合成生物学在活体功能材料构建上的应用

张曦, 李鹏程, 黄建东*, 戴卓君*

中国科学院深圳先进技术研究院合成生物学研究所, 深圳 518055

* 联系人, E-mail: jd.huang@siat.ac.cn; zj.dai@siat.ac.cn

2020-06-15 收稿, 2020-08-07 修回, 2020-08-10 接受, 2020-08-11 网络版发表

国家重点研发计划(2018YFA0903000)、深圳市科技计划(KQTD20180413181837372)和深圳市科技创新委员会项目(KQTD2015033117210153)资助

摘要 材料是人类赖以生存与发展的物质基础, 代表了一个时代科学技术成果的最前沿. 近年来飞速发展的合成生物学, 通过改进现有系统或构建全新的生物体系, 极大地促进了对生物本身的了解, 拓宽了生命科学的应用范围. 将构建的生物体系进一步结合材料科学中的设计工具及方法, 便诞生了活体功能材料这一概念及领域. 与传统材料不同, 活体功能材料以活体细胞为结构单体组装机件, 活体细胞本身成为材料的工程化设计工具以及技术设想和实现途径的基本单元. 将编程后的工程活细胞组装、裁剪成具有生物系统特性的活体功能材料, 将活体细胞的自我修复能力等特性融入材料, 进一步拓展了原有材料的性能. 本文将着重介绍活体功能材料的产生、发展及近年来取得的相关成果, 在此基础上对活体功能材料未来的发展进行展望.

关键词 合成生物学, 活体功能材料, 基因线路, 生物材料

合成生物学作为一门融合了生物学、数理科学和工程学等多学科的新兴科学, 在近20年来取得了重大的发展^[1~3]. 通过在细胞中植入合成基因线路并给予外界信号输入, 工程细胞可以识别、整合和处理输入的信号, 并给予一定的信号输出从而实现特定生物功能^[4~6]. 另外, 生物体本身也是由多种材料自组装形成^[7,8]. 生物作为天然的活体功能材料, 具有高度智能化的特性, 可以对外界的信号进行识别、处理并做出响应. 例如, 细胞在干涸或抗生素存在的情况下, 分泌由多糖和蛋白互相连接形成的生物被膜, 通过降低生物被膜中细胞的代谢和分散系数等抵抗恶劣环境的影响^[9,10]. 海参体壁真皮由天然的高分子组成, 受到外界刺激会快速可逆地改变其机械性能, 在柔性和刚性之间切换^[11,12]. 未来, 材料科学必将朝着智能化, 如适应环境、刺激响应下的功能转换及自愈合等多功能、高

性能的方向发展^[13~15].

合成生物学的快速发展促使各种细胞编辑方法、元器件以及底盘细胞改造方法等的快速积累, 产生了大量的基因线路以及具有特定性能的工程细胞^[16~21]. 而将工程细胞进一步结合材料科学中的设计工具及方法, 便诞生了活体功能材料这一概念及领域. 与传统材料相比, 活体功能材料以活体细胞为结构单体组装机件. 将编程后的工程活细胞组装、裁剪成具有生物系统特性的活体功能材料, 由此将活体细胞的自我修复能力、进化能力、重编程能力、自主排列能力、对环境的响应能力、解毒能力、产生和储存能量的能力等融入材料^[22,23]. 由此可见, 活体功能材料的特性可以极大地扩展现有的材料范围与优势, 势必成为基础科研与实际应用的关注点^[24~27].

综上, 活体功能材料是随着合成生物学发展而诞

引用格式: 张曦, 李鹏程, 黄建东, 等. 合成生物学在活体功能材料构建上的应用. 科学通报, 2021, 66: 341–346

Zhang X, Li P C, Huang J D, et al. Application of synthetic biology in living functional materials (in Chinese). Chin Sci Bull, 2021, 66: 341–346, doi: 10.1360/TB-2020-0475

生,与材料科学紧密交叉的新型学科.在最近几年,已有多个国际知名的科研团队基于工程大肠杆菌、枯草芽孢杆菌乃至哺乳动物细胞等进行活体功能材料的组装及功能的探索.我们将从这些工作出发,探讨其思路 and 独到之处,并进一步展望活体功能材料领域未来的发展.

