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Review

Macroencapsulated bacteria for *in vivo* sensing and therapeutics

Yidan Lyu,[1,10] Hao Huang,[2,10] Yuyan Su,[1,10] Binbin Ying,[3,4] Wen-Che Liu,[1] Kairu Dong,[5] Ningjie Du,[2] Robert S. Langer,[6,7,*] Zhen Gu,[1,8,9,*] and Kewang Nan[1,8,9,*]

SUMMARY

Engineered bacteria are increasingly recognized as sustained and intelligent sources for sensing and therapeutics due to their unique capabilities such as *in situ* multiplication, tissue targeting, and genetic editability. However, the clinical applications of these living agents are hindered by the ineffective immunoisolation, residency, and removal against the complex and dynamic *in vivo* environment. Existing approaches focus on surface decoration and encapsulation of engineered bacteria, or "microencapsulation," but there are limits to what can be achieved with modifications of bacteria themselves. An emerging strategy combines millimeter- to centimeter-scale engineered devices and systems with bacteria, or "macroencapsulation," offering unique advantages such as extending the *in vivo* lifetime and engraftment of bacteria, enhancing immunoisolation, and enabling real-time signal readouts via wireless electronic technologies. In this review, the design rationales for macroencapsulated bacteria toward *in vivo* applications are discussed, and examples in bacterial devices for transdermal and oral applications are highlighted. Since the gastrointestinal tract represents a major site for engineered bacteria, we also summarize and compare various strategies for synthetic engraftment of orally administered encapsulated bacteria.

INTRODUCTION

An ancient Chinese medicine note from the Tsin Dynasty (300–400 AD) revealed a method of using fecal bacteria for the treatment of diseases, the earliest known documented bacterial therapy on humans.[1,2] In the 1990s, the first US Food and Drug Administration (FDA) approval of non-vaccine therapeutic bacteria, bacillus Calmette-Guérin for the treatment of bladder cancer *in situ*, represented significant progress toward massive clinical applications in modern medicine.[3] In recent years, an expanding recognition of engineered bacteria's potential as a promising platform for biosensing, drug delivery, and therapeutics has emerged. On the one hand, bacteria offer unique advantages as medicine, including on-site drug production and penetration within deep tissues, which increase local therapeutic concentrations without a large initial drug load and therefore avoid the effects of systemic toxicity.[4,5] For example, the rapid replication and colonization of therapeutic bacteria in the gastrointestinal (GI) tract offer a cost-effective, volume-saving avenue for the treatment of chronic conditions that often require large drug dosage, such as inflammatory bowel disease[6,7] and colorectal cancer.[1,8] On the other hand, the molecular biology and physiology of bacteria are well characterized and documented, providing virtually limitless design possibilities by leveraging biological diversity.[9–11] Additionally, specific bacterial strains, such as *Escherichia coli* strain Nissle

PROGRESS AND POTENTIAL

Engineered bacterial therapeutics, with promising preclinical outcomes, are advancing in commercialization endeavors. However, their translation into widely accepted clinical products still poses significant challenges, demanding solutions for improved *in vivo* safety and long-term effectiveness. Current approaches focus on surface decoration and single-cell encapsulation (microencapsulation of bacteria), but they face limitations such as strain specificity, high manufacturing cost, and risks of compromised cellular function and uncontrolled growth. Macroencapsulation of bacteria, on the other hand, exploits millimeter- to centimeter-sized materials and devices to encapsulate a group of bacteria, offering advantages such as prolonged *in vivo* lifespan, enhanced immune isolation, and integration with wireless electronic technology for real-time biosignal readouts. This review aims to summarize the current state and challenges of macroencapsulated bacteria toward next-generation clinical implementation of engineered bacterial therapy.

1917 (EcN 1917), have been verified as safe for human use, exhibiting rapid reproduction and ease of storage and transport, rendering them highly promising for clinical translation.[12] These characteristics make bacteria increasingly applicable in the treatment of diabetes,[13] cancers,[14,15] HIV infection,[16] vaginitis,[17] and neurologic diseases.[18,19] Clinical development of bacterial agents is also advancing (Table 1).

Despite these promises, current clinical availability of the engineered bacteria remains limited.[20] First, engineered bacteria are susceptible to harsh *in vivo* conditions, such as the acidic and enzymatic environments of the GI tract, leading to significant loss of bacterial viability after administration.[21] Second, the antigenic proteins located on bacterial surfaces and the excretions produced by bacteria can easily trigger immune responses, leading to excessive inflammation and damage in the host tissues.[22,23] Third, various factors such as colonization resistance, host immune response, and lack of suitable niche limit bacterial engraftment at the targeted sites,[24] thereby diminishing long-term clinical effectiveness unless dosed excessively. Finally, although engineered bacteria can be used as biosensors by responding to metabolic by-products and changes in protein expression, the resulting bioluminescent signals are often weak and unable to directly transmit outside the body, therefore limiting their use as biosensors in real-time *in situ* settings.

Significant research efforts have been devoted to surface decoration and encapsulation of engineered bacteria, or "microencapsulation," to address the above issues. For example, microencapsulation is shown to improve the *in vivo* survival of engineered bacteria by resisting acidity in the GI tract and avoiding clearance by the immune system.[25,26] Bacterial engraftment or adhesion to targeted tissues can be enhanced by surface decoration.[27,28] In addition, functional microencapsulation can improve tissue targeting and responsiveness of bacteria.[29,30] However, the fact that most microencapsulation approaches are strain specific limit their generalization and increase overall cost and complexity during clinical translation. The lack of ability to actively transmit the signals outside the body is also a hindrance to the application of bacterial sensors. Finally, microencapsulated bacteria are challenging to remove completely after treatment as they migrate and penetrate the surrounding tissues, making the potential infection and other side effects uncontainable.

