特约评述

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抗菌肽的生物合成及医学应用

魏岱旭，龚海伦，张旭维
（西北大学生命科学院与医学部，陕西 西安 710069）

摘要：因具有广谱抗菌性和低耐药性，天然抗菌肽成为潜在的抗生素替代品之一，并有望解决长期困扰人类的耐药菌感染问题。除了抗细菌和真菌等微生物病原体，抗菌肽还具备抗癌、抗病毒、抗寄生虫和调节免疫等诸多作用，具有巨大的医学应用前景。本文介绍了抗菌肽的分布和抗性原理，重点归纳抗菌肽的生物合成方法，对比分析依托微生物表达系统的多种抗菌肽生物合成体系的利弊，并介绍合成生物学等新型交叉学科及手段在抗菌肽的设计策略，并总结了抗菌肽在消炎类药品、抗病毒药物、抗寄生虫药品、抗癌药物、医学组织工程、药物递送系统、皮肤护理与医疗美容七大医学领域的应用。同时，本文针对抗菌肽生物合成含量少、分离提取困难、成本高、稳定性差、生物安全不足等潜在问题，提出了潜在的解决方案，包括利用计算机预测与定向基因编辑技术创建新型抗菌肽，以提升其抗菌性能，降低生物毒性；完善抗菌肽生物合成的工业化体系，开发快速回收高纯度抗菌肽的策略；将抗菌肽与现有抗生素联合用药，预防传统抗生素的细菌耐药性；与新型生物材料结合，降低抗菌肽对体内其他组织器官的损伤等。

关键词：抗菌肽；防御素；合成生物学；生物工程；组织工程

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Biosynthesis of antimicrobial peptides and its medical application

WEI Daixu，GONG Hailun，ZHANG Xuwei
（*College of Life Sciences and Medicine*，*Northwest University*，*Xi'an 710069*，*Shaanxi*，*China*）

Abstract: Due to their broad-spectrum antibacterial activity and low incidence of drug resistance, natural antimicrobial peptides have become a potential alternative to antibiotics. In addition to being able to control pathogenic bacteria and fungi, antimicrobial peptides also have many other biological effects, such as anticancer, antiviral, antiparasitic and immunomodulatory activity, exhibiting broad biomedical application prospects. This review introduces the distribution and mechanisms of antimicrobial peptides, and summarizes the biosynthesis methods of antimicrobial peptides. We further compare and analyze the advantages and disadvantages of various antimicrobial peptide biosynthesis approaches relying on microbial expression systems and introduce new interdisciplinary peptide-

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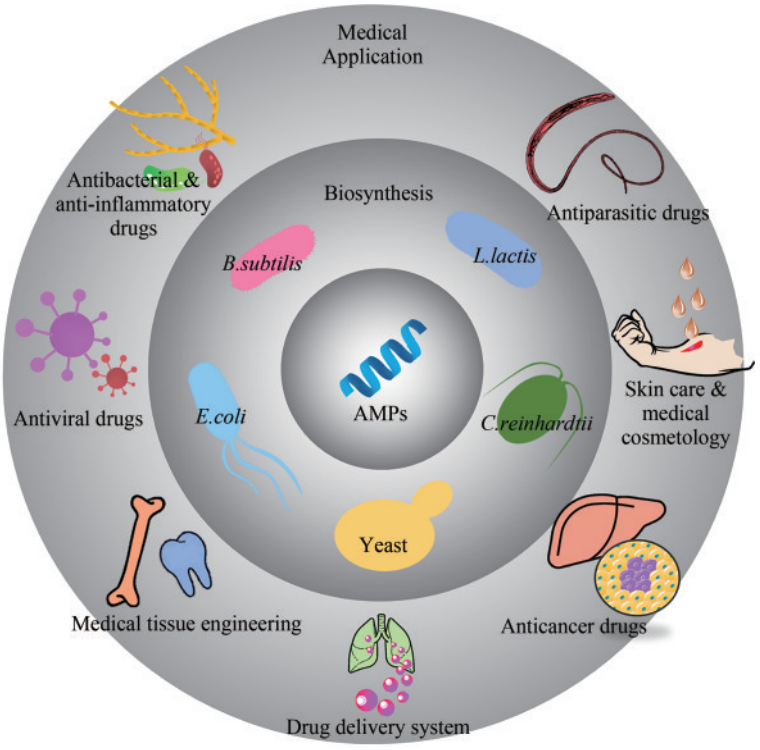
design strategies based on synthetic biology. In addition, we also briefly summarize the applications of antimicrobial peptides. The application prospects of antimicrobial peptides can be classified into seven medical fields, including antiinflammatory drugs, antiviral drugs, antiparasitic drugs, anticancer drugs, medical tissue engineering, drug delivery systems, skin care and cosmetology. Furthermore, we also identify potential problems such as low expression yield, difficulty in extraction, high process cost, poor stability and insufficient biosafety of existing antimicrobial peptides. To solve these issues, computational prediction and directed gene editing technology can be used to create new antimicrobial peptides with improved antibacterial properties and reduced toxicity. It is also important to improve the industrial infrastructure of antibacterial peptide biosynthesis and develop strategies for rapid recovery of high-purity antibacterial peptides. Antimicrobial peptides can also be combined with existing antibiotics to prevent bacterial resistance to traditional antibiotics. Finally, antimicrobial peptides can be combined with new biomaterials to reduce their toxicity to tissues and organs *in vivo*.



