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Review 综 述

基因工程菌的构建、性能研究及其在疾病诊断和治疗中的应用

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摘　要：随着肠道微生物组与宿主关系研究的深入和基因工程的迅速发展，基因工程菌(genetically engineered bacteria, GEB)在医学领域的应用成为研究热点。GEB 是指经基因工程改造而具有高效表达外源蛋白质或分子化合物能力的细菌，相比传统药物具有诸多优势。GEB 的构建过程包括底盘的选择、功能基因的获取、基因转移和重组这几个基本步骤，包裹技术能够提高其存活率和定殖能力，合成基因回路的应用可使其智能化。功能稳定性、有效性和安全性是评价 GEB 的一般指标，也是性能优化过程中需要重点关注的方面。GEB 在炎症性疾病、肿瘤、代谢性疾病、感染性疾病和神经系统疾病等疾病中已有广泛应用，但实现临床转化还有很多问题亟待解决。本文介绍了 GEB 的构建和性能研究方法，总结了近年来其在疾病诊断和治疗中的应用，指出了现存问题并提出了展望。

关键词：基因工程菌；合成生物学；临床应用；活体生物药；益生菌

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Genetically engineered bacteria: construction, performance evaluation, and applications in disease diagnosis and treatment

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Abstract: With the in-depth study of the relationship between intestinal microbiome and host and the rapid development of genetic engineering, the application of genetically engineered bacteria (GEB) in the medical field has become a research hotspot. GEB refer to the bacteria that have been genetically engineered to efficiently express exogenous proteins or compounds to achieve specific goals. Compared with traditional drugs, GEB have a variety of advantages. The construction process of GEB includes the selection of chassis, the acquisition of functional genes, and gene transfer and recombination. At present, the bacteria serving as GEB chassis can be classified into two categories: generally regarded as safe (GRAS) strains and commensal strains. The application of multi-omics facilitates the selection of chassis. Functional genes can be obtained by PCR, DNA synthesis, CRISPR-Cas9 or Red/ET recombination system according to their sizes. Heat shock and electroporation are widely used for plasmid transfer in bacteria. Homologous recombination can directly integrate the target gene into the host's chromosome. The encapsulation technology can improve GEB's survival rate and colonization ability, and synthetic gene circuits can make GEB intelligent. Functional stability, effectiveness, and safety are the general indicators for evaluating GEB. The instability of plasmid is an inherent defect of the GEB constructed by plasmid-mediated gene transfer, while the GEB constructed by gene integration have strong stability. In addition, to achieve long-term stable expression of functional genes, researchers need to evaluate and alleviate the impact of load. The efficacy and safety of GEB need to be evaluated *in vitro*, in animal models and clinical trials. There are some methods that have successfully achieved optimization of the above indicators. GEB have been widely used in the diagnosis and treatment of inflammatory diseases, tumors, metabolic diseases, infectious diseases, neurological diseases, and other diseases, playing a unique role. However, there are still problems regarding construction methods, performance evaluation and optimization, and large-scale production, which limit the clinical application. In this paper, we introduced the methods for construction and performance evaluation of GEB, summarized the application in disease diagnosis and treatment in recent years, pointed out the existing problems, and prospected the development of this field in the future.

Keywords: genetically engineered bacteria; synthetic biology; clinical application; live biotherapeutic products; probiotics

近年来，得益于人体肠道微生物组成和功能相关研究的深入，细菌培养方法、二代及三代测序技术、基因编辑和修饰工具等的革新，特别是合成生物学的迅速发展，基因工程菌(genetically engineered bacteria, GEB)的研究和应用蓬勃兴起。GEB 是指经基因工程改造可高效表达外源蛋白质或分子化合物以实现特定目标的细菌[1]，在生物医学、能源化工、环境保护等诸多领域应用广泛。GEB 具有诸多优势，例如：其可在定殖部位长期发挥作用，无需频繁给药，从而提高患者依从性并减少医疗支出；GEB 治疗的不良反应较少，尤其是口服给药时；对于结构不稳定或环境敏感的化合物，使用 GEB 生产无需药物纯化和低温保存；GEB 可在 1 个菌株中生产多个外源蛋白质或化合物以实现协同治疗，无需多药联用[1]。鉴于这些优势，GEB 在炎症性疾病、肿瘤、代谢性疾病、感染性疾病和神经系统疾病等疾病中已有相当广泛的应用。但构建方法、性能评价与优化、规模化生产等方面仍存在不少问题，限制了 GEB 的临床转化。本文对 GEB 的构建和性能研究方法进行了介绍，总结了近年来其在疾病诊断和治疗中的代表性应用，对现存问题进行了梳理并就此提出了展望。