Macroencapsulation of bacteria, on the other hand, is an emerging set of approaches to improve immunoisolation, navigation, retention, and other functions of engineered bacteria by exploiting bulk encapsulation materials and devices that are thousands to millions of times larger than individual bacteria. The concept was initially applied to cell therapy[31] and later extended to *in vivo* delivery of bacteria. Carriers for macroencapsulation of bacteria include hydrogel matrices,[32,33] microneedles,[34,35] and capsules,[36] which can assist bacterial therapy through transdermal, oral, and other delivery routes. The portability and systematic integration of macroencapsulation with other functional units also make it promising for wearable device development. The incorporation of wireless electronic components allow for the remote control of bacteria as well as the reception of sensory signals.[36] Notably, most macroencapsulation systems are compatible with multiple strains of bacteria and are constructed using approved materials and dimensions,[32,36,37] reducing overall cost and burdens in obtaining FDA approval. Finally, by physically isolating and confining the bacteria, macroencapsulation limits bacterial diffusion into tissues and facilitates bacterial clearance after the treatment, thereby enhancing the safety of bacterial therapy.[32]

In this review, we discuss the design rationales for bacterial encapsulations and compare micro- and macroencapsulation based on different functions. We then

[1]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

[2]College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310058, China

[3]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[4]Department of Gastroenterology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

[5]College of Biomedical Engineering & Instrument Science, Zhejiang University, Hangzhou 310027, China

[6]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[7]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

[8]National Key Laboratory of Advanced Drug Delivery and Release Systems, Zhejiang University, Hangzhou, China

[9]Jinhua Institute of Zhejiang University, Jinhua 321000, China

[10]These authors contributed equally

*Correspondence: rlanger@mit.edu (R.S.L.), guzhen@zju.edu.cn (Z.G.), knan@zju.edu.cn (K.N.)

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Table 1. Clinical trials of bacterial therapies

Trial no.	Type of bacteria	Disease	Status	Route
NCT00004988	*Salmonella* bacteria VNP20009	cancer	phase I	intravenous administration
NCT05764239	engineered *Escherichia coli* Nissle 1917 SYNB1934v1	phenylketonuria	phase III	oral administration
NCT01562626	engineered *Bifidobacterium longum* APS001F	solid tumors	phase I/II	intravenous administration
NCT00936572	*Bifidobacterium longum* BB536/ *Lactobacillus johnsonii* La1	colorectal cancer	phase II	oral administration
NCT00510978	*Bifidobacterium infantis* 35624/ *Lactobacillus salivarius* UCC118	Crohn's disease	phase II/III	oral administration
NCT02766023	*Lactobacillus crispatus* CTV-05	bacterial vaginosis	phase II	topical application
NCT00305227	*Lactobacillus crispatus* CTV-05	urinary tract infection	phase II	topical application
NCT03183128	FMT (SER-109)	*Clostridium difficile* infection	phase III	oral administration
NCT03408548	*Bifidobacterium lactis* HN019	periodontitis	phase II	oral (buccal)
NCT04753944	probiotic	depression	not applicable	oral administration
NCT05523427	*Lactobacillus plantarum* and *Lactobacillus acidophilus*	irritable bowel syndrome	not applicable	oral administration
NCT05871242	*Lactobacillus crispatus* M247	fertility	not applicable	topical application
NCT04383236	probiotic	recurrent aphthous stomatitis	not applicable	oral (buccal)
NCT03151967	*Lactobacillus crispatus* CTV-05	urinary tract infections	phase II/III	topical application
NCT05095350	*Lactobacillus* and *Bifidobacterium*	hypertension	early phase I	oral administration

Data from ClinicalTrials.gov, accessed on January 1, 2024.

review state-of-the-art examples of macroencapsulated bacterial systems for transdermal and oral applications. Recognizing the significance of GI microbiota and their pivotal roles in engineered bacterial therapeutics, we compare different encapsulation strategies that are set to enhance the engraftment and safety of orally administered bacteria in the GI tract. Altogether, we hope to stimulate the medical device and microbiology communities to explore next-generation, integrated bacterial devices toward sensing and therapeutics, a relatively uncharted field with immense scientific and clinical value.

DESIGN RATIONALES FOR MACROENCAPSULATED BACTERIA

We categorize bacterial encapsulations into two general types: microencapsulation and macroencapsulation. The former is defined as surface modification of individual bacteria with a nanomembrane or surface decoration, and multiple encapsulants are delivered simultaneously during treatment. A key advantage of microencapsulated bacteria is that they largely retain the high mobility of individual bacteria for targeted deep tissue delivery, e.g., within a rigid tumor environment. On top of this, microencapsulation can further provide protective or adhesive coatings against harsh environments[25,28] and cell membranes for camouflage effects.[38] Recently, microencapsulated bacteria that integrate physical, biological, and chemical responsiveness have been developed for site-specific targeting or adhesion,[39] magnetic-field-controlled localization,[40,41] responsive protein expression and drug delivery,[42,43] and phototherapy mediated by functional modifications such as photosensitizers.[44] In addition, bacteria and their modifiers can work in synergy to create a "1 + 1 > 2" effect. For example, probiotics modified with artificial enzymes are protected from oxidative damage in inflamed habitats, while the probiotics can in turn promote the targeting and retention of enzymes.[45]

On the other hand, the macroencapsulation of bacteria, despite the limited route of administration due to their bulk sizes, allows for more generalized strain types, more precise control of the localization of bacteria, more extensive *in vivo* residency, and safer removal after treatment. Unlike modifying the bacteria on a molecular basis,

