送药物。因此, 开发不需要皮下注射, 但又可以高效输送药物的透皮给药系统是迫切需要的, 例如用于疫苗接种目的的微针透皮给药。

1 微针技术及其优缺点

微针体系最近成为一个很有前途的透皮给药平台, 可以将任何大分子、小分子药物输送到皮肤内[19,20]。微针可以在不穿透真皮的情况下突破角质层的皮肤屏障, 从而最大限度地减少与经皮给药相关的疼痛或出血[21,22]。微针技术改善了皮肤渗透性, 从而增强了透皮药物的递送, 包括蛋白质、激素、核酸、多肽等多种生物分子[23,24]。微针技术解决了大多数与皮下注射相关的问题, 如针刺伤、针头恐惧症、尖锐废物、血源性病原体的传播等。此外, 微针绕过肝脏首过代谢和避免酶促降解, 提高了药物的生物利用度[25]。由于皮肤上含有丰富的免疫细胞, 微针可以使疫苗在体内产生强大的免疫反应, 并且还减少了药物浪费[26]。微针插入皮肤后引起可逆的破坏降低了皮肤刺激性和感染的风险[27]。使用可生物降解材料降低了炎症反应和生物安全问题的风险[28]。此外, 还可以设计微针以实现快速或缓慢释放药物, 还可以实现同时递送多种药物[29]。最重要的一点, 微针固体形式使一些药物的稳定性得到很大的改善, 无需冷链储存和运输, 减少了成本[30]。

但相应地, 微针也存在一些缺点。比如, 微针负载药量有限, 适用微针制备的材料有限, 且空心微针孔的堵塞会降低针头穿透力和药物输送效率; 此外, 皮肤的黏弹性降低了微针的穿透深度, 皮肤的厚度、水合程度、黏弹性等对实现皮肤渗透深度的一致性也有挑战性; 另外, 不利的制备条件可能导致不耐热药物的失活和降解[6]。

2 用于递送生物技术药物的微针类型

2.1 空心微针 (hollow microneedle) 与皮下注射类似, 空心微针为药物输送到皮肤提供了一个特定通道, 以受控速率和非侵入性方式将药物溶液输注到皮肤层 (图 1A)。空心微针递送药物的显著优势在于它们能够将大量具体的药物输送到皮肤中 (表 1)[31]。空心微针所使用的材料还使它们还拥有较强的机械强度。空心微针的主要问题是在皮肤插入过程中针头断裂或针尖的孔道被皮肤组织堵塞, 针对这个问题, 研究人员设计了在针尖侧面带有偏心孔的空心微针, 以防止孔堵塞并使药物接触到周围的皮肤组织[32]。通过空心微针输送药物的最简单途径是被动扩散, 鉴于药物被动渗透到致密皮肤组织的速度较慢, 可以施加一定程度的压力以促进药物递送。

空心微针可以增强疫苗、卵清蛋白、合成信使核糖核苷酸等多种生物分子的透皮递送 (表 2)[33-72], 它最常见的一个应用是递送胰岛素。有研究人员在体内外和临床研究中使用空心微针来透皮递送胰岛素, 使用被动扩散、压力或电辅助将装载在储液器中的药物驱入皮肤, 与传统皮下注射相比, 通过空心微针皮内注射胰岛素具有更快的吸收速度和更好的治疗效果[33]。Davis 等[73]在糖尿病诱导的大鼠体内利用空心微针进行胰岛素递送, 胰岛素被放置在一个连接微针基底的玻璃室中, 从而形成一个药物储库, 胰岛素通过空心微针上的孔道输送, 与胰岛素放置在完好皮肤上的对照组相比, 血糖水平比治疗前降低了 47%, 而对照组没有明显变化, 这说明通过空心微针递送胰岛素是有效的。有研

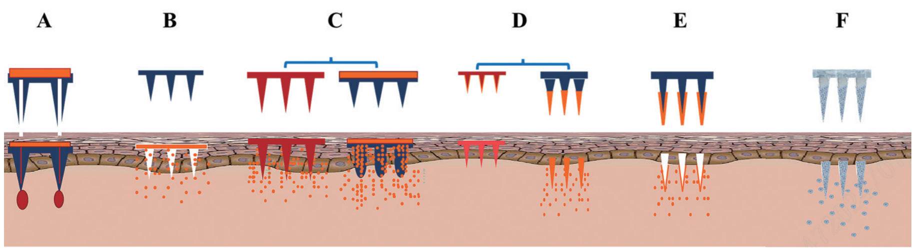

Figure 1　Release modes of different microneedle. A: Hollow microneedle; B: Solid microneedle; C: Swelling microneedle; D: Dissolving microneedle; E: Coated microneedle; F: Cryomicroneedle