1 基因工程菌的构建

1.1 底盘的选择

用于基因工程改造的细菌底盘的选择是构建 GEB 的首要任务。符合以下条件之一的细菌可被考虑作为底盘：在患有与微生物组改变相关疾病的研究对象体内减少的细菌；在特定疾病的粪菌移植(fecal microbiota transplantation, FMT)治疗中取得成功的细菌；已知对微生物组组成或功能具有调节作用的细菌；已知对疾病相关宿主通路或表型有影响的细菌；从菌株库中筛选出的具有预期体外或体内活性的细菌[2]。

多组学技术即基因组学、转录组学、蛋白质组学、代谢组学及功能基因组学等的联合应用可为底盘的选择助力。基因组学可识别含有促进定殖基因的细菌底盘，还可发现底盘与肠道菌群的潜在相互作用；转录组学可发现增强候选底盘竞争优势的生理过程；蛋白质组学可高通量分析能够提高底盘对肠道生理环境适应能力的蛋白质；代谢组学和功能基因组学可揭示候选底盘耐受肠道环境的机制[3]。

目前被用作 GEB 底盘的细菌可分为 2 类——公认安全的(generally regarded as safe, GRAS)菌株(如乳酸乳球菌)和共生菌株(如卵形拟杆菌)[2]。最常用的底盘为大肠杆菌、乳杆菌属和沙门菌属，其他常用底盘有双歧杆菌属、枯草芽胞杆菌、产单核细胞李斯特菌、铜绿假单胞菌、脑膜炎奈瑟菌和霍乱弧菌等[1]。近年来，拟杆菌属、梭状芽胞杆菌属、栖粪杆菌属和阿克曼氏菌属等的一些菌株受到了下一代益生菌(next-generation probiotics, NGPs)研究者的青睐，有望成为构建 GEB 的新底盘，在结肠炎、艰难梭菌感染和代谢紊乱等疾病的治疗中发挥独特的作用[2,4]。

1.2 功能基因的获取

目的基因的大小决定其获取方法。小基因片段(<10 kb)可通过通用或长距离 PCR 法、直接 DNA 合成等方法获取，并进行限制性酶切[1]。Fahnøe 和 Bukh 使用改进长距离 PCR 法成功扩增了丙肝病毒开放阅读框的全长序列，该方法对于 1a 基因型具有高灵敏度和稳定性[5]。大基因片段(>50 kb)的最佳获取方法是 CRISPR-Cas9、Red/ET 重组系统等重组方法[1]。Jiang 等使用 RNA 引导的 Cas9 核酸酶在 2 个指定位点将目的基因组片段从细菌染色体上切割下来，然后通过 Gibson 组装连接到克隆载体上。该技术仅需一步即可实现近乎任意序列、长达 100 kb 的细菌基因组片段的定向克隆[6]。

1.3 基因转移和重组

根据构建方式，GEB 可分为质粒过表达型和基因整合型。质粒过表达型是将功能基因插入质粒载体，再将质粒转移至细菌底盘中；基因整合型是将功能基因直接插入细菌的染色体。

热激发和电穿孔转化被广泛用于大肠杆菌、沙门菌属、苏云金芽胞杆菌和铜绿假单胞菌等细菌中质粒的转移。接合转移和原生质体融合常用于将质粒从供体菌转移至受体菌[7]。

同源重组技术可以将目的基因直接整合到宿主染色体上，主要方法有同源重组、位点特异性重组、转座重组和 CRISPR-Cas9 技术等。转化相关重组、细菌人工染色体、噬菌体重组系统或整合酶介导的重组系统用于实现大基因簇的异源表达[1]。

1.4 包裹技术的应用

GEB 在胃肠道中面临严峻的挑战，如胃酸、酶、胆盐、胃肠蠕动、黏液更新和营养与空间竞争等[3]。包裹技术被广泛使用以提高细菌对胃肠道严酷环境的耐受能力，从而提高其存活率和定殖能力。常用的包裹技术有注射-凝胶化法、复合凝聚法、乳液模板法、静电纺丝技术和凝聚乳化联合法等[8]。例如，用掺杂氧化镁纳米颗粒的注射-凝胶化微凝胶包裹戊糖片球菌 Li05 可抑制氧离子和氢离子进入益生菌，还可中和胃液中的氢离子以减少酸诱导的益生菌降解，从而增强益生菌活性[9]。