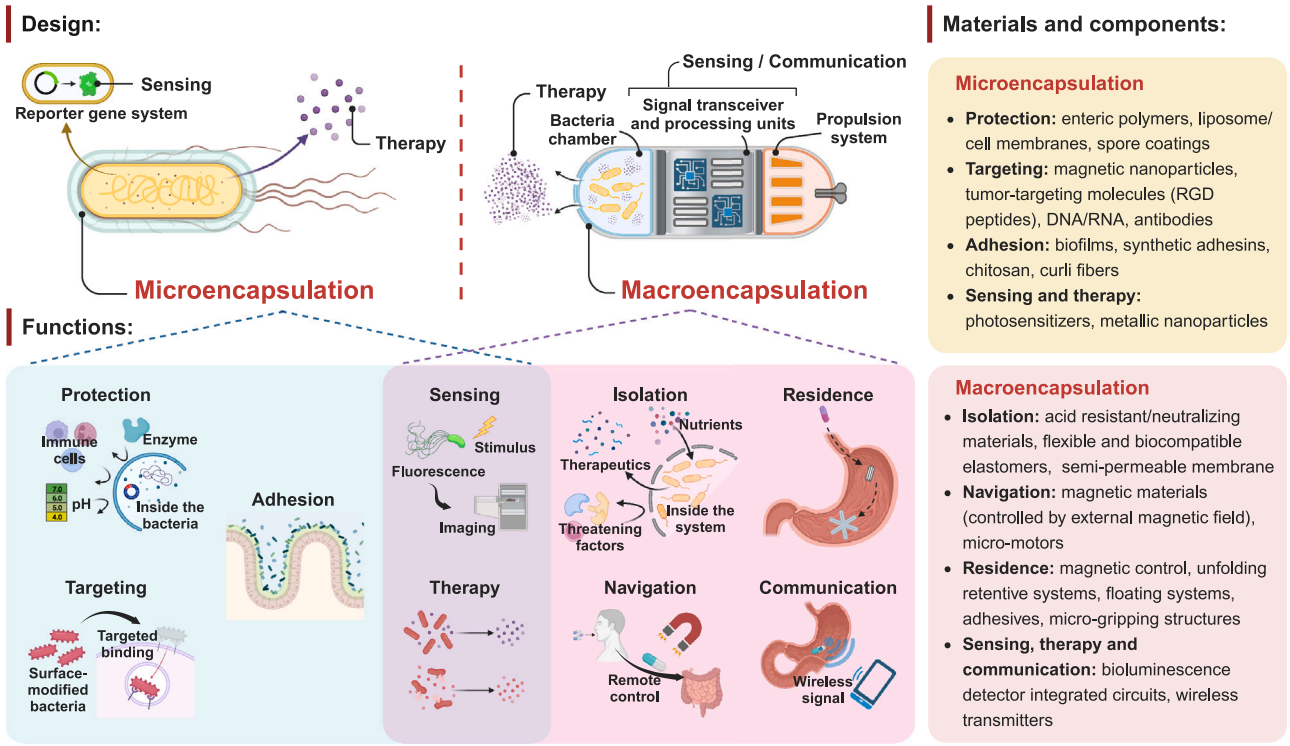

Figure 1. Design rationales for bacterial encapsulations

Summary of the basic design ideas for bacterial micro- and macroencapsulation, as well as the functions that can be realized. In addition to the shared sensing and therapeutic functions, macroencapsulation can realize additional functions such as isolation, residence, navigation, and communication. Materials and components are also enumerated as the basis to help in the realization of these functions.

macroencapsulation provides a systematic, millimeter-to-centimeter-scale platform to store a controlled number of bacteria at a relatively fixed body location.[46,47] Recent advances in microelectronic technologies also allow for the inclusion of wireless signal processing and transmission capabilities,[36,47,48] for the first time allowing real-time readout of bacterial bioluminescent signals from implanted devices.

Despite these promises, research on macroencapsulated bacteria is still in its infancy, and much less literature can be found in comparison to microencapsulated counterparts. The following subsections discuss the functional similarities and differences of micro- and macroencapsulated bacteria. Inspired by existing designs of microencapsulation that are much better studied, we outline some viable design criteria, materials, and components for macroencapsulation (Figure 1). Although largely in the conceptualization and preliminary exploration stage, we envision that macroencapsulation can provide more controllable and otherwise unavailable functions such as isolation, residence, navigation, and communication.

Protection versus isolation

The most fundamental purpose of encapsulation is to protect the bacteria. The protective functions consist primarily of resistance to unfavorable environments (e.g., the acidic environment in the stomach)[49] and avoidance of immune attacks.[38] Microencapsulation achieves these by surface modification, coating, and microcapsules to improve bacterial survival. Taking the unfavorable environment in the GI tract as an example, encapsulations of orally administered bacteria with chemical and biological materials such as enteric polymers,[26] lipid membranes,[50] podocarp polysaccharides,[51] and spore

coatings[52] have shown reduction in the aggression of inappropriate pH and digestive enzymes on bacteria. Dosing a large number of bacteria in a short time can cause acute inflammatory responses as well as rapid clearance by the immune system.[38] In cancer therapy that requires a large dosage, bacteria are encapsulated in erythrocyte membranes as camouflage for the effectiveness and safety of bacterial therapy, which not only reduces the immunogenicity and inflammation produced by the bacteria without affecting their activity but also reduces clearance by the immune system due to the natural anti-phagocytosis ability of the erythrocyte membrane, increasing the enrichment of bacteria at the tumor site.[38,53]

On the other hand, macroencapsulation not only enables protection but also provides an isolated environment that restricts passage of bacteria outside of the encapsulation device. In harsh environments such as the acidic stomach, macroencapsulation can provide protection for millions of bacteria simultaneously using bulk materials, such as acid-neutralizing carbonate[54] and acid-resistant polydimethylsiloxane shells.[36] To establish immune isolation, hydrogels are used to encapsulate a large number of bacteria in the form of relatively large-sized beads, exhibiting the dual functions of protection and preventing their escape.[55] For subcutaneous implants, bacteria are encapsulated with non-degradable hydrogel beads to avoid direct contact with immune cells and extend *in vivo* lifetime.[56] Semi-permeable membranes with pore sizes in the range 220–400 nm have also been exploited to prevent bacterial or cellular leakage while allowing for the entry and exit of nutrients, analytes, and therapeutic products generated by the biologics.[36,47,57] Finally, the systematic removal of bacteria from the human body becomes easier and more controllable, as the macroencapsulation device provides a physical bacterial confinement that effectively isolates the bacteria from surrounding tissues.[47]