Keywords: antimicrobial peptide; defensin; synthetic biology; bioengineering; tissue engineering

抗菌肽（antimicrobial peptides，AMP）是一大类对细菌、真菌、寄生虫和病毒等有害生命体（或病原体）具有抗性功能的活性寡肽[1-3]。因其普遍带有足量的正电荷并常伴随疏水性，可在静电作用下与含有负电的生物膜结合，穿透并破坏膜结构致细胞死亡[4]。与传统抗生素的单一靶点杀菌原理不同，抗菌肽可在病原体进行多靶点破坏，能极大程度降低耐药菌的产生[5]，且具有广谱抗菌性，是未来替代抗生素的最佳选择之一[6]。目前，已有抗菌肽用于致病菌感染、创面愈合、癌症等方面的临床治疗案例，但天然抗菌肽的全面推广也不可避免受到来源、生产成本、生物安全等因素的困扰。随着合成生物学及医学组织工程等交叉学科的兴起，将给抗菌肽的生物设计和合成提供新的思路和技术手段。本文综述抗菌肽的来源、抗性原理及医学应用，并重点探讨基于合

成生物学技术的抗菌肽生物设计及医学应用前景。

1 抗菌肽来源和抗性原理概述

自首次在美国蚕蛹中发现抗菌肽以来，迄今为止，科研人员已经在自然界发现并记录了3791个抗菌肽，来自微生物、植物、无脊椎动物、鱼类、两栖动物、爬行动物、鸟类和哺乳动物[5]；其中动物来源的抗菌肽占比最多（图1）。

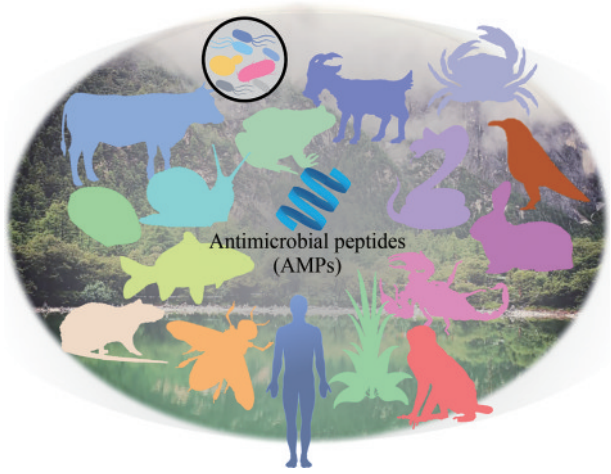


图1 抗菌肽广泛分布于多种生物

Fig. 1 Antimicrobial peptides (AMPs) are widely distributed in many organisms

植物源抗菌肽是植物为了抵御病原体和食草动物的侵害而产生的蛋白质化合物[7]，代表着进化历史上最古老的先天免疫成分之一，为病原体攻击提供了宿主防御的第一道防线[8]。常见的植物源抗菌肽为Hevein[9-10]、Knottin[11-12]、α-Hairpinin[13]和Snakins[14]等家族。此外，还存在富含其他氨基酸的植物源抗菌肽[15]。因微生物来源多样，微生物源抗菌肽的种类也较为丰富且分布广泛，多集中于细菌，少数来自于真菌或其原核细胞。

与其他来源的抗菌肽相比，动物源抗菌肽被发现、分析、改造及应用的报道更多[16]，且人源抗菌肽研究最为系统[17-19]。目前，主要有以天蚕素（Cecropin）为代表的昆虫源抗菌肽[20-21]，以对虾素（Penaeidin）、Polyphemusins等为代表的节肢动物源抗菌肽[22-23]，及以贻贝肽（Myticin）为代表的来自于软体动物源抗菌肽[24-25]。哺乳动物来源抗菌肽主要包括Cathelicidin（抗菌肽）和Defensin（防御素）两大家族。Cathelicidins家族的典型代表是hCAP-18/LL-37，也是唯一存在于人体的抗菌肽[26-28]，但其抗菌能力主要来自C末端结构域的37个氨基酸（LL-37）[29]，且对大肠杆菌、伤寒杆菌、金黄色葡萄球菌、铜绿假单胞菌、表皮葡萄球菌、李斯特氏菌甚至部分高危耐药菌球都具有相当的杀伤能力[17]。Defensin是一类富含精氨酸而显正电荷的寡肽，常由18～54个氨基酸组成[30-31]，能杀伤绝大部分需氧细菌，对真菌、病毒也具有抑制作用[18-19]，也常与不同的生物材料结合使用，用于解决人工骨及牙等辅助修复过程中的厌氧菌抑制问题[32-34]。

目前，主流观点认为抗菌肽的作用靶点是细菌的细胞膜，为非特异性。阳离子型抗菌肽通过与带负电荷的细胞膜成分［如革兰氏阴性菌的脂多糖（lipopolysaccharide，LPS）、革兰氏阳性菌的脂磷壁酸和真菌的甘露聚糖］相互作用，且抗菌肽普遍具有较强的疏水性和两亲性结构，也增加了细胞膜的通透性，破坏其稳态，致细胞膜溶解，促进细胞内容物释放，实现抗菌或其他抗性功能[35]。基于现有的研究数据，针对阳离子型抗菌肽，提出了一些膜腔形成的4种常见的假设模型[36-40]：桶板模型（barrel-stave）[37]、环孔模型（toroidal）[38]、地毯式模型（carpet）[39-40]和聚集体模型（aggregate）[41]（图2）。阳离子型抗菌肽不同，阴离子型抗菌肽的作用机制仍不清楚，但也存在类似的现象[42-48]。

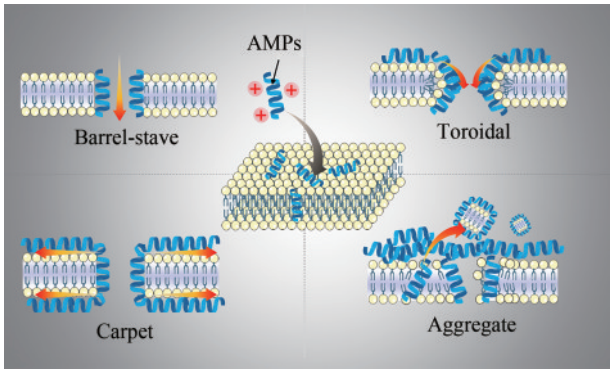


图2 阳离子型抗菌肽抗菌机制模型

Fig. 2 Antibacterial mechanisms models of cationic AMPs

除了抗细菌，大部分抗菌肽还对真菌[49-50]、病毒[51-54]、寄生虫[55-59]乃至癌细胞[60-63]也有抗性或抑制作用。部分抗菌肽主要是通过破坏、阻止真菌细

胞壁合成的方式来消灭真菌；也有间接干扰真菌胞内的大分子合成从而破坏真菌脂膜等结构抑制真菌的生长[49-50]。抗病毒类抗菌肽主要通过破坏病毒包膜、抑制病毒附着和病毒-细胞膜融合、抑制病毒复制等方式来消灭病毒[64]。目前，抗寄生虫类抗菌肽的抗性机制制尚不清楚，但有研究发现少数抗菌肽可通过膜透化与去极化等膜溶解机制杀死寄生虫[57]。