1.5 合成生物学的应用

GEB 大多组成性或在简单诱导系统下表达单一蛋白质，而近年来复杂合成基因回路的应用实现了基因表达的精准调控[10]。基因回路的结构一般分为 3 个基本模块，即输入模块、操作模块和输出模块。输入模块用于检测生物或非生物信号并将其转化为分子信号；操作模块计算来自输入模块的信号并决定适宜的细胞行为，布尔逻辑门用于处理单个或多个输入信号以产生所需基因表达效应；最后，输出模块将计算后的信号转化为预期细胞反应[11]。

合成生物学的发展推动了元件设计、构建和测试的快速自动化以及大型 DNA 回路构建和变异方法的革新。这些工具使长达数百 kb 的合成 DNA 元件的编辑成为可能，从而使具有复杂生物合成通路的细菌药物的制备得以实现。快速突变测试、定向蛋白质进化及测试、递送计算机所设计肽的潜力使人们不再局限于天然治疗因子的设计，转向全新治疗功能的探索。合成生物学也为使用光等新输入信号控制回路创造了机会[10]。

2 基因工程菌的性能研究

2.1 功能稳定性

在充满压力和竞争的环境如肠道中，选择压力和突变率可能处于最高水平。随着给药时间和定殖时间的延长，GEB 功能丧失的风险逐渐增加[10]。对质粒过表达型 GEB 而言，质粒的不稳定性是其固有缺陷，但也有一些实现质粒长期保留的案例[12-13]。相比之下，基因整合型 GEB 的稳定性较强。此外，功能基因的引入难免会增加细菌的负荷，为实现功能基因的长期稳定表达，必须评估和减轻负荷的影响。目前已有一些预测和评估负荷水平的方法[14-15]，调节总表达量和相对表达量可以减轻负荷的影响[16-18]。Riglar 等利用具有长期定殖能力的菌株 *Escherichia coli* NGF-1，将合成基因回路整合至细菌染色体，减轻了负荷并实现了回路的长期保留，体内外功能验证和全基因组测序的结果证实了该 GEB 的功能稳定性[19]。

2.2 有效性和安全性

GEB 的有效性和安全性需在体外、动物模型和临床试验 3 个层面进行评价。基因组测序可

用于筛选可传播的抗生素抗性基因和可能存在的毒素等毒力因子,酶动力学实验和细胞模型可用于确认与预期效果相关的表型[2]。需要指出的是，体外测试环境应最大程度模拟体内生理环境。已有一些较好的体外测试系统，如人体肠道微生物生态系统模拟器[18]、芯片上的器官微流体[20]、体外器官生长技术[21]，但其使用不够方便。在动物模型中，除验证 GEB 的定殖能力和诊断或治疗特定疾病的有效性外,还要关注安全性和毒性相关指标。临床试验分为 3 个阶段：第一阶段试验用于确认安全性和确定剂量范围;第二阶段试验将在较小群体中围绕主要研究终点进行；第三阶段试验将在较大群体中进行，用于检测疗效、副作用和相对获益[2]。

目前有许多提高 GEB 安全性的方法，主要包括毒力基因的敲除或突变、细菌数量的调节以及治疗结束后 GEB 的清除[1]。自杀开关、基因防火墙是防止 GEB 在靶部位外生长的有效方法,但它们也存在较强选择压力导致编码死亡基因的回路突变的问题。合成营养缺陷型是迄今为止防止 GEB 从限制措施中逃脱的唯一成功方法，但其使用依赖足量非天然底物的服用，临床应用的方便性有待提高[10]。