1 活体功能材料在近年来的发展

1.1 基于大肠杆菌的活体功能材料研究

大肠杆菌生物被膜中的淀粉样蛋白纤维Curli由成核蛋白CsgB和纤维单元CsgA等自组装组成,是最早被广泛研究的活体功能材料之一^[28,29].2014年,Lu实验室^[30]通过敲除大肠杆菌自身的CsgA基因,引入可控诱导表达的CsgA基因,实现了大肠杆菌表面蛋白纤维Curli的可控组装.His-tag修饰后的CsgA,可进一步与纳米金颗粒特异性结合,为Curli表面增加一层纳米金颗粒修饰,使大肠杆菌具有导电的性质和功能.同时,他们构建了aTc-CsgA-His和AHL-CsgA的正交诱导体系工程菌群,在菌群共培养的过程中,改变两种菌初始浓度的比例以及诱导物的浓度,得到了一种近似于嵌段共聚物的复杂结构,巧妙地展现了活体功能材料可编程的特性,并利用有机(细胞)-无机体(纳米金颗粒)的结合提高了材料的性能并拓展了应用的范畴.该文章是活体功能材料领域的一篇代表性文章,发表不久后,Chen等人在*ACS Synth Biol*发表综述文章“Engineering Living Functional Materials”^[31],进一步定义了活体功能材料,即利用细胞本身作为单元进行材料组装这一概念.文中特别提到其与传统生物材料,如蛋白材料的不同.在传统的蛋白材料组装中,细胞只是作为工厂出现在蛋白生产的环节,而之后材料的组装都是利用纯化后的蛋白单体在体外进行.该综述进一步阐述活体功能材料区别于传统材料的特性,包括可进化性(evolvability)、可自主排列(self-organization)、针对环境的响应性(responsiveness to environment)以及可与无机材料相结合(bridging the function gap with inorganic materials)等.

2017年,You实验室^[32]通过基因线路编辑细菌自组装形成核壳结构,并在基因线路下游引入CsgA-His基因^[33],使得大肠杆菌菌落形成半球状结构时,蛋白纤维Curli大量分布在半球的壳表面.通过anti-His抗体与标记了纳米金的二抗结合,形成有机-无机杂化核壳结构.

当受到外力挤压时,两个相向的核壳被压在一起,所受的压力强度及持续时间不同导致金纳米颗粒局部分布发生不同变化,继而引发电导率的变化,使三维自组装材料实现了压力传感器的功能.这一工作巧妙地利用基因线路编辑细菌,使其自发形成(self-organization)功能器件的基础三维结构,实现了基因线路编辑细菌自组装形成功能材料的突破.

2018年,Voigt实验室^[34]设计了一个由18个基因组成的基因回路:其中3个是编码光敏蛋白的基因,可分别感应红(波长705 nm)、绿(波长535 nm)、蓝(波长470 nm)三种波长的光(RGB系统).不同波长光可以启动细菌合成不同色素.在此基础上,2019年,该课题组将光响应的下游基因替换成3种不同修饰的CsgA,在不同波长光的启动下诱导不同功能特性的Curli组装.通过调节不同波长的光的组合,实现不同功能的Curli的逐层可控组装^[35].该工作成功利用光控手段实现了活体功能材料可控组装,充分体现了活体功能材料针对环境的响应性.

利用活体功能材料不仅可以在体外实现导电、传感等多种用途,也可以进一步延伸至医药应用领域.2019年底,Joshi课题组^[36]通过编辑一种属于益生菌的大肠杆菌(Nissle1917),将可治疗炎症性肠病(inflammatory bowel disease)的多肽Trefol factors(TFFs)与CsgA基因融合表达.结果表明,将工程益生菌灌胃给老鼠后,工程益生菌形成的治疗性材料会贴附在肠道的黏膜上,有效地抑制由DSS(dextran sodium sulfate)诱导的肠炎.