Table 1　Microneedle types for transdermal drug delivery

Microneedle type	Advantage	Disadvantage
Solid microneedle	Good mechanical performance	Possible safety hazards caused by broken or damaged needles
	Mature preparation technology	Inaccurate dosing
	Versatile drug formulations	Sharp waste disposal
		Fast pore closure
		Inconvenient two-step usage method
Coated microneedle	Precise dosing	Uneven and limited drug coating
	Short usage time	The coating is prone to peeling off
	High delivery efficiency	Difficulty in controlling dissolution kinetics
	Good long-term stability	High preparation cost
	Rapid dissolution of coatings to release drugs	Non reusable
		Risk of fracture
		Sharp waste disposal
Dissolving microneedle	Biodegradation	Temporary foreign body reaction
	High safety	Drugs affect the mechanical strength of microneedles
	High biocompatibility	Limited range of applicable materials
	High drug loading	
	Short usage time	
	Control drug release rate	
	Simple one-step application	
	No sharp waste	
	Room temperature storage	
Hollow microneedle	Delivering high-dose drugs	Possible blockage by skin tissue
	Controllable administration rate	Difficulty in processing
	High delivery efficiency	Complex administration
	Use for molecules with high and low potency	Inconvenient two-step usage method
	Good mechanical performance	Sharp waste disposal
		Possible drug leakage
Swelling microneedle	Continuous or controlled release	Slow release rate
	Regulating delivery characteristics	Continuous attachment required
	Can be removed after administration	No accurate dosing
	Room temperature storage	Limited range of swelling materials
	Simple one-step application	
	No sharp waste	
	High safety	
	High biocompatibility	
	Delivering high-dose drugs	
Cryomicroneedle	Delivering live cells	Extremely low storage temperature (−80 ℃ or lower)
	Low invasiveness	Limitations on cell delivery volume
	Maintaining cellular activity	Immature technology
	Short usage time	

究团队使用3D打印和液晶显示方法开发了两种空心微针, 他们使用扫描电子显微镜表征了微针的尺寸, 使用微焦点计算机断层扫描表征了微针和微通道的体积特性, 使用有限元分析模拟表征了机械性能和皮肤穿透效率, 制造的空心微针可以促进胰岛素在体外向皮肤输送[74]。此外, 一项针对I型糖尿病儿童的研究表明, 与传统注射方法相比, 通过空心微针注射胰岛素可实现更快的愈合和更少的疼痛[75]。