2 抗菌肽的生物合成及生物表达系统

除了按某种特定氨基酸序列进行多肽的化学合成技术[65-67]，越来越多的抗菌肽通过生物合成获得。对比从动植物及微生物体内直接分离、纯化得到的抗菌肽的天然提取法，基因工程法及升级的合成生物学法都存在操作程序简单、成本低、污染小等优势[68]，这已经成为获取抗菌肽的最主要途径之一。如表1所示，除少数采用动、植物表达系统外[100]，大部分抗菌肽采用依托微生物表达系统实现生物合成；且随着合成生物学的快速发展，科研人员也正在尝试利用新型底盘细胞和多种合成生物学工具对抗菌肽的微生物合成表达进行改良。

2.1 利用原核表达系统生物合成抗菌肽

因大肠杆菌具有生长速度快、培养成本低、生物背景清晰等优势，最早用于抗菌肽的基因工程法生物合成[101]。最常用的大肠杆菌为*E. coli* BL21（DE3）菌株[102]，常用的表达载体有pET、pGEX等。

早在20世纪90年代，已利用*E. coli*重组表达Cecropin及其衍生肽，与提取的天蚕素一样，重组表达的Cecropin能有效裂解细胞膜、杀死多种细菌[21，69-72]。为了获得大量的来自亚洲海生蛤蜊的抗菌肽Perinerin，将其编码序列克隆到pET32a（+）载体中，作为融合蛋白的一部分在*E. coli* BL21（DE3）中表达且纯化，抗菌实验显示重组Perinerin具有与天然Perinerin相似的抗菌活性[73]。腺调素（Adenoregulin，ADR）是从树蛙皮肤中分离获得的由33个氨基酸组成的抗菌肽，对丝状真菌和致病微生物具有广泛的致死作用，将ADR合成基因克隆到pET32a载体中并在*E. coli* BL21（DE3）中表达，其蛋白表达量高达全细胞总蛋白20%，其抗菌活性与之前报道的化学合成ADR相似[74]。Kim等[75]用pKSEC1和6HisSUMO-abaecin为载体，*E. coli* BL21（DE3）为宿主表达了从蜜蜂中分离出的抗菌肽Abaecin，展现出较好的抗菌能力且不影响宿主细胞的活力。Da Costa等[76]也利用*E. coli* BL21（DE3）菌株重组表达Hep-A200，对革兰氏阳性及阴性细菌均具备抗菌活性，且无细胞毒性。目前，利用大肠杆菌表达系统合成的

表1 常见的抗菌肽生物表达系统

Tab. 1 Expression system of antimicrobial peptides in this review

Expression system and engineered microorganism	Antimicrobial peptides	References
Prokaryotic		
E. coli	Cecropin	[21, 69-72]
	Perinerin	[73]
	Adenoregulin	[74]
	Abaecin	[75]
	Hep-A200	[76]
	MLH	[77]
	Hal18	[78]
	Plectasin	[79]
	Lactoferricin	[80]
	Buforin II	[81-82]
B. subtilis	Cecropin AD	[83]
	Cathelicidin-BF	[84]
	T9W	[85]
	CiMAM	[86]
Eukaryotic		
S. cerevisiae	CecropinXJ	[87]
	Cecropin P1	[88]
P. pastoris	Cecropin AD	[89]
	PaDef	[90]
	Melittin	[91]
	Fowlicidin-2	[92]
	HKABF	[93]
	Mytichitin-A	[94]
	ABP-CM4	[95]
C. reinhardtii	3×Mytichitin-A	[96]
	Bacteriocin LS2	[97]
	ToAMP4	[98]
	Mytichitin-CB	[99]

抗菌肽的数量最多，分布最广，且技术最成熟，还实现了LL37衍生肽MLH[77]、Hal18[78]、Plectasin[79]、Lactoferricin[80]、Buforin Ⅱ[81-82]等抗菌肽的生物合成。

此外，有些抗菌肽也常用枯草芽孢杆菌（*Bacillus subtilis*）表达系统合成[83]。与大肠杆菌相比，*B. subtilis*表达系统可将抗菌肽直接分泌到胞外，有利于收集、分离和纯化目标蛋白；但也存在分泌蛋白酶、产物易降解、产量低且外泌抗菌肽杀死宿主等缺陷。如抗菌肽Cathelicidin-BF[84]、T9W[85]、Cecropin AD[83]、CiMAM[86]都曾利用枯草芽孢杆菌进行高效表达，且抑菌效果显著。但该系统主要用来植物病害的治疗，在人类健康领域应用较少。

但所有的原核表达系统都也面临着一些挑战：①具备生物活性的大部分抗菌肽自身对原核宿主菌株存在广谱性的杀伤能力；②因绝大多数抗菌肽具有正净电荷，易被内源性蛋白酶降解，但通过融合蛋白策略可以适当克服此问题[103]。

2.2 利用真核表达系统生物合成抗菌肽

真核表达系统的代表是酵母菌表达系统，包括酿酒酵母（*S. cerevisiae*）、毕赤酵母（*P. pastoris*）、莱茵衣藻（*C. reinhardtii*）等，其表达载体有pPICZa、pPIC9K、pGAPZa等[104]。相对于原核表达系统，酵母菌表达系统具有抗菌肽产物毒性低、活性高、易分离等优点，还可促进抗菌肽细胞外表达和能够翻译后修饰（促进二硫键形成、*O*-糖基化和*N*-糖基化等）[89, 105]；但也存在细胞生长慢、产量低、部分抗菌肽（如蜂毒素）对宿主菌株有害等不足之处。

酿酒酵母（*S. cerevisiae*）是最常用的真核表达系统，可使用强启动子以获得抗菌肽的高效表达量。Xia等[87]将抗菌肽基因*cecropinXJ*克隆到pYES2/CT/α因子表达载体中，并在*S. cerevisiae* INVSc1菌株中成功表达重组蛋白CecropinXJ，且占总蛋白的79.45%。Jiang等[88]在*S. cerevisiae*中成功合成了具有抗病毒和抗菌双重性能的抗菌肽Cecropin P1。由于*S. cerevisiae*在生物合成过程中产生副产物乙醇，必定会影响菌群数量和抗菌肽的最终产量。同时，*S. cerevisiae*还存在过度糖基化、信号肽加工不完全等先天缺陷，这也限制了抗菌肽的规模化生产。