3 基因工程菌在疾病诊断和治疗中的应用

GEB 的临床前研究和临床试验已有很多，本文述评了部分具有代表性的研究(表 1)。

3.1 炎症性疾病

炎症性肠病(inflammatory bowel disease, IBD)是胃肠道最常见的慢性炎症性疾病[22-23],近几十年来中国 IBD 的发病率呈上升趋势[24]。传统诊断方法如结肠镜检查是有创的，会给患者带来身体和心理上的痛苦，不利于疾病的长期监测[25]。口服具有疾病标记物检测与记录功能的 GEB 并通过粪便检测追踪疾病进展为 IBD 的诊断提供了新的选择。Riglar 等利用一株共生鼠源大肠杆菌构建了一个由 cro 触发元件与记忆元件组成的工程菌 PAS638，其可保存炎症状态下产生的连四硫酸盐的暴露记忆，供粪便检测分析。在超过 6 个月的时间内,研究者使用 PAS638 在不同小鼠肠炎模型中检测到了连四硫酸盐，且合成基因回路长期保持遗传稳定性和预期功能[19]。PAS638 作用的长效性展示了 GEB 用于慢性疾病诊断的巨大潜力。Zou 等设计的智能工程益生菌 i-ROBOT 实现了诊断治疗二合一。i-ROBOT 以大肠杆菌 Nissle 1917 (*Escherichia coli* Nissle 1917, EcN)为底盘，可对炎症标记物硫代硫酸盐做出响应,激活碱基编辑系统以产生可遗传基因组 DNA 序列，并产生比色信号。同时，硫代硫酸盐水平的波动会驱动免疫调节剂 AvCystatin 的可调节释放。给予结肠炎小鼠 i-ROBOT 可在处理过的粪便和结肠样本中产生分子记录信号，并有效缓解疾病[26]。i-ROBOT 为胃肠道疾病的无创实时诊断和可调节治疗提供了范例。

免疫调节细胞因子的紊乱在 IBD 的发生发展中扮演着重要的角色，因此，恢复细胞因子网络的稳态是 IBD 的一大治疗策略[27]。Steidler 等在 2 个小鼠模型中发现用分泌抗炎细胞因子白细胞介素-10 (interleukin-10, IL-10)的乳酸乳球菌灌胃可减少 IL-10 的治疗剂量，使葡聚糖硫酸钠(dextran sodium sulfate, DSS)处理的小鼠结肠炎减轻 50%，并预防 IL-10(−/−)小鼠结肠炎的发生[28]。黏膜愈合在 IBD 的治疗中起着至关重要的作用。Praveschotinunt 团队对 EcN 进行基因改造,使其产生具有原位促进肠上皮修复能力的纤维基质。这种基质由与三叶因子(trefoil factor family, TFF)融合的卷曲纳米纤维组成，TFF 能促进肠道屏障功能和上皮修复。此工程化 EcN

表 1　GEB 在疾病诊断和治疗中的应用

Table 1　Applications of GEB in disease diagnosis and treatment

Bacterial chassis	Functional gene	Gene transfer and recombination method	Function	Indication	Research stage	Reference
Escherichia coli NGF-1	Circuits for tetrathionate detection and recording	P1vir transduction	Detecting and recording the signal of the inflammatory biomarker, tetrathionate	IBD	Mouse model	[19]
Escherichia coli Nissle 1917	Circuits for thiosulfate detection and recording, tunable AvCystatin release system	Homologous recombination	Detecting and recording the signal of the inflammatory biomarker, thiosulfate and releasing the immunomodulator, AvCystatin, in a tunable manner	IBD	Mouse model	[26]
Lactococcus lactis (strain unknown)	IL-10 gene	Electroporation	Secreting the anti-inflammatory cytokine, IL-10	IBD	Mouse model	[28]
Escherichia coli Nissle 1917	Gene of the curli fiber protein fused with TFF3	Electroporation	Producing a fibrous matrix composed of curli fibers fused with TFF3 to promote intestinal barrier function and epithelial repair	IBD	Mouse model	[29]
Escherichia coli Nissle 1917	Genes of superoxide dismutase and catalase	Electroporation	Expressing superoxide dismutase and catalase to reduce reactive oxygen species	IBD	Mouse model	[32]
Escherichia coli Nissle 1917	*dacA*	λ-red recombination	Producing STING agonists that activate STING in APCs to produce type I interferons	Metastatic malignancies	Mouse model Clinical trial initiated	[33]
Escherichia coli DH5αZ1	Circuits for glucose detection and recording	Electroporation	Detecting and recording glucose levels in blood and urine	Diabetes	Patient sample detection	[37]
Lactobacillus gasseri ATCC 33323	GLP-1 gene	Homologous recombination	Secreting GLP-1 and inducing intestinal epithelial cells to differentiate into functional glucose-responsive insulin-producing cells	Diabetes	Rat model	[38]

(待续)

(续表 1)