Table 2 Microneedle types for biotechnology drugs

Microneedle type	Biotechnology drug	Drug type	Dosage	Microneedle material	Advantage	Ref.
Hollow microneedle	Insulin	Protein	2 U	/	Faster absorption speed	[33]
	mRNA	Nucleic acid	5 μg	Ceramics	Improving medication acceptance	[34]
	Dendritic cells	Cell	/	Vat photopolymerization	Delivering cells to target sites	[35]
	Polio virus	Vaccine	10 μL/5 DU	Silica capillaries	Enhancing immune effects	[36]
Solid microneedle	Insulin	Protein	0.3 U	Polylactic acid	Accelerating drug absorption	[37]
	Ovalbumin	Protein	10.5 μg	Dermaroller microneedle	Inducing a strong immune response	[38]
	Tetrapeptide, hexapeptide, acetyl hexapeptide, oxytocin	Peptide	90 $mmol \cdot L^{-1}$	Silicon wafers	Enhancing transdermal peptide delivery	[39]
	Hepatitis B virus	Vaccine	50 μg	Carbomer	Enhancing immunity	[40]
Swelling microneedle	Integrin alpha V beta 6-blocking antibodies	Recombinant protein	/	Polyvinyl alcohol, polyethylene glycol diacrylate	Rapid response	[41]
	Mesenchymal stem cell	Exosomes	/	Polyvinyl alcohol	Regulatory release	[42]
	miRNA	Nucleic acid	300 μg	Gelatin	Anti fibrotic	[43]
	Alpha-melanocyte-stimulating hormone	Peptide	20 mg	Dextran methacrylate, cyclodextrinadamantane based host-guest supramolecules	Increasing pigmentation	[44]
	Insulin	Protein	18.2 wt%	Hyaluronic acid polymers functionalized with dopamine and 4-amino-3-fluorophenylboronic acid	Intelligent release	[45]
	Platelet rich plasma	Protein	20 wt%	Gelatin methacryloyl	Promoting hair regeneration	[46]
	Growth hormone	Protein	185 μg	Silk	Improving clinical treatment effectiveness	[47]
	Fibroblast-derived exosomes	Exosomes	/	Hyaluronic acid methacrylate, polyvinyl alcohol	Promoting healing	[48]
	Insulin	Protein	5/20 U	Silk fibroin	Sustained release	[49]
	Bevacizumab	Antibody	2.5 mg	Gantrez S-97, PEG 10000	Improving bioavailability	[50]
Dissolving microneedle	Lysozyme	Protein	2 μg	Hyaluronic acid, polyvinyl alcohol, sodium carboxymethyl cellulose	Maintaining enzyme activity	[51]
	DNA	Nucleic acid	/	Chitosan oligosaccharide	Storage stability and enhancing immunity	[52]
	Plasmid	Viral chimera	20 μg	Hydroxyethylcellulose, sucrose, polyvinyl alcohol, polyvinylpyrrolidone	Enhancing immunity	[53]
	Insulin	Protein	0.2 U	Polygamma glutamic acid, polyvinyl alcohol, polyvinylpyrrolidone	Rapid drug delivery	[54]
	Endotoxin	Protein	50 μL	Polyvinylpyrrolidone, sodium carboxymethyl cellulose, sorbitol	Enhancing immunity	[55]
	Vascular endothelial growth factor	Growth factor	/	Chitosan	Intelligent delivery	[56]
	Recombinant human epidermal growth factor	Growth factor	0.2 μg	Hyaluronic acid	Response release	[57]
	Antigen	Vaccine	10 μg	Maltodextrin, sucrose, fish gelatin	Enhancing immunity	[58]
	mRNA	Nucleic acid	1 μg	Polyvinyl alcohol, polyvinylpyrrolidone	3D printing	[59]
	Ovalbumin	Protein	/	Baiji polysaccharide	Good stability and cell compatibility	[60]

Continued

Microneedle type	Biotechnology drug	Drug type	Dosage	Microneedle material	Advantage	Ref.
	Liraglutide	Peptide	7/8 μg	Hyaluronic acid	Weight loss	[61]
	Extracellular vesicles	Exosomes	50 μg	Hyaluronic acid	Long lasting effect	[62]
	Inactivated virus	Vaccine	5 μg	Polyvinylpyrrolidone	Enhancing immunity	[63]
Coated microneedle	Recombinant adenovirus, vaccinia virus	Vaccine	6.5 μL	Silicon	Vaccine storage stability	[64]
	Human growth hormone	Protein	167 μg	Titanium	Drug storage stability and fast delivery	[65]
	Insulin	Protein	/	Stainless steel sheets	Process stabilization	[66]
	Peptide A	Peptide	196.8 μg	Quid crystalline polymer	High bioavailability and drug storage stability	[67]
	Parathyroid hormone 1-34	Protein	40 μg	Titanium	Drug storage stability	[68]
Cryomicroneedle	TNF-α siRNA	Nucleic acid	39.6 ± 1.29 μg	Trehalose	Drug storage stability	[69]
	Mesenchymal stem cells, melanocytes, antigen-pulsed dendritic cells	Cell	~220 cells·mm^{-2}	Hyaluronic acid methacrylate	Accurate delivery of therapeutic cells	[70]
	Nanovesicles	Exosomes	/	Hyaluronic acid methacrylate/ polyvinyl alcohol	Keeping the bioactivity of the nanovesicles	[48]
	Nanozyme	Protein	/	Iron ion, tannic acid, polyvinyl pyrrolidone	Delivering the nanozyme inside the cornea	[71]
	Insulin	Protein	1.03 ± 0.045 U	Hyaluronic acid, mannitol	Drug storage stability	[72]

2.2　实心微针 (solid microneedle)　实心微针是通过插入皮肤被移除后, 在皮肤上留下短暂的微通道, 将载药的局部制剂如凝胶、乳膏、软膏等或透皮贴剂应用于微通道以递送药物[76] (表 1)。产生的微通道允许使用的药物被动扩散到皮肤层, 经毛细血管吸收后进行全身循环, 在治疗部位应用药物制剂后, 该微通道的药物递送可以持续到药物耗尽或通道关闭 (图 1B)。