1.2 基于其他微生物的活体功能材料构建

枯草芽孢杆菌是一种革兰氏阳性菌,因出色的分泌能力及被FDA认证的安全性在食品和制药工业中被广泛使用.Zhong课题组^[37]利用合成生物学和材料科学交叉手段,将His-Tag、SpyTag、Mefp(adhesive mussel foot proteins derived, *Mytilus edulis*)等蛋白或结构域与TasA融合表达并分泌,实现了多种功能的胞外TasA纳米纤维的组装.研究结果进一步展示了这些活体功能材料的性能,如融合了有机磷酸酶的TasA纳米纤维与无机纳米金颗粒相结合,通过新型的催化级联反应,将剧毒的有机磷农药降解为低毒害化学品.此外,课题组发现敲除枯草芽孢杆菌本身的TasA表达系统,外源诱导表达形成的生物被膜疏水性更强,展现出更适宜生物制造的凝胶胶体性质,可以通过3D打印精确地制成不同的三维形状.2019年,课题组进一步探索,发现将

Mefp5蛋白与TasA融合后形成的Mefp5-TasA纤维,其黏弹性能与未融合的生物膜相比提高了60%,改造后的枯草芽孢杆菌被膜以其更优良的特性和安全性,为作为细胞胶水帮助伤口愈合提供了可能^[38]。

另一方面,基于微生物的产电材料研究也有极大的进展。2014年, Song课题组^[39]用水溶性石墨烯自组装形成的纳米片层将希瓦氏菌捕获,继而培养形成内外向电流显著提高的电活性三维生物膜,提高了产电菌和电极之间的电子传递效率。2018年,该课题组继续通过模块化合成生物学手段,找出希瓦氏菌中NAD⁺生物合成途径相关的5个关键基因,即*ycel*、*pncB*、*nadM*、*nadD*及*nadE*,并将其组装至希瓦氏菌中过表达,重组后的希瓦氏菌产生的最大电量提高4倍,显示出NAD⁺生物合成途径的改造对提高希瓦氏菌电子转移能力的巨大潜力^[40]。

1.3 基于哺乳动物细胞的活体材料构建

2016年, Parke小组^[41]使用钛金属模具制作了一只机器鳐鱼,机器鳐鱼由4层结构构成:第一层是三维PDMS弹性层,第二层通过热蒸镀法制作了一层金骨架,第三层是薄的有机硅弹性间层,最后一层打印了大鼠胚胎心肌细胞。心肌细胞中引入光敏基因线路,在470 nm蓝光激发下ChR2离子通道开放并激活细胞收缩。通过控制蓝光照射的频率和位置,可以使这条鳐鱼的运动速度和运动方向发生变化。这项工作将组织工程和软体机器人技术结合,为未来发展具有自主和适应能力的人造生物处理多个感官输入并产生复杂行为奠定了基础。

2 总结与展望

活体功能材料是合成生物学和材料科学领域交叉的新兴研究方向,其发展受到合成生物学学科本身发展产生的极大支撑,如大量的调控元件、基因线路工具,光控、温控等启动子库的构建^[42,43],开关、振荡器等被开发并成功地运行于不同的模式生物,丰富了细

胞编辑的能力。同时,随着CRISPR^[44]的发展及普及、基因合成成本的降低^[45]、生物编辑的成本及难度大幅下降^[46]等,这些基因编辑工具都为编辑活体功能材料的基本构成单元——细胞提供了丰富的手段和工具。从工程细胞到组装具有多功能、自我修复、自我调节及信号处理能力的活体功能材料也成为合成生物学造物致用的一个重要方向。与此同时,其他工程技术的快速发展,如3D打印技术、微电子技术等,也极大地提升了活体功能材料的组装、加工以及整合水平,促使活体功能材料的研究有了新的发展基础和空间。