Targeting versus navigation

Microencapsulated bacteria typically retain the high motility and chemotactic ability of natural bacteria to achieve targeted accumulation in specific parts of the body. For example, parthenogenetic anaerobic bacteria have high selectivity and colonization ability at hypoxic tumor sites.[58] Bacteria can also be genetically engineered or surface modified to acquire unique chemotactic ability. Specifically, they can be programmed to release from encapsulation in response to certain pH values or presence of enzymes, or they can target specific disease sites after modification by targeting factors or aptamers.[59] Remote control of bacteria can also be achieved by external stimuli including light[60] and magnetic fields.[43,61] Among these, magnetic fields are frequently used as non-invasive and tissue-penetrating driving forces for directing and concentrating therapeutic bacteria modified with magnetic nanomaterials into targeted tissues.[62,63] However, the unfocused nature of magnetic fields limits the *in vivo* localization accuracy of magnetic tweezers. Recently, acoustic tweezers have emerged as a promising tool for biological particle manipulation due to their low damage, high tissue penetration, and relatively high spatial precision at the micrometer scale. Bacteria genetically modified to produce gas vesicles in the cytoplasm have higher acoustic sensitivity and can be driven directionally in the circulatory system under ultrasonic manipulation.[64]

While macroencapsulation restrains the inherent tropism and chemotactic ability of bacteria, such limitation is counterbalanced by providing bulk navigation, i.e., movement of the entire device under external control, similar to a robot. For example, the ingestible magnetic hydrogel containing living bacteria can be controlled by an external magnetic field to navigate through the GI tract.[32] In addition, various electric motors that fit oral capsule sizes have been developed and

demonstrated in ambulating porcine models.[65] In addition to oral administration, wearable sensors[66] and skin wound dressings[67,68] are also common hosts for macroencapsulated bacteria. Often using hydrogels as matrices, these epidermal or transdermal devices provide a portable and easily removable chassis for bacteria.[37]

Adhesion versus residence

Apart from maintaining high bacterial vitality, extending the physical presence of bacteria *in vivo* is equally important to ensure their clinical efficacy. The majority of the reports on *in vivo* retention of microencapsulated bacteria is based on tissue adhesion. Modifying bacterial surface proteins or extracellular appendages such as curli fibers through genetic engineering can improve the mucosal adhesion properties of bacteria.[69] In addition, chemical decoration of catechol functional groups can enhance bacterial adhesion to the intestinal epithelia.[70] Self-produced biofilms of bacteria can be used as biological encapsulation to enhance the survival and adhesion of bacteria in the intestine.[71] Microencapsulation may also reinforce the engraftment of foreign bacteria by facilitating their connection to native intestinal bacteria.[72] Similarly, macroencapsulation can contribute to the *in vivo* residence of bacteria through mucosal adhesion. Bacteria encapsulated in poly(vinyl alcohol) films with tunable formulations can achieve both mucosal adhesion and controlled release after being escorted to the small intestine by enteric capsules.[46]

An approach unique to macroencapsulation that can significantly extend the *in vivo* residency of bacteria is by constructing bacteria-loaded, bulk retentive systems. Miniaturized capsules with a porous structure can be fabricated by emulsification/internal gelation to achieve retention by suspension while protecting the bacteria within the upper GI tract.[73] Gastric retentive systems containing living bacteria can be realized by hydrogels that polymerize *in situ* in the stomach. Specifically, by successive consumption of gel components such as crosslinking agents and alginate, hydrogels with a certain volume and toughness can be created in the stomach, thereby enabling the gastric residence of bacteria for multiple hours.[54] GI retentive systems for sustained drug delivery can also inspire long-term bacteria delivery.[74] For example, unfolding retentive systems or low-density floating systems that can be deployed in the stomach can achieve gastric residence for several months[75,76]; bioinspired adhesive and microgripping structures can enable attachment to the lining of the GI tract[77,78]; and ingestible permanent magnets coupled with external magnetic fields have the potential to enable longer-term immobilization of bacteria-containing devices.[32]

Additional functions

The main function of microencapsulated bacteria is as therapeutics.[79] Living bacterial therapeutics can be based on each of the following principles: bacteria that can release therapeutic agents in a sustained or responsive manner[80,81]; bacteria that can achieve the treatment of diseases by regulating the homeostasis of the human flora,[82,83] for example by inhibiting the growth of harmful bacteria and by preying on the disease-causing organisms; bacteria that promote health through metabolic pathways not encoded in the human genome, such as bacteria modified to metabolize phenylalanine (Phe) in the gut, thereby lowering Phe levels in patients with phenylketonuria[84]; and bacteria that can prevent and treat diseases by influencing the immune system, such as by enhancing the activity and function of immune cells.[85] Among these, the function of bacteria as "living drug factories" has received much attention. Genetically engineered bacteria are capable of releasing therapeutic metabolites—small and large molecules that function as drugs.[80,86,87] Encapsulation of bacteria ensures that these therapeutic molecules are released properly

while avoiding the clearing of the immune system and the threat of bacteria to human safety.

The principle of bacterial biosensors is based on bacterial bioluminescence, fluorescence, and other biological indicators that can be converted to electrical signals.[88,89] The ultra-low detection limit of bacteria and their wide spectrum of analytes enabled by genetic engineering make them increasingly attractive in next-generation health monitoring and disease diagnosis.[90] However, bacteria alone are incapable of actively transmitting signals out of the body without external detection hardware, and thus are not applicable for real-time *in situ* monitoring. One of the key advantages of macroencapsulation is the ability to integrate functional electronic components. Integrating stimuli-responsive bacteria with technologies including on-board fluorescence detectors and wireless signal transmitters, it is possible to monitor trace amounts of biochemicals in previously inaccessible organ parts on a real-time basis, such as hemoglobin in the stomach[36] and inflammatory biomarkers in the intestine.[47] These and other exciting advances make it possible to construct a closed-loop system in which responsive therapeutics are dictated by sensors and their communications are enabled using on-board wireless electronic technologies.[91,92]