实心微针的制备重点是选择微针的尺寸、密度、几何形状、材料以提供足够的机械强度, 减少插入皮肤时的阻力[77]。实心微针已由多种材料制成, 包括硅、陶瓷[78]、金属[79]、不可降解聚合物 (如光刻胶[80]、聚碳酸酯[81]和聚甲基丙烯酸甲酯[82])、可降解聚合物 (如聚乳酸-乙醇酸共聚物、聚乙醇酸[83]和聚乳酸[37])。实心微针的常见设计包括实心阵列式、柔性贴片式或滚轮式。

许多生物分子都受益于实心微针预处理对皮肤的破坏 (表 2)。Wu 等[84]利用了实心微针来辅助药物及有效成分的吸收, 体内外经皮结果表明微针预处理后胰岛素的渗透量都显著增强, 并在去除给药体后持续数小时增加, 可以显著降低血糖水平。Kumar 等[38]发现用实心微针预处理后可以向皮肤渗透结合着卵清蛋白的纳米粒子, 与单独使用卵清蛋白相比, 用纳米粒子携带的抗原在微针处理过的皮肤区域进行经皮免疫可能会引起更强烈的免疫反应。Zhang 等[39]使用了固体微针辅助多肽进行经皮递送, 结果表明皮肤预处理后显著增强了低分子量肽在皮肤上的渗透, 并在 24 h 内提供了肽的持续释放, 且渗透率随着多肽分子量的增加而降低。因此, 借助实心微针进行皮肤预处理, 显著增强了大多数大分子的透皮递送。

2.3　溶胀微针 (swelling microneedle)　溶胀微针是一种由水凝胶形成的新型微针, 通常是由交联聚合物材料制成 (表 1)。当插入皮肤时, 这些针头会迅速吸收皮肤组织液并膨胀, 从而形成膨胀的多孔结构, 作为药物扩散的通道[85] (图 1C)。并且可以将膨胀的微针完好无损地从皮肤上移除, 仅在皮肤中留下很少的聚合物残留物, 它还具有可调节的药物释放速率[86]。通常, 形成水凝胶的微针在其结构中不含药物, 而是药物被储存到位于微针基底的储液器中。这种新技术的发展还克服了硅或金属微针的生物相容性问题, 这些材料具有潜在毒性, 所以水凝胶在应用过程中不会对患者产生影响, 并且会通过自然过程在体内降解。

溶胀微针可以有效地将各种生物技术药物输送到皮肤中 (表 2)。载胰岛素的丝素蛋白溶胀微针可在体内持续释放和输送药物。结果显示针尖可持续释放胰岛素 12 h, 能有效控制糖尿病大鼠的血糖水平, 因此, 这种微针体系可实现长期降糖治疗[49]。Seong 等[87]开发了针尖具有膨胀功能的双层微针, 药物负载在膨胀针尖材料里, 得以使负载的胰岛素长期释放, 还能保持胰岛素的生物活性, 且该体系还增强了与大鼠皮肤的黏附强度。Courtenay 等[50]展示了用于递送贝伐单抗的溶胀微针, 他们将单抗输送到啮齿动物体内, 并使用

酶联免疫吸附试验检测血清和淋巴结样本中单抗的浓度, 结果显示可以持续释放药物7天, 并报告了淋巴结处的聚积, 这说明为淋巴瘤和继发性转移瘤提供了新的治疗选择。溶胀微针可以递送多种药物, 又不在体内留下残余的聚合物成分, 具有良好的生物相容性和可降解性, 并且无细胞毒性, 消除了使用过程中的风险, 是未来很有前景的经皮给药方式。

2.4 可溶性微针 (dissolving microneedle) 与涂层微针相比, 现已开发可溶性微针可以完全溶解在皮肤中, 从而在使用后不会留下生物危害性尖锐物 (表1)。这些微针通常是由水溶性材料制成, 插入皮肤后, 这些载药微针溶解在皮肤组织液中以释放药物 (图1D)。该体系可以提供持续的药物释放动力学, 具体取决于聚合物材料的溶解速率和微针的贴敷时间[88]。可溶性微针的制备方法一般是将含药聚合物溶液浇筑在模具中, 等待干燥后从模具上剥离出来, 获得可溶性微针。适用于可溶性微针的可生物降解和水溶性聚合物一般有羧甲基纤维素钠、麦芽糖、葡聚糖、壳聚糖、硫酸软骨素、丝素蛋白、聚乙烯醇、透明质酸、聚乙烯吡咯烷酮等。此外, 温和的制备条件有利于保护生物技术药物的活性, 且可以增加储存稳定性。