综上,本文点评了几个活体功能材料领域的代表性工作,如利用工程大肠杆菌、枯草芽孢杆菌、希瓦氏菌或真核细胞等组装具有导电、传感、医疗、发电等多种功能的活体功能材料。这些研究本身极具新颖性,也同时具备极大的拓展空间。首先,当前的活体功能材料大部分是在其制造中利用了活体细胞的能力,如利用光控实现蛋白纤维组装以及引入可编程细菌图案的基因线路组装菌落三维结构等,但还未将活体细胞的优势运用到材料的性质上,而这恰好是最能体现活体功能材料优势的部分。另一方面,生物作为材料本身具有一些局限,如较差的机械性能以及生物材料性质的批次间差异等,生物编辑与传统高分子材料相结合能否突破这些局限,或其他解决方法都亟待设计、开发与验证。除此以外,还有众多领域对活体功能材料有极大需求,如极端条件(雨林或极地、深海、外太空等)、偏远地区等对机动性构筑材料的需求。生物体本身的快速生长、复制以及自组装可以极好地满足这些需求,使得活体功能材料极有可能成为解决这些问题的最佳选择。我们相信,未来活体功能材料在解决材料可降解、环境监测及生命健康问题,如类器官、生物胶水、极端条件下的材料快速构建等诸多方面会有更多的应用前景。随着合成生物学以及其他工程学科的发展,我们也相信越来越多的新技术、新方法会支持活体功能材料领域在学术和产业上迈向更加新颖、广阔的未来。

参考文献

- Way J C, Collins J J, Keasling J D, et al. Integrating biological redesign: Where synthetic biology came from and where it needs to go?. *Cell*, 2014, 157: 151–161
- Ackers G K, Johnson A D, Shea M A. Quantitative model for gene regulation by lambda phage repressor. *Proc Natl Acad Sci USA*, 1982, 79: 1129–1133
- Smanski M J, Zhou H, Claesen J, et al. Synthetic biology to access and expand nature's chemical diversity. *Nat Rev Microbiol*, 2016, 14: 135–149