Bacterial chassis	Functional gene	Gene transfer and recombination method	Function	Indication	Research stage	Reference
Lactococcus lactis MG1363	Genes of human proinsulin and IL-10	Double homologous recombination	Secreting human proinsulin and IL-10	Diabetes	Mouse model	[39]
Escherichia coli Nissle 1917	Genes of phenylalanine-ammonia-lyase and L-amino acid deaminase	λ-red recombination	Converting phenylalanine into trans-cinnamic acid salt to lower blood phenylalanine levels	Phenylketonuria	Mouse model Phase 1/2a clinical trial	[41-42]
Escherichia coli Nissle 1917	Deleting the gene of the negative regulator of I-arginine synthesis and adding the gene of a feedback-resistant I-arginine biosynthetic enzyme	λ-red recombination	Converting ammonia to I-arginine to block ammonia accumulation in the blood	Hyperammonemia	Mouse model Phase 1 clinical trial completed	[45]
Lactococcus lactis MG1363	GLP-1 gene	Electroporation	Secreting GLP-1	Obesity	Mouse model	[46]
Bacillus subtilis SCK6	Inserting butyryl CoA: acetic acid CoA transferase gene and disrupting *sdpC* and *skfA*	CRISPR-Cas9 system	Promoting butyric acid production	Obesity	Mouse model	[47]
Lactococcus lactis MG1363	Circuits for detection and recording of *Vibrio cholerae* quorum sensing molecules	Electroporation	Specifically detecting *Vibrio cholerae* quorum sensing signals	Cholera	Mouse model	[48]
A wild-type *Escherichia coli* strain	Circuits for detection of *Vibrio cholerae* quorum sensing molecules and Art-085 release system	Transformation (details unknown)	Specifically detecting *Vibrio cholerae* with quorum sensing molecules and killing *Vibrio cholerae* by self-cleaving and releasing killer proteins	Cholera	*In vitro* detection	[49]
Escherichia coli Nissle 1917	N-acyl homoserine lactone detection circuit and pyocin S5 and dispersin release system	Transformation (details unknown)	Sensing *Pseudomonas aeruginosa* by detecting N-acyl homoserine lactone and clearing pathogenic bacteria by releasing the bacteriocin pyocin S5 and dispersin through autolysis	*Pseudomonas aeruginosa* intestinal infection	*Caenorhabditis elegans* and mouse model	[50]

(待续)

(续表 1)

Bacterial chassis	Functional gene	Gene transfer and recombination method	Function	Indication	Research stage	Reference
Lactococcus lactis MG1363	GLP-1 gene	Electroporation	Secreting GLP-1	AD	Mouse model	[51-52]
Lactococcus lactis MG1363	GLP-1 gene	Electroporation	Secreting GLP-1	PD	Mouse model	[52-53]
Escherichia coli Nissle 1917	GLP-1 gene	CRISPR-Cas9 system	Secreting GLP-1	PD	Mouse model	[54]
Lactococcus lactis NZ9000	Blue light switches and genes related to gamma-aminobutyric acid (GABA) /granulocyte-colony stimulating factor (GCSF)/GLP-1 secretion	Electroporation	Producing GABA/GCSF/GLP-1 upon blue light exposure	Anxiety behavior/PD/vagal afferent regulation	Mouse model	[55]
Bacteroides ovatus ATCC 8483 ΔBAVOVA_03071 (tdk), *Lactobacillus Plantarum* BAA-793	Loss of BO1194/PAD gene function	Homologous recombination/CRISPR system/mutagenesis in conjunction with selection or screening	Inhibiting the production of 4EP and/or 4EPS	Anxiety and/or ASD	Mouse model Clinical trial	[56]
Escherichia coli BW25113Δ*tnaA*	Genes related to 5-HTP synthesis	Transformation (details unknown)	Producing 5-HTP	Depression, insomnia, obesity, chronic headache, etc.	*In vitro* production	[57]
Escherichia coli BL21(DE3)	Genes related to valerena-4,7(11)-diene synthesis	Transformation (details unknown)	Producing valerena-4,7(11)-diene	Attention deficit hyperactivity disorder, etc.	*In vitro* production	[58]
Escherichia coli Nissle strains, *Bacillus subtilis* 168	Genes related to 5-HTP, 5-HT, TRM or melatonin synthesis	Transformation (details unknown), CRISPR-Cas9 system	Producing 5-HTP, 5-HT, TRM or melatonin	5-HTP, 5-HT, TRM or melatonin-related central nervous system, enteric nervous system, gastrointestinal and metabolic diseases	Mouse model Clinical trial	[59]
Escherichia coli Nissle 1917	Circuits for heme detection and recording	Details unknown	Detecting heme, generating a fluorescent signal and activating adjacent circuits to generate a wireless signal	Gastrointestinal hemorrhage	Swine model	[62]
Lactobacillus plantarum NC8	Genes of tuna frame protein and yellowfin sole frame protein	Electroporation	Synthesizing angiotensin-converting enzyme inhibitory peptides	Hypertension	Rat model	[63]