可溶性微针作为载体可透皮递送多种生物技术药物 (表2), 载有干扰素-α-2b的可溶性微针与肌内注射具有生物等效性, 表明微针可以成为传统肌内给药的可靠替代方案[89]。Yang等[90]开发了一种葡萄糖响应闭环微针系统, 用于透皮递送胰岛素和胰高血糖素, 药物的释放可以根据血糖水平的变化自动调节, 这种体系对诱发I型糖尿病的小鼠和小型猪长期有效。同样, 有研究还开发了一种“智能胰岛素贴片”, 其交联透明质酸基质含有葡萄糖反应性囊泡, 可有效快速降低糖尿病小鼠的血糖水平[91]。Fakhraei Lahiji等[51]在4 ℃温度下制备了负载溶菌酶的可溶性微针, 并在制剂中加入稳定剂, 在室温环境下干燥, 结果可以维持12周的99%药物生物活性。含有血管内皮生长因子的壳聚糖微针可促进伤口愈合中的胶原蛋白快速沉积、炎症减少和组织再生[56]。Chen等[92]利用氧氟沙星和碱性成纤维细胞生长因子的双相释放动力学制备了用于伤口愈合的溶解型聚乙烯吡咯烷酮微针, 微针基底先快速溶解释放氧氟沙星, 然后将针尖负载生长因子的PLGA微球递送到伤口区域, 随着微球逐渐降解缓慢释放生长因子, 促进伤口愈合。Zhou等[60]用天然材料白芨多糖成功制备了可溶性微针, 这些微针提供了优于透明质酸和聚乙烯醇微针的机械强度和稳定性, 且具有良好的细胞相容性、无感染、没有皮肤刺激性, 此外, 圆二色谱结果显示卵清蛋白在微针中能保持21天二级结构稳定。还有研究人员设计了可分离微针阵列, 以实现药物快速释放和较短的贴敷时间。有报道从聚丙烯酸 (PAA)/$NaHCO_3$-丝素蛋白开发了可分离的可溶性微针, 用于重组人生长激素的透皮递送, 温和的制备工艺提高了生长激素的稳定性和生物利用度, 这个体系可以持续递送药物7天, 并提供与每日皮下注射生长激素相当的效果[47]。

2.5 涂层微针 (coated microneedle) 使用实心微针增强药物透皮递送的改进策略是将含有活性化合物和水溶性赋形剂的制剂涂在针尖表面上, 即得到涂层微针 (表1)。当药物涂层微针插入皮肤时, 涂层会遇到皮肤中存在的组织液, 进而引发涂层从微针表面脱离并迅速溶解释放药物 (图1E)。依据包衣赋形剂的水溶性, 涂层的分离可以在几秒到几分钟内完成, 涂层微针不仅可以将涂层有效递送至皮肤, 还可用于输送到其他组织[93]。与实心微针的两步使用过程相比, 这种涂层微针技术更加高效、可控和方便, 大多数经皮大分子递送的体内研究都使用了涂层微针。目前, 已经开发出不同的涂层方法, 包括浸涂、气体喷射干燥、喷涂、基于电流体动力雾化工艺和压电喷墨印刷[94]。涂层微针的一个缺点就是针尖表面上只能携带非常少量的药物, 过多的涂层可能会导致微针的机械强度和锋利度降低, 因此, 涂层微针有利于相对较低治疗剂量的生物分子。

一些大分子可以通过涂层微针有效地递送到皮肤内 (表2), 进入体内循环。van der Maaden等[95]将灭活的流感疫苗涂敷在金属微针上进行经皮递送, 该方式可诱导强烈的细胞免疫反应, 能够像肌肉注射免疫一样有效地提供针对病毒攻击的保护, 疫苗涂层金属微针提供了一种新颖且高效的免疫方法。Vrdoljak等[64]将重组腺病毒和经修饰的安卡拉痘苗病毒载体涂覆到固体微针阵列上, 成功地递送到皮肤内, 诱导抗体产生, 与注射免疫获得相当的抗体。Ameri等[65]制备了负载重组人生长激素的涂层微针, 研究表明微针贴片包被的人生长激素以高效率递送, 其绝对生物利用度与皮下注射制剂相似, 且微针贴片可以在40 ℃下保持稳定6个月, 聚集体百分比没有显著变化。Zhou等[19]开发了一种新型的凝胶包封重组人干扰素α-1b涂层微针, 可以缓慢释放药物, 该体系具有生物安全性和室温下6个月的储存稳定性, 微针制剂与皮下注射具有生物等效性。Kapoor及其同事[67]将多肽涂覆在固体微针上, 载药量可以精确地得到控制, 涂层微针透皮递送实现了与皮下注射相似的绝对生物利用度, 且肽的稳定性也得到显著改善, 对皮肤刺激性也很小。Ross等[66]使用喷涂技术将胰岛素聚合物涂层应用在金属微