所以与*S. cerevisiae*相比，更多的抗菌肽是利用毕赤酵母（*P. pastoris*）进行重组表达。Meng等[90]成功利用*P. pastoris*重组表达分泌型的PaDef，在最佳条件（28 ℃、pH 6.0、1.5%甲醇）下培养72 h，PaDef的积累量高达79.6 μg/mL。Moridi等[91]采用*P. pastoris* GS115表达系统制备了蜂毒抗菌肽Melittin，具有广谱抗菌活性。以毕赤酵母X-33为表达系统，pPICZα-A为载体，Xing等[92]成功重组表达Fowlicidin-2，其表达纯度为85.6 mg/L，具有广谱抑菌性，但重组Fowlicidin-2具有溶血性。Wang等[93]利用*P. pastoris*重组表达HKABF肽，并对金黄色葡萄球菌和腐生葡萄球菌具有较低浓度的抑菌活性，且其热稳定性好，溶血活性低。为了大量生产Mytichitin-A，Meng等[94]利用6 His标签的Mytichitin-A插入pPICZαA中，通过电穿孔将质粒转化*P. pastoris* GS115，使其分泌重组的Mytichitin-A，其纯度为97.8%。Zhang等[95]采用pPICZaA为表达载体，利用*P. pastoris*重组表达的抗菌肽ABP-CM4能分泌至培养基中，且纯化后的ABP-CM4与天然提取的抗菌肽蛋白在功能上没有显著区别。Ahmad等[89]也成功在甲基营养型*P. pastoris*中表达出抗菌肽Cecropin AD。

莱茵衣藻（*C. reinhardtii*）是一种生长周期短、生长迅速且能自主光合作用的单细胞生物，具有“光合酵母”美誉。与*P. pastoris*和*S. cerevisiae*相比，*C. reinhardtii*表达异源蛋白更稳定。Dong等[96]利用*C. reinhardtii*表达抗菌肽3×Mytichitin-A，其产量占总可溶性蛋白的0.28%，且连续传代6个月后表达水平稳定。利用*C. reinhardtii*还成功合成Bacteriocin LS2[97]、ToAMP4[98]、Mytichitin-CB[99]等，这也意味着*C. reinhardtii*可作为新的合成生物学底盘细胞和微生物合成平台。

3 抗菌肽的分子设计与合成生物学改造策略

利用微生物表达系统生产天然抗菌肽，有时

会出现产率过低、宿主毒性过大、抗性活性不足等缺陷，有的抗菌肽溶血性显著，难以推广。为了解决上述问题，研究人员尝试进行截取有效序列、氨基酸残基替换、改造结构参数等基因工程技术对天然抗菌肽基因序列进行1.0版本改造。将天然抗菌肽Chensinin-1蛋白序列中的Gly替换成Trp[106]，或增加原肽中Trp的占比[107-108]，增强了革兰氏阴性菌的抗性。重新设计抗菌肽LGR16的亲疏水区域，可调节其溶血活性和抗性[109]。

随着合成生物学的发展及人工智能（artificial intelligence，AI）的兴起，利用计算机对天然抗菌肽序列的数据进行分析和学习，可建立预测算法并利用该算法找出相关特征和规律，有望推广至未知抗菌肽的预测及精准设计[110]，实现抗菌肽改造的2.0版本（图3）。目前，已经有团队基于一个基于支持向量机（support vector machine，SVM）平台来研究α-螺旋抗菌肽的功能共性和序列同源性，依托该平台可根据抗菌肽的理化性质设计出具有α-螺旋结构的新型抗菌肽[111]。基于上述平台，对286个α-螺旋抗菌肽和286个非抗菌肽数据进行训练，以识别出决定抗菌肽活性的关键特性，并以此为依据进行筛选，其精度高达91.9%，特异性和敏感性也分别达到93.0%和90.7%。目前通过该模型已发现了数种通过自然进化或点突变难以产生的α-螺旋结构抗菌肽[111]。2021年，Das等[112]使用基于变分推断的自编码器（variational autoencoder）学习所有通用蛋白质资源（universal protein resource）中报道的约170万条多肽，并将抗菌肽单独取出分为抗菌能力强弱两类，通过建立多肽抗菌能力的“分类器”，经“假设-拒绝采样”，最终获得新的高活性抗菌肽。

CRISPR/Cas9基因编辑系统是一种在合成生物学范畴的序列特异精准突变方法，也是最新的基因编辑技术。CRISPR/Cas9介导的基因沉默、基因敲除或特定DNA序列的操作也能增多异源抗菌肽的产生，使其正确/更快地折叠或能够对其进行适当的翻译后修饰[102]。Park等[113]利用CRISPR/Cas系统，将Cas9核酸酶和特异的RNA导入家蚕卵巢的BM-N细胞，使其内源性基因功能减弱，并对起抗感染的Toll通路进行负调控，成功编辑家蚕Cactus基因，激活家蚕免疫应答信号通路，分泌Moricin和Lebocin等抗菌肽（图4）。目前，利用CRISPR/Cas9等合成生物学领域的基因编辑技术指导抗菌肽合成和杀菌的研究较少，但未来潜力巨大。