在体内外均能分泌纤维基质，且无致病性。其对DSS诱导的小鼠结肠炎的保护作用增强，机制涉及黏膜愈合和免疫调节[29]。治疗IBD的另一策略是增加肠道中的超氧化物歧化酶或过氧化氢酶，从而减少炎症关键因素活性氧自由基[30-31]。Zhou等通过基因工程改造使EcN表达超氧化物歧化酶和过氧化氢酶，并通过逐层静电自组装方法用壳聚糖和海藻酸钠包裹工程化EcN，得到ECN-pE(C/A)$_2$。在小鼠IBD模型中，ECN-pE(C/A)$_2$能有效减轻炎症并修复结肠上皮屏障[32]。这些研究结果表明，GEB是一种安全有效的IBD治疗药物，有望作为传统药物的补充。

3.2 肿瘤

GEB在肿瘤中的应用十分广泛，在肿瘤免疫治疗中更是具有独特优势。例如，Leventhal等将干扰素基因刺激因子(stimulator of interferon genes, STING)激动剂生成酶基因*dacA*导入EcN，构建了工程菌SYNB1891。SYNB1891可促进肿瘤微环境中吞噬性抗原提呈细胞(antigen presenting cells, APCs)的STING激活，产生I型干扰素，引发长期抗肿瘤免疫。另外，其内设有生物防逃逸装置(营养缺陷型)，满足生产和法规要求[33]。研究者已启动临床试验以评估肿瘤内注射SYNB1891对转移性恶性肿瘤的疗效。GEB在肿瘤中的更多应用在其他综述中已有详细总结归纳[34-36]，这里不再赘述。

3.3 代谢性疾病

糖尿病的诊断和治疗也是GEB应用较多的领域。Courbet团队将数字放大基因开关和逻辑门整合到藻酸盐聚合物包裹的大肠杆菌中，用于检测和记录糖尿病患者血液和尿液中的葡萄糖水平。这种具有更强计算和放大能力的新一代全细胞生物传感器能够满足临床需求，并为医学诊断提供新方法[37]。分泌胰高血糖素样肽-1(glucagon-like peptide-1, GLP-1)的格氏乳酸杆菌可诱导肠上皮细胞分化为功能性葡萄糖反应性胰岛素分泌细胞，改善糖尿病大鼠的葡萄糖控制[38]。低剂量抗CD3抗体与分泌人胰岛素原和IL-10的乳酸乳球菌的联合治疗，治愈了66%的新发糖尿病小鼠[39]。

使用GEB在胃肠道中捕获有害物质从而避免其在血液中蓄积是一种治疗代谢性疾病的有效策略。苯丙酮尿症是一种先天性代谢障碍，患者体内缺乏苯丙氨酸羟化酶，因此食物来源的苯丙氨酸无法代谢，在血液中积聚，造成神经损伤[40]。基因组中插入苯丙氨酸解氨酶和L-氨基酸脱氨酶基因的EcN (SYNB1618)可在胃肠道中将苯丙氨酸转化为反式肉桂酸盐，有效降低小鼠血液苯丙氨酸水平[41]。临床试验证实了SYNB1618的安全性和耐受性，并在受试者的血浆和尿液中观察到菌株特异性苯丙氨酸代谢物的剂量响应性增加，从机制上证明了其有效性[42]。在健康个体中，胃肠道来源的氨在肝脏中通过尿素循环代谢为尿素，然后通过尿液排出。在患有肝脏疾病(如肝硬化)[43]或罕见代谢病尿素循环障碍[44]的个体中，氨在血液中蓄积，导致高氨血症和神经损伤。通过敲除I-精氨酸合成的负调控因子基因并添加抗反馈I-精氨酸生物合成酶基因，EcN成功实现了氨向I-精氨酸的转化，从而阻断体内氨积累，减缓高氨血症进程，已在健康志愿者中完成I期临床试验[45]。