IN VIVO APPLICATIONS OF MACROENCAPSULATED BACTERIA

Various forms of bacteria already exert sensing and therapeutic effects through oral, subcutaneous, and intratumoral routes. Among the various routes of administration, macroencapsulated bacteria are mainly applied orally (ingestibles) or epidermally (wearables). Macroencapsulated bacteria are being used much less on tumors compared to intratumorally or intravenously injected microencapsulated bacteria. This may be due to the loss of tumorigenicity (autonomous motility) of macroencapsulated bacteria that are key to the anti-tumor efficacy of bacterial treatment. However, recent studies have used macroencapsulated cells to treat tumors, which could imply potential applications of bacteria in tumor treatment.[31] In this section, we review the sensing and therapeutic applications of macroencapsulated bacteria in different delivery routes (Figure 2). The corresponding encapsulation materials and delivery forms are also summarized in Table 2. We further discuss the challenges associated with long-term implantable bacterial devices (implantables).

Wearables

The epidermal and transdermal routes are heavily studied targets for bacterial sensing and therapy due to minimal invasiveness,[99] controllable removal,[93] and access to bioluminescent signal readout.[66] In these applications, macroencapsulations provide portable carriers for protection, isolation, and enhancement of sensing and therapeutic functions.

For wearable biosensing devices, biocompatible hydrogels show potential as ideal matrices for living materials. Hydrogels of specific composition can exhibit high mechanical toughness and stretchability, which facilitates the fabrication of wearable devices that conform to the skin. Liu et al. designed a set of living materials and devices based on hydrogel-elastomer composites for hosting various types of genetically engineered bacteria.[93] Such composites utilize high stretchability and robustness to prevent bacterial leakage while providing nutrients and breathability to keep them alive. Through genetic engineering, stretchable living sensors were developed that demonstrated a range of applications, including wearable patches to detect chemical secretion from the skin and chemical-sensing wearable gloves

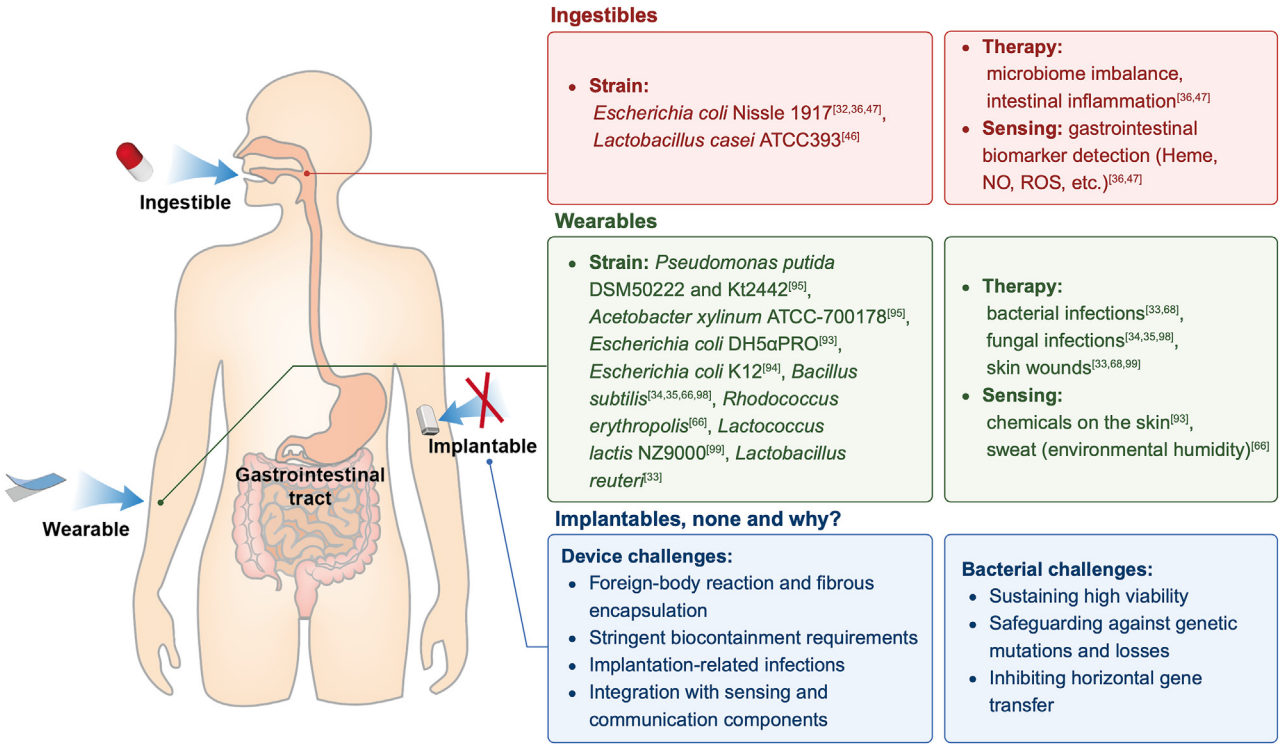

Figure 2. Commonly used macroencapsulated bacterial strains for different delivery routes and their applications

Two routes of delivery of macroencapsulated bacteria, ingestibles and wearables, are illustrated. The strains used in the two delivery routes are listed separately, and their sensing and therapeutic applications are outlined. In addition, challenges associated with long-term implantable bacterial devices are presented. NO, nitric oxide; ROS, reactive oxygen species.