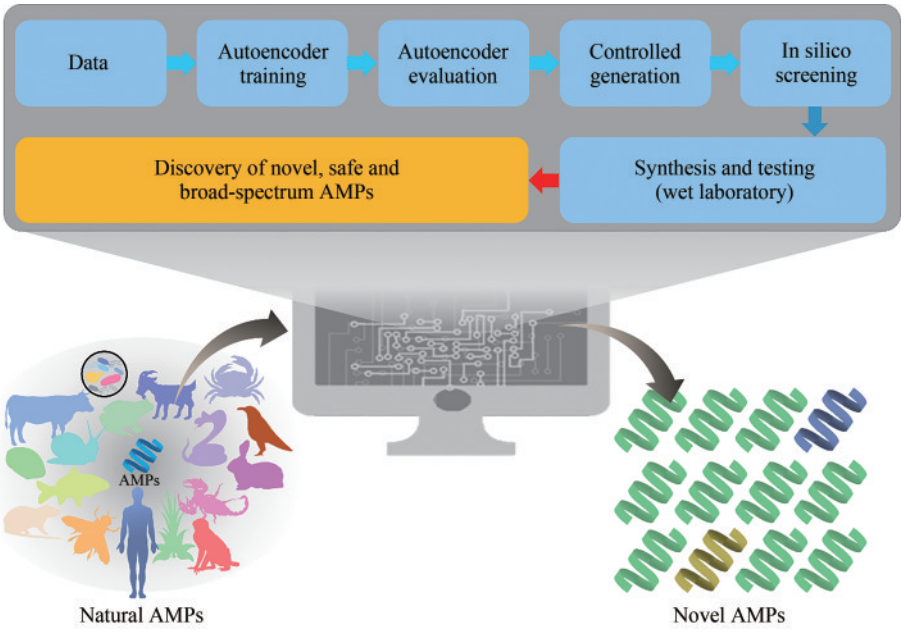


图3 计算机辅助及人工智能加速新型抗菌的设计

Fig. 3 Overview of the proposed computer-assisted and AI-driven approach for accelerated novel antimicrobial peptides (AMPs) design

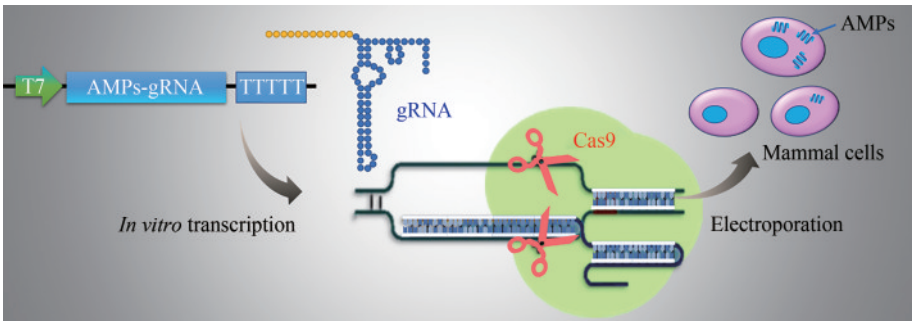


图4 CRISPR/Cas9基因编辑系统促进细胞分泌抗菌肽

Fig. 4 Overview of the CRISPR/Cas9 gene editing system promoting the secretion of antimicrobial peptides in mammal cells

4 抗菌肽的医学应用

抗菌肽主要对有害病原体的细胞膜进行非特异性的快速杀伤和细胞内干扰，并不针对特定的分子途径，这大大降低细菌对抗菌肽产生耐药性的概率[35]。因此，将抗菌肽应用于抗菌药物的临床研究具有重要意义。绝大部分情况下，抗菌肽自身常作为抗菌消炎类药物的原药（非载体），可通过涂抹或注射等方式进行最终目的为人体治疗的研究测试。目前，Daptomycin、Vancomycin、Dalbavancin（BI397，Dalvance，Xydalba）和Colistin 4种抗菌肽已被美国食品及药物管理局（FDA）批准采用静脉注射的形式用于抗菌治疗（表2）[6, 114-116]，另外多种抗菌肽正在临床开发中。除了最初的抗菌效应，越来越多的抗菌肽展现出多重作用，包括抗癌、免疫调节、抗病毒及抗寄生虫等，也开始与医用生物材料相结合，服务于医学组织工程、药物递送与医疗美容，这些尝试为抗菌肽的应用开辟了新的应用领域（图5）。

4.1 抗菌消炎类药品

绝大部分抗菌肽都曾作为潜在的抗菌消炎类药品进行研究开发。细菌素（Bacteriocin）是从细菌中分离出来的一类由30～60个氨基酸残基形成的阳离子抗菌肽[117]。Nisin是该家族的一员，对多种革兰氏阳性菌甚至革兰氏阴性菌都有很高的抗菌活性[118-119]，还可联合精油肉桂醛和乙二胺四乙酸（EDTA）控制产肠毒素大肠杆菌的生长[120-121]。也有报道称，当青霉素或氯霉素联合Nisin使用时能提高粪肠杆菌的抑菌效果，但单药单独效果欠佳[122]。同样，从蛙皮中提取的Esculentin-1a抗假单胞菌肽具有杀死假单胞菌细胞的活性，且不诱导耐药性[123]。因此，抗菌肽也可作为治疗耐药细菌的新选择，无论是单独或协同应用。

除了具备抗菌能力，部分抗菌肽还可直接或间接参与宿主的免疫调节，保护宿主免受感染[124-125]。防御素是另一大类抗菌肽家族，具有多个成员，广泛存在于中性粒细胞[126]、巨噬细胞[127]、淋巴细胞[128-129]、NK细胞[130]等免疫细胞中，且具有强大的促炎功能（pro-inflammatory function）[131]。其中，人源防御素HBD2、HBD3和HBD4皆可刺激人角质形成细胞中IL-6、IL-10等免疫因子的表达；还发现这些防御素能诱导EGFR、STAT 1和磷酸化STAT3等参与角质形成细胞迁移和增殖的细胞内信号分子，促进角质细胞迁移和增殖参与伤口愈合[132-133]。

表2 美国食品及药物管理局（FDA）批准的抗菌肽的名称、治疗的适应证、给药途径及临床试验标识号

Tab. 2 Name, indication, deliever and clinical trial identification of antimicrobial peptides approved by US Food and Drug Administration (FDA)[6, 114-116]

AMPs	Indication	Deliever	Status	Clinical trial identifiers
Daptomycin	Bacterial skin infections	Intravenous	Approved	NCT01211470
Vancomycin	Staphylococcal infections	Intravenous	Approved	NCT00175370
Dalbavancin (BI397, Dalvance, Xydalba)	Acute bacterial skin infections	Intravenous	Approved	NCT03233438
Colistin	Multidrug-resistant Gram-negative infections	Intravenous	Approved	NCT03397914