GEB还可用于肥胖的治疗。陈廷涛团队构建了一种组成性分泌GLP-1的工程化乳酸乳球菌MG1363-pMG36e-GLP-1，可在小鼠模型中改善高脂饮食诱导的肥胖，机制可能与其促进脂肪酸氧化和增加肠道微生物多样性有关[46]。另一项研究通过对枯草芽胞杆菌SCK6进行基因改造提高了其丁酸产量，改造后的菌株可降低高脂饮食小鼠的体重、体重增量和摄食量，改善其生化指标[47]。

3.4 感染性疾病

Mao 等利用乳酸乳球菌构建了一种 GEB，其可在肠道中特异性检测霍乱弧菌的群体感应信号并触发报告基因的表达，表达产物易在粪便样本中测得[48]，从而辅助霍乱的诊断。Jayaraman 等用合成生物学方法改造野生大肠杆菌，使其通过群体感应分子 CAI-1 特异性检测霍乱弧菌，并表达裂解蛋白 YebF-Art-085，从而自我裂解以释放杀伤蛋白 Art-085 来杀伤霍乱弧菌。当霍乱弧菌上清液存在时，该工程化大肠杆菌能够有效抑制霍乱弧菌细胞生长[49]，实现霍乱的治疗。工程化 EcN 可通过检测 N-酰基高丝氨酸内酯感应铜绿假单胞菌，并通过自溶释放细菌素 pyocin S5 和一种抗生物膜的酶分散蛋白，最终清除肠道中的致病菌。此工程菌在铜绿假单胞菌肠道感染的秀丽隐杆线虫和小鼠模型中均表现出预防和治疗活性，展示了 GEB 在预防和治疗肠道感染方面的应用潜力[50]。

3.5 神经系统疾病

MG1363-pMG36e-GLP-1 除应用于肥胖外，还在神经系统疾病中展现出不俗的疗效。补充 MG1363-pMG36e-GLP-1 恢复了脂多糖(lipopolysaccharides, LPS)引起的小鼠空间学习记忆障碍，抑制了胶质细胞活化和 Aβ 积累，下调了炎症因子的表达，表明 MG1363-pMG36e-GLP-1 可作为一种安全有效的非吸收口服药物治疗阿尔茨海默病(Alzheimer's disease, AD)等神经炎症相关疾病[51-52]。在 1-甲基-4-苯基-1,2,3,6-四氢吡啶(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP)诱导的帕金森病(Parkinson's disease, PD)小鼠模型中，MG1363-pMG36e-GLP-1 可能通过抑制铁死亡改善小鼠的运动障碍和相关组织病理学改变，还能改善氧化应激状况[52-53]。陈廷涛团队还利用 CRISPR-Cas9 双质粒系统将 GLP-1 基因整合到 EcN 的染色体上，获得了一株无抗性标记的工程菌 EcN-GLP-1。EcN-GLP-1 可以改善 PD 小鼠的运动缺陷，增加酪氨酸羟化酶阳性神经元，抑制小胶质细胞和星形胶质细胞活化，减轻脑和结肠炎症，改善结肠屏障功能，还能恢复肠道菌群的平衡[54]。Pan 等设计了 3 种蓝光响应性乳酸乳球菌，由上转换光遗传微纳米系统进行时空递送和控制。这种微纳米系统促进了外源乳酸乳球菌的小肠靶向和生物活性分子生产，实现了对焦虑行为、帕金森病的长期缓解和对迷走神经传入的精确操控。这种非侵入性和实时的益生菌干预策略使得从肠道微生物到宿主的交流更加可控，从而使工程微生物准确有效地调节宿主健康[55]。BO1194 基因功能缺失的卵形拟杆菌和/或酚酸脱羧酶(phenolic acid decarboxylase, PAD)基因功能缺失的植物乳杆菌可抑制受试者体内 4-乙基苯酚(4-ethylphenol, 4EP)和/或 4-乙基苯基硫酸盐(4-ethylphenyl sulfate, 4EPS)的产生，从而改善焦虑和/或自闭症谱系障碍(autism spectrum disorder, ASD)相关症状[56]。将大肠杆菌作为细胞工厂可以高效生产 5-羟色氨酸(5-hydroxytryptophan, 5-HTP)、缬草-4,7 (11)-二烯等，用于抑郁、失眠、慢性头痛、注意力缺陷多动症等的治疗[57-58]，但细胞工厂在体内的疗效尚未得到证实。产生 5-HTP、5-羟色胺(5-hydroxytryptamine, 5-HT)或色胺(tryptamine, TRM)的 EcN 可用于预防和/或治疗相关中枢神经系统、肠神经系统疾病。产生褪黑素的 EcN 可用于治疗抑郁、痴呆和睡眠障碍等[59]。本课题组前期研究结果表明，精神分裂症(schizophrenia, SCZ)患者的粪便菌群可通过调节色氨酸-犬尿氨酸代谢通路诱发小鼠的 SCZ 样行为异常[60]；宏基因组关联研究鉴定出了多个 SCZ 相关菌种，移植 SCZ 富集菌可使小鼠出现社会行为缺陷和外周组织神经递质水平改变[61]。可见肠道菌群在 SCZ 的发生发展中扮演着重要的角色，GEB 有望为该病的治疗提供富有潜力的