(Figure 3A).[93] To address the issue of low bacterial vitality during device manufacturing and storage, Nguyen et al. proposed the use of freeze-dried, cell-free synthetic circuits instead of living bacteria that were bound to lightweight, flexible substrates and textiles. The resulting wearable materials constructed with cell-free synthetic biology sensors could be used for the detection of small molecules, nucleic acids, and toxins from biological fluids (Figure 3B).[37]

In addition to living hydrogels, engineered bacteria can also be deposited onto specific materials including latex, polystyrene, and cotton to form biohybrid films. Taking advantage of the hygroscopic and biofluorescent behavior of living bacteria, Wang et al. designed a film that can rapidly and reversibly change shape and biofluorescent intensity in response to changes in environmental humidity.[66] The properties of such films can guide the design of sweat-responsive wearables that dynamically adjust ventilation according to the body's cooling needs. In addition, Moser et al. exploited optogenetic control of biofilm formation to pattern EcN 1917 onto different materials, extending the design of responsive living materials.[97]

For transdermal drug delivery involving bacteria, the main forms of macroencapsulation include hydrogel patches and microneedles. Bacterial hydrogel patches are often used to promote wound healing by carrying bacteria that can secrete therapeutic substances, such as antifungal agents[98] and vascular endothelial growth factors.[99] These patches may use thermoresponsive hydrogels[98,99] to control the timing of gelation so that a gel with low viscosity at room temperature undergoes a phase change at body surface temperature and thus adheres better to the skin

Table 2. *In vivo* applications of macroencapsulated bacteria

Route	Type of bacteria	Encapsulation material	Delivery form	Application	Reference
Wearable	*Escherichia coli* DH5αPRO	hydrogel-elastomer hybrids	skin patches, gloves-based sensors	living sensors, interactive genetic circuits, living wearable devices	Liu et al.[93]
	Escherichia coli K12-derivative, NEB Turbo	a thin coating of aerosolized hydrogel, customized resin formulations	3D-printed hybrid living materials	wearable therapeutics or monitoring devices, customizable consumer products	Smith et al.[94]
	Pseudomonas putida DSM50222 and Kt2442, *Bacillus subtilis* DSM675, *Acetobacter xylinum* ATCC-700178	a hydrogel composed of biocompatible hyaluronic acid (HA), κ-carrageenan (κ-CA), and fumed silica (FS)	3D-printed bacteria-functionalized structures with complex shapes	3D cellular structures for bioremediation, complex-shaped synthetic skin scaffolds for biomedical applications	Schaffner et al.[95]
	Escherichia coli DH5αPRO	ink containing Pluronic F127-DA, photoinitiator, Luria-Bertani broth, antibiotic, chemical inducer, bacterial cell pellets, and deionized water	3D-printed living materials	logic gates, spatiotemporally responsive patterning, wearable devices	Liu et al.[96]
	Escherichia coli MG1655, *Bacillus subtilis* (pLS19) (pLS20) wild-type isolate, *Rhodococcus erythropolis*, *Pseudomonasnitroreducens* HBP1, baker's yeast *Saccharomyces cerevisiae*	a bilayer-structured biohybrid film (produced by microprinting microbial cells on the latex surface)	wearable running suits, a fluorescent shoe prototype with bioflaps	moisture-responsive wearables	Wang et al.[66]
	Escherichia coli JF1 (Δcsg)	material coated with bacteria (use light to pattern bacteria onto diverse materials by controlling the expression of curli fibers that anchor the formation of a biofilm)	3D-printed materials, plastics (polystyrene), and textiles (cotton) with cell patterns	wearable devices with responsive living bacterial coatings	Moser et al.[97]
Epidermal or transdermal	*Bacillus subtilis*	growth medium, water, and a thermoresponsive polymer (Pluronic F-127)	thermoresponsive hydrogel	treatment of fungal skin infections	Lufton et al.[98]
	Lactobacillus reuteri	a piece of hydrogel with bacteria-encapsulated hydrogel microspheres immobilized within it	photo-crosslinking hydrogel	clinical management of infected wounds	Ming et al.[33]
	Lactococcus lactis NZ9000	a heparin-poloxamer hydrogel formed by conjugating the heparin with the monoamine-terminated poloxamer	thermoresponsive hydrogel	promoting diabetic wound healing	Lu et al.[99]
	Bacillus subtilis 3610	hydrogel-based porous microneedles	living microneedles	treatment of fungal skin infections	Wang et al.[34]
	Bacillus subtilis 3610	ice microneedles formed by freezing water-containing materials (hydrogel)	living microneedles	treatment of fungal skin infections	Zhang et al.[35]
Ocular	*Bdellovibrio bacteriovorus*	cryomicroneedles prepared by freezing a cryoprotectant medium containing bacteria	living microneedles	treatment of eye infections	Cui et al.[100]
Ingestible	*Lactobacillus casei* ATCC393	polymeric films with sustained release and mucoadhesive functions	oral capsules loaded with multiple films containing bacteria	treatment of gastrointestinal infections and inflammatory bowel diseases	Qiu et al.[46]
	Escherichia coli Nissle 1917	hydrogels containing silica-coated NdFeB microparticles	magnetic living hydrogels	long-term monitoring of the digestive system (gastrointestinal bleeding)	Liu et al.[32]
	Escherichia coli Nissle 1917	integration of bacteria (biosensors) with an electronic sensor and wireless transmission platform in a molded capsule	an ingestible micro-bioelectronic device	ingestible gastrointestinal diagnostics (gastrointestinal bleeding)	Mimee et al.[36]
	Escherichia coli Nissle 1917	a miniaturized device that integrates genetically engineered probiotic biosensors with a custom-designed photodetector and readout chip	a miniaturized wireless bioelectronic pill	ingestible gastrointestinal diagnostics (detecting labile inflammatory biomarkers *in situ*)	Inda-Webb et al.[47]