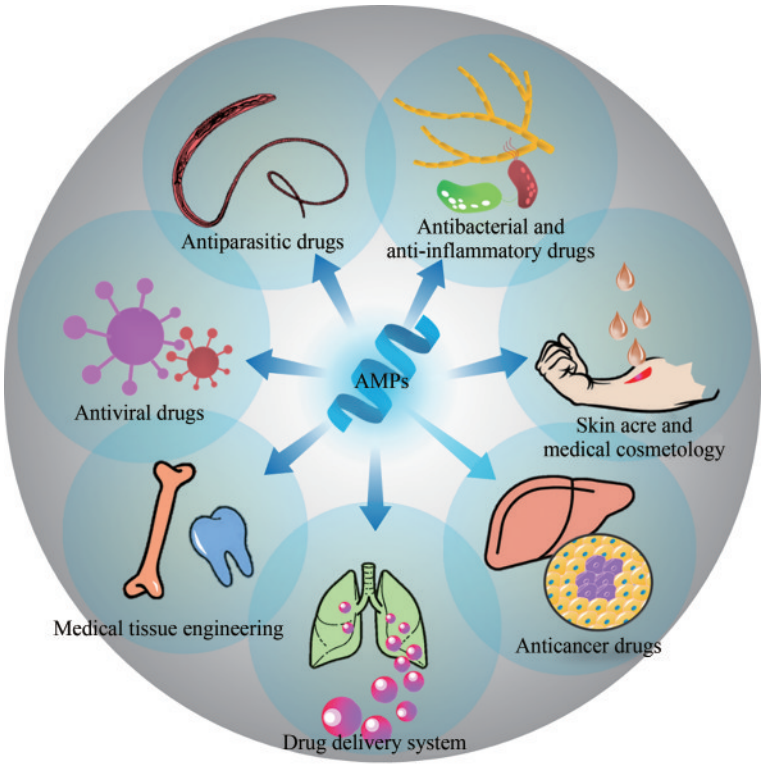


图5 抗菌肽的医学应用

Fig. 5 Medical applications of antimicrobial peptides (AMPs)

4.2 抗病毒药品

除了抗菌消炎和免疫调控，有的抗菌肽对病毒还具有广谱抗性[51]，LL-37最为典型。Barlow等[134]评估了LL-37可降低流感病毒感染模型小鼠的疾病程度和病毒复制能力，其治疗效果与特异性抗病毒药物扎那米韦（Zanamivir）相似；Tripathi等[135]发现LL-37可直接对流感病毒的膜结构进行破坏而实现病毒抑制。LL-37还可抑制其他具有膜结构的病毒，如人类免疫缺陷病毒（HIV）[52]、牛痘病毒[53]、单纯性疱疹病毒[51]和登革病毒[51]等。此外，Todorov等[136]分离得到一种3950 Da大小的新型广谱抗菌肽，灭活诸多有害菌的同时对单纯疱疹病毒、脊髓灰质炎病毒和麻疹病毒均有抗性。牛抗菌肽-13（APB-13）对猪传染性肠胃炎病毒的抑制率高达74.1%[137]；经透射电镜确认，Temporin B能直接破坏1型单纯疱疹病毒的膜，抑制其在宿主细胞内大量扩增[138]。最近的研究表明，抗菌肽Nisin也可以与新冠病毒SARS-CoV-2的刺突蛋白靶向的细胞受体相互作用[139]，从而开启了使用AMPs对抗新型大流行冠状病毒的前景[140-141]。

4.3 抗寄生虫药品

与上述临床研究相比，关于抗菌肽的抗寄生虫类报道较为少见，多集中于抗疟原虫、抗利什曼原虫，抗锥虫的研究。Halictine-2[55]、Dragomide E[56]、Temporin-Sha[57]、BmajPLA2-Ⅱ[59]等不同来源的抗菌肽都具备较强的抗利什曼原虫能力。此外，大黄鱼来源的Lc-P5L4能使刺激海水鱼类寄生虫隐核虫细胞膜破裂[58]；蛇抗菌肽的衍生的肽LZ1可通过特异性抑制疟原虫感染红细胞中三磷酸腺苷（adenosine triphosphate，ATP）的产生，实现对血期恶性疟原虫的强烈抑制[56]。

4.4 抗癌药品

最近研究发现，部分抗菌肽对某些癌细胞也具有抗性，可作为潜在的新型抗癌药物进行研发[142]。与正常细胞相比，癌细胞膜表面的阴离子组成是抗菌肽进攻的特异性靶点；抗菌肽通过静电作用更易与癌细胞表面解除并选择性地杀死癌细胞，但大部分抗菌肽更偏爱调节癌细胞的代谢通路

或细胞因子实现抗癌。KillerFLIP可以通过静电和疏水驱动力的微调平衡来选择性杀死癌细胞[60]；鳄梨来源的防御素PaDef对慢性髓系白血病K562细胞株具有细胞毒性（IC_{50}=97.3 μg/mL）；[61]；LL-37的类似物FF/CAP18定位于结肠癌细胞的细胞质，增强了生长抑制miRNAs的表达，抑制癌细胞增殖[62]；调节抗菌肽Myristoly-CM4的浓度可直接干扰白血病细胞（K562、MDR、Jurkat）的凋亡或坏死行为，进行特异性毒杀[63]，且对乳腺癌细胞系（MCF-7、MDA-MB-231、MX-1）具有抑制作用[143]；KT2和RT2可以通过诱导细胞的周期停滞以及凋亡等方式对人结肠癌HCT-116细胞进行特异性抑制，减少癌细胞的转移[144]；蛋氨酸脑啡肽（methionine enkephalin）能抑制B16黑色素瘤细胞的生长并且诱导其凋亡[145]。这些研究都为研究抗癌药物的研发提供了思路。

4.5 医学组织工程

随着组织工程学和医用生物材料的不断发展[146-152]，组织工程支架的抗菌性研究和新型抗菌材料已经是未来重点发展的方向之一。由于抗菌肽不易产生耐药性，具有对于细菌、真菌、寄生虫、病毒等多种病原体的广谱抗性。与传统抗生素和纳米金属类新型抗菌剂对比，抗菌肽毋庸置疑是下一代抗菌剂的主力军。为了避免免疫原性等因素，应用于医学组织工程及再生医学领域的抗菌肽多为人类来源的LL-37和人防御素（human defense）系列，且多为化学固定或物理混合的形式与各种医疗器械、体内植入物、药物制剂载体、医疗耗材、外伤敷料相结合。目前，与抗菌肽结合的组织工程支架已经应用于骨[153-155]、牙[34]、皮肤[156]、眼角膜[157]等诸多器官和组织的修复。