针上以进行透皮给药, 涂层中胰岛素的α螺旋和β折叠在聚乙烯己内酰胺–聚乙酸乙烯酯–聚乙二醇和海藻糖中都得到了很好的保存。有研究将带有不同分子(蛋白质、不混溶分子和纳米颗粒) 的涂层涂覆在金属微针上, 以实现从单个微针阵列中提供多种疗法[96]。

一种新颖的涂层微针体系是Macroflux微针阵列, Macroflux系统可以将各种生物分子 (即生物制剂、肽、蛋白质和疫苗) 涂覆到固体微针表面上。其中, 甲状旁腺激素1-34是一种用于治疗绝经后骨质疏松症的药物, 在临床前和临床试验中受到了极大的关注[97]。重要的是, 涂覆在微针上的甲状旁腺激素在25 ℃下储存两年后在成品中保持稳定, 从而消除了冷链或特殊储存的需求[68]。

2.6　冷冻微针 (cryomicroneedle)　冷冻微针主要是通过模具法对生物技术药物的低温介质进行冷冻成型, 属于溶解微针的一种类型 (表1)。当插入皮肤时, 这些针头会和溶解微针一样迅速吸收皮肤组织液以释放药物 (图1F)。冷冻微针可以在皮内精确递送治疗细胞、纳米酶、siRNA、纳米囊泡等生物技术药物, 它最主要的优势是能够长久保持所负载细胞的活性, 同时兼具良好的机械强度, 能够通过负载不同种类的治疗细胞, 实现不同的治疗效果, 为细胞治疗提供了一种简便、安全、高效和微创的策略。

冷冻微针作为载体主要用于递送生物技术药物(表2), Wen等[69]通过微成型制备了负载由外泌体包裹的TNF-α siRNA的低温微针, 其负载效率高达21%, 该微针具有良好的生物相容性, 可有效缓解类风湿性关节炎。有研究将间充质干细胞、治疗白癜风的黑素细胞、抗原脉冲树突状细胞负载在冷冻微针里, 进行了临床相关的体外和体内检测, 有望可以实现常温运输, 这将促进微针技术在细胞治疗和再生医学领域的应用[70]。相关研究制备出了新型负载纳米酶的冷冻微针, 它能够在角膜内可控地递送纳米酶以治疗角膜炎, 并且冷冻过程可以大大提高微针的机械强度, 与传统的滴眼液相比, 该制剂在大鼠眼部感染模型中表现出优越的治疗潜力[71]。

3　微针中生物技术药物的稳定性

与小分子化药相比, 生物技术药物通常更容易被极端条件如pH、温度和湿度等降解[98]。在生物技术药物制剂制备、储存、运输和给药等各个步骤中都可能会使生物技术药物活性降低[99]。有机溶剂, 例如二氯甲烷、乙酸乙酯、碳酸二甲酯, 会促进蛋白质分解。当接触到水时, 生物技术药物通常会发生聚集、变性和沉淀[100]。生物分子结构变化会导致不好的效果, 例如药物疗效降低、生物活性丧失、药物安全性受损及产生免疫原性。通常, 二级结构发生变化可导致蛋白质聚集, 特别是形成不溶性和富含β-折叠的淀粉样蛋白原纤维, 天然生物制剂不会通过人类B和T细胞识别异源表位来触发免疫反应, 然而, 这些聚集体包含多个氨基酸结构域, 这导致抗原呈递细胞的摄取增强, 从而产生免疫原性[101]。表3中列出了不同微针类型中影响药物稳定性的降解机制和因素。开发任何制剂都应在深入了解生物分子的理化特性和稳定性方面的情况下开始, 最佳生产工艺、材料选择和配方开发应确保药物的完整性、稳定性和有效性。