- 4 Wu F, Bethke J H, Wang M, et al. Quantitative and synthetic biology approaches to combat bacterial pathogens. *Curr Opin BioMed Eng*, 2017, 4: 116–126
- 5 Ausländer S, Wieland M, Fussenegger M. Smart medication through combination of synthetic biology and cell microencapsulation. *Metab Eng*, 2012, 14: 252–260
- 6 Ye H, Baba M D E, Peng R W, et al. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science*, 2011, 332: 1565–1568
- 7 Nguyen P Q, Courchesne N D, Duraj-Thatte A, et al. Engineered living materials: Prospects and challenges for using biological systems to direct the assembly of smart materials. *Adv Mater*, 2018, 30: e1704847
- 8 Bohm C F, Harris J, Schodder P I, et al. Bioinspired materials: From living systems to new concepts in materials chemistry. *Materials*, 2019, 12: 1–19
- 9 Limoli D H, Jones C J, Wozniak D J. Bacterial extracellular polysaccharides in biofilm formation and function. *MicroBiol Spectrum*, 2015, 3: 1–9
- 10 Kovács Á T, van Gestel J, Kuipers O P. The protective layer of biofilm: A repellent function for a new class of amphiphilic proteins. *Mol MicroBiol*, 2012, 85: 8–11
- 11 Takehana Y, Yamada A, Tamori M, et al. Softenin, a novel protein that softens the connective tissue of sea cucumbers through inhibiting interaction between collagen fibrils. *PLoS One*, 2014, 9: e85644
- 12 Liu Y, Liu Z, Song L, et al. Effects of collagenase type I on the structural features of collagen fibres from sea cucumber (*Stichopus japonicus*) body wall. *Food Chem*, 2019, 301: 125302
- 13 Capadona J R, Shanmuganathan K, Tyler D J, et al. Stimuli-responsive polymer nanocomposites inspired by the sea cucumber dermis. *Science*, 2008, 319: 1370–1374
- 14 Wong T S, Kang S H, Tang S K Y, et al. Bioinspired self-repairing slippery surfaces with pressure-stable omniphobicity. *Nature*, 2011, 477: 443–447
- 15 Wang J, Sun L, Zou M, et al. Bioinspired shape-memory graphene film with tunable wettability. *Sci Adv*, 2017, 3: e1700004
- 16 Segall-Shapiro T H, Sontag E D, Voigt C A. Engineered promoters enable constant gene expression at any copy number in bacteria. *Nat Biotechnol*, 2018, 36: 352–358
- 17 Kyllis N, Tuza Z A, Stan G B, et al. Tools for engineering coordinated system behaviour in synthetic microbial consortia. *Nat Commun*, 2018, 9: 1–9
- 18 Vilaboa N, Fenna M, Munson J, et al. Novel gene switches for targeted and timed expression of proteins of interest. *Mol Ther*, 2005, 12: 290–298
- 19 Gao C, Hou J, Xu P, et al. Programmable biomolecular switches for rewiring flux in *Escherichia coli*. *Nat Commun*, 2019, 10: 3751
- 20 Novák B, Tyson J J. Design principles of biochemical oscillators. *Nat Rev Mol Cell Biol*, 2008, 9: 981–991
- 21 Potvin-Trottier L, Lord N D, Vinnicombe G, et al. Synchronous long-term oscillations in a synthetic gene circuit. *Nature*, 2016, 538: 514–517
- 22 Cremaldi J C, Bhushan B. Bioinspired self-healing materials: Lessons from nature. *Beilstein J Nanotechnol*, 2018, 9: 907–935
- 23 Liu Z, Zhang Z, Ritchie R O. On the materials science of nature's arms race. *Adv Mater*, 2018, 30: e1705220
- 24 Gonzalez L M, Mukhitov N, Voigt C A. Resilient living materials built by printing bacterial spores. *Nat Chem Biol*, 2019, 16: 126–133
- 25 Nussbaumer M G, Nguyen P Q, Tay P K R, et al. Bootstrapped biocatalysis: Biofilm-derived materials as reversibly functionalizable multienzyme surfaces. *ChemCatChem*, 2017, 9: 4328–4333
- 26 Morales N M, McClean M N. Engineered bacteria self-organize to sense pressure. *Nat Biotechnol*, 2017, 35: 1045–1047
- 27 Bittihn P, Din M O, Tsimring L S, et al. Rational engineering of synthetic microbial systems: From single cells to consortia. *Curr Opin MicroBiol*, 2018, 45: 92–99
- 28 Chapman M R. Role of *Escherichia coli* curli operons in directing amyloid fiber formation. *Science*, 2002, 295: 851–855
- 29 Gilbert C, Ellis T. Biological engineered living materials: Growing functional materials with genetically programmable properties. *ACS Synth Biol*, 2019, 8: 1–15
- 30 Chen A Y, Deng Z, Billings A N, et al. Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat Mater*, 2014, 13: 515–523
- 31 Chen A Y, Zhong C, Lu T K. Engineering living functional materials. *ACS Synth Biol*, 2015, 4: 8–11
- 32 Cao Y, Ryser M D, Payne S, et al. Collective space-sensing coordinates pattern scaling in engineered bacteria. *Cell*, 2016, 165: 620–630
- 33 Cao Y, Feng Y, Ryser M D, et al. Programmable assembly of pressure sensors using pattern-forming bacteria. *Nat Biotechnol*, 2017, 35: 1087–1093
- 34 Fernandez-Rodriguez J, Moser F, Song M, et al. Engineering RGB color vision into *Escherichia coli*. *Nat Chem Biol*, 2017, 13: 706–708
- 35 Moser F, Tham E, González L M, et al. Light-controlled, high-resolution patterning of living engineered bacteria onto textiles, ceramics, and plastic. *Adv Funct Mater*, 2019, 29: 1–11
- 36 Praveschotinunt P, Duraj-Thatte A M, Gelfat I, et al. Engineered *E. coli* Nissle 1917 for the delivery of matrix-tethered therapeutic domains to the