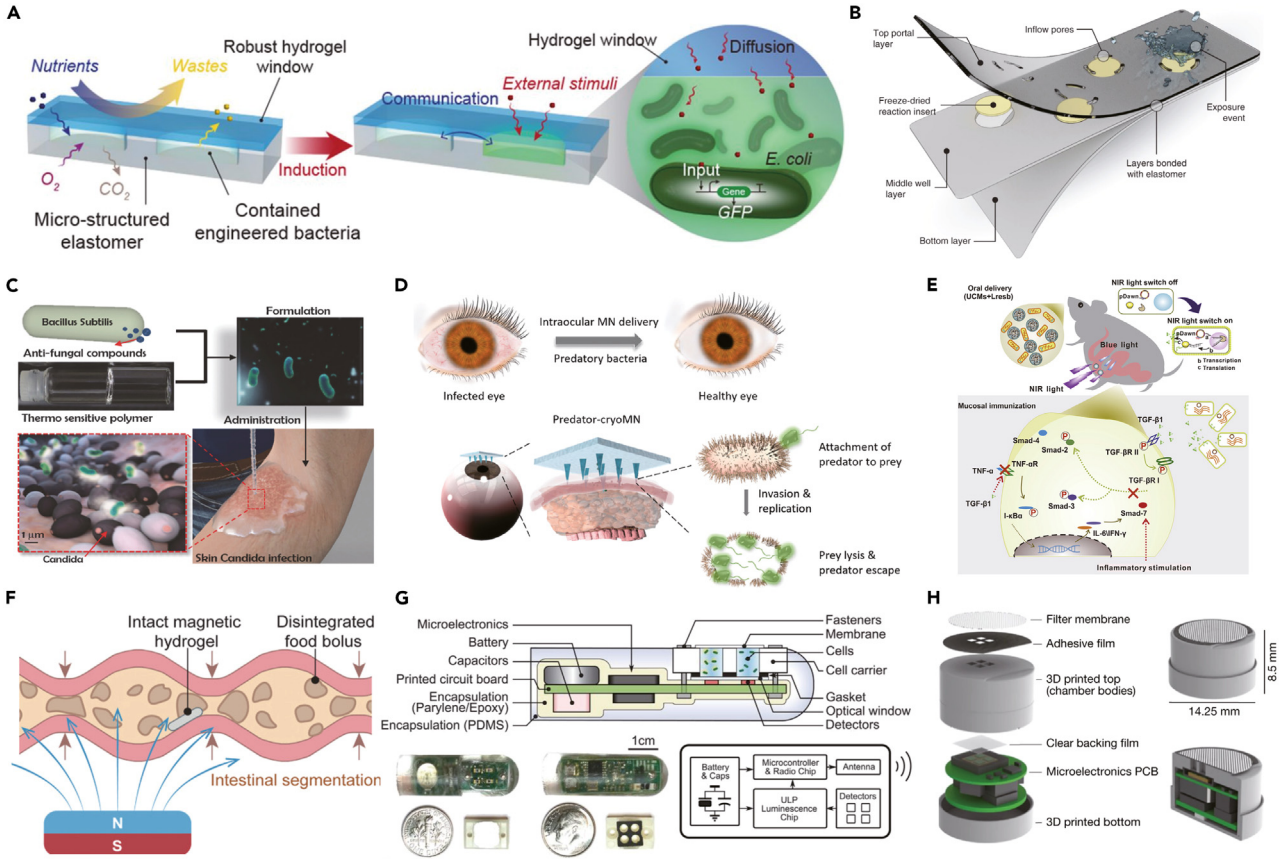

Figure 3. Applications of macroencapsulated bacteria

(A) Hydrogel-elastomer hybrids hosting engineered bacteria. From Liu et al.[93] Copyright 2017, Proc. Natl. Acad. Sci. USA.

(B) Wearable material with embedded synthetic biology sensors. From Nguyen et al.[37] Copyright 2021, Springer Nature.

(C) Thermoresponsive gel with living bacteria. From Lufton et al.[98] Copyright 2018, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

(D) Cryomicroneedles for the ocular delivery of predatory bacteria. From Cui et al.[100] Copyright 2021, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

(E) Live bioglue coatings with NIR light-responsive bacteria for precise colonization. From Cui et al.[39] Copyright 2021, Springer Nature.

(F) Magnetic hydrogel with living bacteria for intestinal residence and diagnosis. From Liu et al.[32] Copyright 2021, Wiley-VCH GmbH.

(G) Bacteria-electronic capsule to detect gastrointestinal health, capable of communicating with external devices. From Mimee et al.[36] Copyright 2018, AAAS.

(H) Miniature capsule integrating bacteria sensor and electronics for diagnosis. From Inda-Webb et al.[47] Copyright 2023, Springer Nature.

and locks the bacteria within (Figure 3C). Light-induced crosslinking has also been investigated to generate *in situ* hydrogel networks at the wound site.[33] Living hydrogel systems incorporating photodynamic antimicrobial properties are also being developed.[101] The extracellular matrix (ECM) loaded with photosynthetic bacteria (PSB) manufactured by Zhao et al. acted as both a photothermal and an anti-inflammatory agent.[102] PSB can convert light into heat to kill bacteria under near-infrared irradiation (NIR) and secrete anti-oxidant metabolites. When combined with the growth factors and nutrients supplied by the ECM, this dual action promotes both expedited wound healing and wound sterilization.[102] In another example, the oxygen-producing bilayer hydrogel developed by Zhu et al. provides the dual function of monitoring infection and repairing tissue for the treatment of refractory anaerobic wounds.[68] The inner hydrogel was laced with the photosensitizer to enable photodynamic therapy (PDT) and incorporated pH indicator to visualize bacterial infections. The outer hydrogel was loaded with photosynthetic cyanobacteria for continuous oxygen production under natural light to alleviate tissue hypoxia and enhance PDT.[68] Other studies utilized bacteria as nitric oxide donors by containing them in

hydrogels to alleviate chronic skin inflammation.[103] In addition, living microneedles using hydrogel[34] or ice[35] as matrices were used to load functionalized bacteria that secrete antimicrobial substances for the treatment of fungal infections. Notably, Cui et al. developed cryomicroneedles to enable ocular delivery of the predatory bacteria *Bdellovibrio bacteriovorus* that effectively inhibited the growth of gram-negative bacteria in an ocular infection model (Figure 3D).[100]

Little work has described bacteria-loaded microneedles for disease monitoring. However, a microneedle sensor array containing a glucose-responsive fluorescent monomer has been developed for continuous and precise monitoring of glucose concentration in tissue fluids.[104] Compared with epidermal sensors, microneedles gain access to transdermal biofluids including blood, which can greatly extend the applications to the detection of blood-specific analytes such as interleukin-6 and tumor necrosis factor α.[105]

Finally, 3D printing of living materials, which combines the fields of 3D printing and synthetic biology, was also explored, enabling the creation of functional structures using living materials containing engineered bacteria.[94–96] This technology offers numerous potential applications in biosensing, drug delivery, tissue engineering, and more. The presence of engineered bacteria could, for instance, help in the production of therapeutic molecules or support the growth and differentiation of surrounding cells in these printed tissues.[96]

Overall, macroencapsulated bacteria are well suited for applications through epidermal or transdermal pathways, as they provide a portable platform that can be accessed by external electronics and safely removed after use. The examples provided above also show that existing systems are currently limited to performing either sensing or treatment, but not both simultaneously. Therefore, the development of closed-loop bacterial devices that integrate monitoring and treatment through algorithms is a promising future direction.