炎症性骨丢失的修复和再生仍然是一个骨科临床挑战。LL-37除了抗菌消炎，在细胞迁移、细胞因子产生、细胞凋亡和血管生成中发挥重要作用[158]。通过对间充质干细胞和LPS诱导的小鼠颅骨溶解性骨缺损模型进行体外和体内的研究，发现LL-37在体外可有效抑制LPS诱导的破骨细胞形成和有害细菌的活性[154]。同样，通过聚多巴胺涂层可实现三维PLGA多孔支架的骨形态发生蛋白2（bone morphogenetic protein-2，BMP-2）和Ponericin G1功能化修饰，该支架能有效调节前成骨细胞的成骨分化，同时抑制致病微生物，从而提高生物活性[153]。除了PLGA，也可将抗菌肽LF1-11修饰于钛合金表面植入物，在大力提高间充质干细胞的黏附能力（因添加RGD序列）的同时，还对金黄色葡萄球菌具有有效的抗菌作用[155]。为解决在临床假牙或钛合金骨支架常存在的细菌感染及成骨效率低双重困局，Liu等[34]开发了一种双功能钛合金纳米羟基磷灰石涂层，能够持续释放重组的HBD-3和BMP-2。该涂层能同步释放两种活性物质，并能在抑制细菌生长的同时促进骨组织再生，在骨或牙组织工程中具有很大的临床潜力。Lin等[156]将抗菌肽Tet213固定于海藻酸、透明质酸和胶原蛋白的创面敷料中，该创面敷料对大肠杆菌、MRSA和金黄色葡萄球菌3种病原菌都具有抗菌活性，并能促进NIH3T3成纤维细胞的增殖；此外，与市售银基敷料相比，该创面敷料的伤口愈合效果更佳。采用基于磁力的组织工程技术，可制备出能过表达HBD-3基因的多层角化细胞抗菌薄片，为感染性疾病提供一种新的基因治疗策略[157]。

4.6 药物递送系统

由于大部分抗菌肽的本质是蛋白质或多肽，在用药途中（如口服）极易受蛋白酶降解而失效，且有些抗菌肽具备非特异性的细胞毒性，能同时抑制甚至杀死肿瘤细胞和正常细胞，这一特性也限制了该类种抗菌肽的临床推广[159-160]。可参考药物递送系统[161-163]，利用纳米尺寸的载体可负载浓缩抗菌肽，以降低运输过程中药物的释放并在一定程度避免蛋白酶降解，提升抗菌肽对癌细胞抗性及靶向性[164]，也能提升部分抗菌肽对细菌[165]或真菌[166]的抗性。目前，与抗菌肽结合的纳米载体包括可降解的生物高分子、脂质体，及不可降解的磁性纳米颗粒等[165，167-169]。

与游离的LL-37抗菌肽治疗相比，磁性纳米颗粒连接的LL-37能导致癌细胞活力下降和凋亡率上升，这表明磁性纳米颗粒化的LL-37是潜在的结肠癌治疗方案之一[166]。该系统还可用于抗真菌治

疗，且不干扰成骨细胞的增殖和活力，具有较高的生物相容性[170]。Falciani等[168]报道了一种基于非天然抗菌肽SET-M33肽与单链葡聚糖纳米颗粒的新型纳米复合体（M33-NS），改复合体能抵抗生物液体中的降解，对临床分离出的耐药性革兰氏阴性病菌存在治疗效果，且在肺炎、败血症和皮肤感染的临床前感染模型中也有生物效应。Lam等[165]展示了一类被称为“结构纳米工程抗菌肽聚合物”的复合抗菌剂，对所有耐药性革兰氏阴性细菌都表现出较好的杀伤性，同时显示出低的细胞毒性。纳米尺度的高分子微凝胶（microgel）与多肽的相互作用以静电为主。通过调节电荷密度，微凝胶能结合或释放抗菌肽KYE28，杀死大肠杆菌并对人单核细胞起到抗炎作用[169]。

虽然利用基于纳米载体的抗菌肽传递系统能有效解决部分抗菌肽的毒性和靶向性，但引入的纳米尺寸的载体自身的生物相容性和纳米粒子沉积等问题也不可忽视。有研究发现，当LL-37肽暴露在碳纳米颗粒中，其抗菌功能严重损失；纳米颗粒可以改变宿主防御肽结构和功能[171]，这也表明纳米颗粒的引入可能干扰抗菌肽的生物活性，增加疾病的感染性。

4.7 皮肤护理与医疗美容

除了抗菌消炎，一些抗菌肽能诱导内皮细胞生长和促血管生成因子的分泌，削弱因衰老而造成的血管萎缩，促进皮肤损伤后创面愈合，具有潜在的医疗美容和皮肤护理功效[172-180]。人瘢痕疙瘩组织中，LL-37也被发现具有缓解皮肤胶原蛋白生成，具备抗纤维化的特性[174]。LL-37还可通过LPS和巨噬细胞的活化诱导内皮细胞增殖、迁移和形成小管样结构，增加了血管化和再上皮化[175]。角质形成细胞释放银屑病素可抑制成纤维细胞胶原、纤维连接蛋白-1、平滑肌肌动蛋白和转化生长因子的表达[176]；菌肽AG-30能以诱导内皮细胞生长和分泌促血管生成因子的方式，削弱人因为衰老而造成的血管减少的结果，使皮肤获得足够的营养[177-178]；DRGN-1除了抗菌作用外，还可以通过清理感染菌的生物膜和促进角质形成细胞迁移和增殖用于加速伤口闭合，形成无疤痕的有效组织再生[179]；CopA3可以抑制紫外线辐射引起的人纤维细胞分泌Ⅰ型前胶原的能力，减少皮肤的老化[180]，因此，抗菌肽在黑色素瘤、痤疮、糖尿病足溃疡和银屑病等多种皮肤疾病治疗中起到作用，也可作为新型外用皮肤护理型化妆品的添加剂。

5 抗菌肽的缺陷与医学应用瓶颈

虽然抗菌肽具有广谱抗菌、低耐药性、抗癌、调节免疫等诸多优点，但也存缺陷，其主要为抗菌肽的合成成本较高、体内的稳定型和有效性不足及部分抗菌肽毒副作用大。这限制了抗菌肽在医学应用的发展，也增大了其临床验证和审批程序的难度[181-182]。