这些生物分子在普通环境中以液体或冻干制剂形式储存时会容易失去其生物活性和功效, 将药物包封在微针中可以使生物分子热稳定性增加, 对生物制品的储存有了极大的帮助。皮肤作为一个强大的免疫器官, 主要安全问题是免疫原性, 因此, 在负载生物技术药物的微针制剂中, 分析其产生的聚集体和亚可见颗粒很有必要性[102]。在微针的制备过程中, 应尽量避免一些高温、有机溶剂、紫外线照射等极端条件, 使用较温和的条件来制备微针。很多研究都表明, 将生物分子包封在微针制剂中可以显著提高药物的稳定性。Prausnitz团队[103]先是开发了一种包封流感疫苗的耐热微针制剂, 通过对流感疫苗稳定性的评估, 筛选了微针配方、干燥和储存条件, 其中筛选了61种不同配方赋形剂及其一些组合, 最后用精氨酸和葡萄糖酸钙制备的可溶性微针在室温下储存6个月期间对3种季节性流感疫苗毒株都没有显著的活性损失。接着, 又对这些微针制剂进行了长期稳定性检测, 发现含有海藻糖/蔗糖、蔗糖/精氨酸和精氨酸/庚葡糖酸盐组合的制

Table 3　Biomolecules degradation in microneedles

Type	Factor	Degradation form
Drug-device interface	Pinholes, material properties, surface morphology, and drug formulation	Aggregation, adsorption, unfolding
Drug concentration	Coating and injection molding formulation	Aggregation
Temperature	Material polymerization, transition temperature, and drying process	Aggregation, chemical degradation, unfolding
Metal catalysis	Formulation ingredients and microneedle materials	Aggregation, oxidation
Air exposure	Storage conditions	Aggregation, adsorption, oxidation, unfolding
pH	Composition and characteristics of coatings and injection molding formulation	Aggregation, chemical degradation, unfolding

剂在25 ℃下储存24个月的过程中成功保留了大部分疫苗活性, 同时这些制剂在暴露于60 ℃条件下4个月、多次冻融循环和电子束照射后没有失去明显的活性[104]。该团队还研究了二价麻疹和风疹疫苗在微针制剂中的热稳定性, 发现在干燥过程中, 中性pH的磷酸钾缓冲液对两种疫苗都是最佳的, 还筛选了43种赋形剂, 得到pH为7.5的蔗糖−苏氨酸−磷酸钾缓冲制剂作为最佳制剂, 该微针制剂在1个月后没有明显的疫苗滴度损失, 并且在3～4个月后在5、25、40 ℃储存下能保持在一个log (10) 滴度损失的临界值内[105]。该团队还提出了一种热稳定、易于使用的负载催产素微针贴片, 发现赋形剂中聚丙烯酸最能稳定催产素, 用含有海藻糖的柠檬酸盐缓冲液和乙醇混合物的制剂制备的微针在40 ℃下储存12个月后催产素效力保持75%, 而对照组商业催产素产品的效力降低至35%[30]。Ameri等[68]研究了涂在实心微针上的甲状旁腺激素的稳定性, 金属微针中存在的金属元素会加速药物氧化过程, 在涂层组合物中加入蔗糖会使甲状旁腺激素聚集物从7%显著降到0.5%, 当保持在室温和60%相对湿度条件下时, 甲状旁腺激素生物活性可保存长达18个月。You等[106]设计了一个有3个功能层结构的蛋心微针, 可以在微针制备过程中保持负载药物的最大活性, 并将其完全递送到皮肤中, 壳层设计是通过模仿鸡蛋中白蛋白的作用来保持药物活性, 在糖尿病小鼠模型中, 微针制剂同注射剂一样表现出相似的降糖效果, 与传统的利拉鲁肽注射剂相比, 微针制剂中利拉鲁肽在制备过程中甚至在储存2个月后仍保持稳定。Migalska等[107]制备了负载胰岛素的可溶性微针, 制备过程没有对胰岛素的活性造成影响, 其二级结构没有发生变化。将胰岛素包封在可溶性微针中可以在−80到40 ℃温度下储存1个月维持药物活性[108]。最佳配方的微针制剂可以在冷链外的长期储存期间和其他环境压力期间保持生物制剂的活性, 这说明微针制剂可能在不需要冷藏储存的情况下出现在药房货架上, 这有望降低生物制剂的成本。