新选项。这些研究结果提示，开发工程益生菌平台用于肠-脑轴相关疾病的治疗是一个前景广阔的研究方向。

3.6 其他疾病

Mimee 等开发了一种由工程菌和微电子器件构成的可摄入微型生物电子装置(ingestible micro-bio-electronic device, IMBED)，用于原位生物分子检测。其中的工程菌可以检测血液中的出血标记物血红素并产生荧光信号，随后激活相邻的电路以产生无线信号，实现了猪消化道出血的准确诊断。该检测平台具有模块化和可扩展特性[62]，为疾病诊断提供了新思路。

将编码金枪鱼框架蛋白和黄鳍原基蛋白的基因导入植物乳杆菌 NC8 中可赋予该益生菌合成血管紧张素转换酶抑制肽的功能。口服此工程菌(RLP)显著降低了自发性高血压大鼠模型的收缩压，且在体内尚未观察到明显的副作用或细菌移位，表明 RLP 用于高血压治疗安全有效。这项研究也有一些缺陷，比如抗生素选择标记物的存在[63]，有待后续研究完善。

4 困境与展望

鉴于广阔的应用前景，GEB 吸引了众多 NGPs 研究者的注意力，微生态药物公司也纷纷在此领域抢占高地。虽然 GEB 相关研究层出不穷，但还有不少问题需要解决。

在构建方法方面，现有技术满足大多数 GEB 构建需求，但构建可靠、高效表达外源蛋白质或化合物的 GEB 仍然富有挑战性，种间差异就是一个需要跨越的障碍[1]，CRISPR-Cas9 系统、机器学习[64]、单细胞和空间组学[65-66]等新兴技术的应用可优化 GEB 的设计与构建，新底盘的发掘及相应编辑工具的完善可能赋予 GEB 前所未有的强大功能；目前 GEB 使用的基因回路较为简单，进入临床试验阶段的 GEB 更是如此，复杂合成控制回路(如记忆回路、逻辑门、基因振荡器)的综合应用有望带来更好的控制效果和生物学效应[10]。

在性能评价及优化方面，GEB 诊断、预防、治疗疾病的有效性尚无官方评价标准，需要制定统一的标准来控制其质量；若无额外保护措施，GEB 口服时存活率不超过 50%[3]，使其作用效果大打折扣，因此包裹技术的应用很有必要；许多 GEB 在体内工作较短时间后即被清除，定殖能力较差[67]，可通过选择定殖能力强的底盘、导入促进定殖的基因或对细菌进行表面修饰[68]等途径来克服；评价 GEB 性能时，适宜的给药剂量难以确定，有必要设置剂量梯度对其进行探索；潜在的致病性、免疫原性[69]、基因转移[10]等安全性方面的问题阻碍了 GEB 的临床转化，使用 GRAS 底盘、敲除有害基因、应用生物限制措施等方法有望提高安全性。

此外，还需考虑规模化生产相关问题。益生菌(包括 GEB)的生产一般需经历发酵、冻干、研磨、质控和包裹这几个步骤，这一系列技术阶段给众多旨在开发厌氧肠道共生菌新型疗法的初创公司带来了巨大挑战[2]，应使用对氧气敏感性较低的菌株或在生产过程的各个环节做好氧气隔绝措施；如何储存 GEB 使其长久保持活性也是一道难题，后续研究可着眼于此，提出有效储存方法。

放眼未来，GEB 在医学领域必将大有可为。

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