与成熟的抗生素合成工业相比，目前抗菌肽的生产基本处于实验室阶段，还没有工业化概念和成熟的产业体系，因此其合成成本普遍较高，是常规抗生素的5～20倍[182]。

与传统抗生素或金属纳米抗菌剂相比，抗菌肽因其特殊的多肽或蛋白质结构更易与人体的蛋白酶亲和而降解，同时在极端pH或高温的环境下也更易失活。因此大部分抗菌肽的稳定性不佳，体内有效性难以维持，这也导致部分抗菌肽的药效及纳米药物递送应用的结果可能低于预期[183-184]。

除了作用于细菌、真菌等微生物的细胞膜，抗菌肽中的正电荷也可与人体或哺乳动物细胞膜表面的负离子相互作用，形成寡聚体破坏细胞结构[185]，导致不同程度的溶血性、干扰细胞信号肽，引起细胞凋亡、肥大细胞脱粒或细胞外DNA转移等不良临床现象[186-188]。目前，只有少数具有药用价值的抗菌肽进行了系统的溶血性、免疫原性和毒性等生物安全性评估。Pini等[189]报道M6是一种体内使用的低毒抗菌肽，其小鼠的腹腔注射半数致死量（LD_{50}）为125 mg/kg，其静脉注射LD_{50}为37.5 mg/kg。JH-3是一种新型抗菌肽，经小鼠腹腔注射的LD_{50}为180 mg/kg[190]。Li等[191]通过腹腔和尾静脉注射不同剂量的Hp1404对小鼠的急性毒性测定。结果表明，Hp1404对BALB/c小鼠的LD_{50}为89.8 mg/kg（静脉注射），表现出低毒性。TP1是一种典型的阳离子β-发夹型抗菌肽，具有膜溶作用机制，但由于其保守的β-发夹二级结构基

序，TP1在小鼠和人血浆中具有非常高的稳定性[192]。

除了上述3种普遍存在的缺陷，部分抗菌肽对病原体的亲和能力也可能反向促进感染。志贺氏菌以人肠道防御素HD5为靶点，但HD5能促进志贺氏菌的吞噬和繁殖并导致巨噬细胞死亡，最终释放大量的细菌感染肠道细胞[193-194]。

6 小结与展望

自青霉素问世以来，抗生素已经在人类医疗系统中频繁使用挽救了无数生命；但随之产生了多种人体耐药菌增加了相关治疗的难度，给人类健康和生态环境带来严重挑战，这成为21世纪公共卫生威胁之一。抗菌肽因具有独特的抗菌性质，广谱抗击微生物（含耐药菌）、病毒、寄生虫等各种病原体，且不易产生耐药性病原微生物等诸多优点，可能成为未来抗生素的替代品；同时，强大的免疫调节和抗癌特性也增大抗菌肽的医药应用前景。但成本高、稳定性差、生物毒性等缺点也限制了抗菌肽的临床医学发展。随着合成生物学和人工智能等新型交叉学科的快速发展，可能给抗菌肽的设计、合成提供新的技术平台，也在不同层面和角度推动抗菌肽的转化和应用。其中主要的可行的发展策略包括：

（1）利用计算机对天然抗菌肽结构进行分析，了解抗菌肽序列中氨基酸残基的作用与功能，预测抗菌肽的抗菌活性、空间构象、突变体等性能，实现抗菌肽的高通量筛选，设计出兼具广谱抗性和结构稳定的新型抗菌肽，满足更多市场需求，提高抗菌肽的利用率。

（2）基于合成生物学技术，利用CRISPR/Cas9等新型的基因编辑技术对天然抗菌肽基因进行定向编辑，例如增加、替换、删除某些氨基酸残基，减少抗菌肽对生产菌株及人体细胞的毒性，增加抗菌肽的抗菌性能。

（3）筛选优化新型生物合成菌株系统（或底盘细胞），传统的原核宿主细胞（如大肠杆菌）虽均有生长速度快、培养成本低、生物背景清晰的优点被广泛应用，但其生产的抗菌肽一般还需与融合蛋白或包涵体等分离，纯化程序复杂。其他新型的表达系统及生物合成菌株或许能调控合成菌株的氨基酸代谢流，增强抗菌肽的体内积累。

（4）利用微生物形态工程技术优化宿主细胞的形态或者分裂方式基因，使菌株由短杆状转变为长杆状、椭圆状等形态。此外，通过改变菌株的分裂方式，破坏细菌传统的二分裂方式，使一个细菌细胞可同时分裂成多个细胞，有利于菌体内产物的积累，提高生物合成效率，降低生物合成成本。

（5）完善抗菌肽生物合成的工业化体系，开发快速回收高纯度抗菌肽的工艺。细菌作为宿主细胞生产抗菌肽时，融合蛋白被用于掩盖抗菌肽，降低其对宿主细胞的杀伤，可利用融合蛋白组分等电点或溶解度差异回收抗菌肽，有利于降低成本，促进抗菌肽的大规模生产。

（6）与现有抗生素联合用药，增强药效，避免耐药性。除了作为单一抗菌药物，抗菌肽还能与青霉素、氯霉素、四环素、环丙沙星、头孢菌素等常规抗生素联合使用，通过多种杀菌方式联合破坏细菌细胞膜的稳定性，引起细胞膜破裂或者溶解，使细菌死亡，发挥更高效的抗菌作用，有利于降低抗生素或抗菌肽的毒副作用，并预防细菌耐药性。

（7）与生物材料结合，提高抗菌肽稳定性，降低生物毒性。开发新型抗菌类植入物及手术器材，这类抗菌植入物或手术器材具有双重杀菌性能，增强治疗效果，同时生物材料还能将抗菌肽递送到作用部位，并缓慢释放抗菌肽，避免抗菌肽大量降解，确保抗菌肽的有效抗菌性能，延缓给药时间，降低药物对体内其他组织器官的损伤。

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第一作者及通讯作者：魏岱旭（1986—），男，博士，教授，博士生导师。研究方向为生物材料、合成生物学、组织工程与再生医学、医用微纳米器件和智能递药系统、医学美容及化妆品、生物力学及空间生物学。

E-mail：weidaixu@nwu.edu.cn；
daviddxwei@163.com