- gut. *Nat Commun*, 2019, 10: 5580
- 37 Huang J, Liu S, Zhang C, et al. Programmable and printable *Bacillus subtilis* biofilms as engineered living materials. *Nat Chem Biol*, 2019, 15: 34–41
- 38 Zhang C, Huang J, Zhang J, et al. Engineered *Bacillus subtilis* biofilms as living glues. *Mater Today*, 2019, 28: 40–48
- 39 Yong Y C, Yu Y Y, Zhang X, et al. Highly active bidirectional electron transfer by a self-assembled electroactive reduced-graphene-oxide-hybridized biofilm. *Angew Chem Int Ed*, 2014, 53: 4480–4483
- 40 Li F, Li Y X, Cao Y X, et al. Modular engineering to increase intracellular NAD(H⁺) promotes rate of extracellular electron transfer of *Shewanella oneidensis*. *Nat Commun*, 2018, 9: 3637
- 41 Park S J, Gazzola M, Park K S, et al. Phototactic guidance of a tissue-engineered soft-robotic ray. *Science*, 2016, 353: 158–162
- 42 Ong N T, Olson E J, Tabor J J. Engineering an *E. coli* near-infrared light sensor. *ACS Synth Biol*, 2018, 7: 240–248
- 43 Mahr R, von Boeselager R F, Wiechert J, et al. Screening of an *Escherichia coli* promoter library for a phenylalanine biosensor. *Appl Microbiol Biotechnol*, 2016, 100: 6739–6753
- 44 Suzuki Y, Kouzuma A, Watanabe K. CRISPR/Cas9-mediated genome editing of *Shewanella oneidensis* MR-1 using a broad host-range pBBR1-based plasmid. *J Gen Appl Microbiol*, 2019, 1: 41–45
- 45 Hughes R A, Ellington A D. Synthetic DNA synthesis and assembly: Putting the synthetic in Synthetic Biology. *Cold Spring Harb Perspect Biol*, 2017, 9: 1–18
- 46 Ausländer S, Ausländer D, Fussenegger M. Synthetic Biology—The synthesis of biology. *Angew Chem Int Ed*, 2017, 56: 6396–6419

Summary for “合成生物学在活体功能材料构建上的应用”

Application of synthetic biology in living functional materials

Xi Zhang, Pengcheng Li, Jiandong Huang^{*} & Zhuojun Dai^{*}

Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

^{*} Corresponding authors, E-mail: jd.huang@siat.ac.cn; zj.dai@siat.ac.cn

The fast development of synthetic biology has greatly promoted the understanding of biology and broadened the application of engineered biological systems. Numerous cell-based circuits are assembled using modular biological building blocks. These circuits are further utilized to program the living organisms to attain multiple well-defined functions. Especially, the field of biological engineered living materials (ELMs) emerged at the intersection between synthetic biology and material science. Actually, living organisms themselves are made of materials with remarkable properties including self-organization and adaption to the stimulus. In nature, various patterns form autonomously during the development of the animal, and the bacteria can secrete biofilm made of polysaccharides and proteins to protect themselves when treated with antibiotics. The existing material systems typically lack these “smart” properties, which are urgently requested by the next generation materials. A key difference between the ELMs and classical material is that ELMs are composed of living cells that form or assemble the material itself. The living cells act as building blocks, modulate and direct the formation and function of the desired material. Therefore, the ELMs exhibit living properties, such as evolvability, self-repair and responsiveness to environment. Synthetic biological circuits can further program the cells spatially and temporally, and brought various functionalities into the ELMs. The most pioneering efforts in ELMs development have focused on engineering functional amyloids, which are secreted and assembled into nanofibrous structure on the cell surface during biofilm formation. Among them, the curli produced by *E. coli* is the most well-studied system. During the curli formation, the membrane curli-specific gene B (*CsgB*) proteins functioned as nucleators and guided the polymerization of the major protein subunit CsgA into curli fibrous networks extracellularly. The system is highly engineerable by fusing heterologous protein domains with specific functions to CsgA, including the curli that can be designed to bind inorganic materials and become electrically conductive by fusing functional tags. Besides *E. coli*, other organisms spanning from prokaryotic systems to eukaryotic cells are also engineered and adopted for ELMs assembly. By integrating with the tools and technologies (3D printing, microfluidics, etc) from the material engineering and other disciplines, these rewired cells can be further engineered into materials with complex functions such as disease detection and bioremediation. Here we review the efforts on ELMs development, with an emphasis on its implementation and production using engineered bacterial systems, and also discuss the current limitation and future challenges of the ELMs.

synthetic biology, living functional materials, gene circuit, biomaterialsdoi: [10.1360/TB-2020-0475](https://doi.org/10.1360/TB-2020-0475)