4　微针的安全性和可接受性

微针在插入皮肤后, 会造成皮肤上短暂性和可逆的微通道, 因此, 用于制造微针的材料必须具有惰性、非免疫原性、生物相容性和稳定性, 评估材料的生物相容性和安全性是人类转化研究的基础。微针可以由多种材料制成, 但是其中一些材料可能会对皮肤或人体造成刺激、毒性和其他不良反应。硅和玻璃是易碎的材料, 在按压微针过程中容易断针, 留在皮肤中一些细小的碎屑, 这些材料的生物相容性尚未得到充分证明, 可能会引起严重的炎症[109]。与可溶性微针和膨胀微针不同, 实心、空心和涂层微针所使用的材料是不可生物降解的, 还会产生一些尖锐废物, 从而产生交叉污染和疾病传播的风险[110]。但是, 微针的这种风险明显低于传统皮下注射针。大多数聚合物是可生物降解的, 具有良好的生物相容性, 这比其他材料更有优势, 它们提供了可控的生物降解速率。而且聚合物微针通常具有高韧性, 避免脆性使用时断针出现, 聚合物微针在使用时是安全的。研究表明使用微针最明显的不良反应是皮肤刺激性与轻度红斑, 无创微针治疗不会引起疼痛, 最多只会引起轻微刺激, 而皮肤会在数小时内完全恢复, 在使用过程中不会引起疼痛是微针的一个最大优势[111]。

微针产品最终商业成功不仅取决于它们按设计运行的能力, 还取决于它们对患者的可接受性, 以及公众对产品的兴趣和信心。给药产生的疼痛与患者的依从性和接受度密切相关, 与传统皮下注射相比, 大众更加偏向于使用微针给药系统。由于微针针体没有到达真皮层, 几乎触碰不到神经末梢, 穿刺产生的疼痛感较小。此外, 微针的使用更加便利, 它不需要由专业的医疗人员指导或执行, 可以在家安全有效地自行使用, 自我管理, 且方便携带。Prausnitz团队[112]进行了使用微针对局部皮肤反应、使用可靠性和患者可接受性的研究, 有15名志愿者参与了该试验, 结果表明, 可溶性微针在应用部位没有任何疼痛或肿胀, 只有轻微的红斑, 但在7天后也全部都消退了, 表现出良好的耐受性, 并被受试者强烈接受。

5　展望

微针作为一种前沿的创新技术, 具有广阔的发展前景。然而, 在微针产业化进程中, 仍面临诸多挑战, 包括药物稳定性、良好生产规范合规性、质量控制、剂量限制、规模化和自动化。微针作为一种新型的药物递送系统, 其载药量和药物释放性能直接受药物稳定性的影响。由于微针所使用的材料、制备工艺和储存条件等因素, 可能会对生物技术药物的稳定性产生影响。微针的生产过程需要遵循GMP以确保产品的质量和安全性。然而, 由于微针技术的特殊性, 其生产过程和质量控制标准尚未得到全面规范。微针的质量控制涉及多个方面, 包括微针的尺寸、形状、机械强度、药物含量、释放性能、贴敷时间、使用安全性等, 而国内目前针对微针的法规相对空白。由于微针尺寸小、结构复杂, 导致质量控制难度较大, 且载药量有限。微针产品在使用过程中还可能面临各种风险和挑战, 如不正确的使用方法会使微针刺入深度不同, 导致递送药物剂量不准确, 还有可能会对皮肤产生刺激, 有感染的风险, 这都需要得到充分的评估和解决, 才能进入临床使

用。其中, 还有最重要的微针的规模化和自动化生产问题, 面临着技术难度大、设备成本高和人员培训需求大等一系列挑战。另外, 企业在研发和生产时受到的政策激励不够, 微针产品的审评又蒙着一层不确定性, 部分生物医药企业对于这方面的合作也持着一种相对保守的态度, 所以未来想要实现产业化依然面临着较多的阻碍。

因此, 在产业化进程中, 应采用合适的药物负载技术、优化微针材料和制备工艺, 及改善储存条件, 以提高药物的稳定性。加强GMP培训, 建立符合微针生产特点的GMP体系, 确保生产过程的合规性。同时, 与监管机构密切合作, 推动微针相关法规的制定和完善。采用先进的质量检测技术和设备, 对微针进行全面的质量控制。同时, 建立严格的质量控制标准和流程, 确保每批产品都符合质量要求。通过优化微针的设计和制备工艺, 提高载药量和药物释放速度的可控性。加大技术研发投入, 开发适用于微针生产的先进技术和设备。还应开发微针给药器, 实现患者自主精准给药, 降低递送剂量不准确的风险。同时, 加强人员培训和技术交流, 提高生产人员的技能水平。

总之, 微针产业化的成功是一个复杂而长期的过程, 需要不断加强技术研发、优化生产流程、提高产品质量和推动产业标准化发展, 来推动微针产业的健康、可持续发展。

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