Ingestibles

At a total number of $\sim 10^{13}$–10^{14} that roughly equals the total cell count of the human body,[106] the gut microbiota exists symbiotically with the digestive system and plays a crucial role in human health and well-being such as digestion,[107] nutrient absorption,[108] and immune system regulation.[109] Recent studies in animal models also reveal increasing linkages between gut microbiota and mental health,[110] brain functions,[111] and neurodegenerative diseases[112,113] through the gut-brain axis. The pivotal role of the microbiota in the human digestive system has stimulated interest in engineered bacteria approaches to diagnose and treat diseases. Aligned with the convenience and non-intrusiveness of oral administration, the concept of "bacteriophage therapy" via the oral delivery of live biotherapeutic products has become an emerging area of pharmacological research.[114] Bacteria that are microscopically modified or macroscopically encapsulated both offer advantages over direct delivery of live bacteria throughout the oral delivery process.

Before describing the macroencapsulation of orally administered bacteria, we find it useful to first review existing microencapsulated bacteria approaches to enable stimuli-responsive drug delivery. Stimuli-responsive bacteria generated by surface modification or genetic editing can respond to external energy such as magnetism, light, heat, and ultrasound. These properties enable external control of the location of bacteria as well as the triggering of drug production by bacteria. Buss et al. developed a composite material consisting of magnetized bacteria and microscale magnetic particles that can be orally administered to enhance the *in vivo* localization, retention, and colonization of

therapeutic bacteria by an external magnetic field.[115] To overcome the highly viscous GI environment that impedes bacterial movement, their solution was to amplify the external magnetic field with co-administered magnetic particles, which resulted in a ~50-fold increase in *E. coli* retention in the small intestine of mice. In addition, light-responsive strains using optogenetic engineering potentially provide a viable approach to GI bacterial delivery. Cui et al. designed an up-converting optogenetic microsystem to optically control the state of engineered bacteria to achieve precise colonization in the host GI tract (Figure 3E).[39] The rod-shaped blue-light-emitting nanoparticles within the up-converting microgel can emit localized visible blue light upon excitation via the exogenous NIR light to activate the engineered bacteria, which in turn secretes autotransporter 43 adhesin antigen (AG43) to mediate intestinal adhesion upon activation. They successfully demonstrated *in vivo* therapeutic effects using dextran sulfate sodium-induced colitis mouse models.

Existing reported macroencapsulating materials for oral bacterial delivery include viscous polymer films,[46] biphasic emulsions,[116,117] and hydrogels,[32] but most of them lack the ability to interact with external stimuli. To address this limitation, Liu et al. added magnetic particles to the hydrogel and loaded it with bacteria to make magnetic living hydrogels capable of residing in specific locations in the intestine under the control of a wearable magnet (Figure 3F).[32] Furthermore, ingestible electronics and robots, as a result of advances in miniaturization of electronic components and energy-storage units, are being increasingly exploited as a functional chassis for engineered bacteria. For example, Mimee et al. reported an ingestible micro-bioelectronic device that combined bioluminescent bacteria with electronic capsules for real-time biomarker detection in the stomach (Figure 3G).[36] Bioluminescence generated by the bacteria was processed by an ultra-low-power photodetection chip and could be further transmitted wirelessly to external displays. Using heme-sensitive probiotics, the platform demonstrated real-time detection of GI bleeding in porcine models.[36] A key obstacle to bacteria-loaded electronic capsules is reducing the size of the overall system to prevent GI tract obstruction while maximizing the space for energy-storage units such as silver oxide batteries. Liu et al. addressed this challenge by implementing a custom integrated circuit that could detect ultra-low-intensity light signals from bioluminescent bacteria at a nanowatt power budget, thereby significantly reducing the size of key components.[48] This work demonstrated the first millimeter-scale bacterial-electronic ingestible capsule with a total capsule size of 6.5 mm by 12 mm. Using this chip technology, Inda-Webb et al. developed an ingestible electronic capsule of <1.4 cm^3 that integrates bacteria sensors, a multi-channel photodiode array, and a signal processing and wireless transmission chip (Figure 3H).[47] This platform demonstrated *in situ* detection of unstable transient biomarkers such as nitric oxide and reactive oxygen species in the small intestines. Some of these bacteria-loaded capsules have been successfully tested in porcine models that have GI characteristics similar to those of human adults, thus holding the potential to translate to human-scale diagnoses.

Despite these advances and other progress, during the writing of this section it has come to our attention that very little literature has demonstrated long-term (>24 h) retention of engineered bacteria in the GI tract, despite being the host of the largest microbial community in the body. An important reason behind this is that orally administered bacteria are exposed to an extremely harsh environment, rapid turnover of the GI tract, and immune responses from host microbiota and cells, resulting in a rapid diminution of living bacterial count. Recognizing it as a crucial challenge as well as the importance of oral administration of engineered bacteria, in the next section we review and compare existing approaches to enhancing the *in vivo* engraftment of orally administered bacteria.

Box 1. Encapsulation of probiotic products

Probiotics are a group of live microorganisms that are beneficial to the health of the host when administered in sufficient quantities. To ensure that enough live bacteria reach the colon and effectively colonize after oral administration, proper encapsulation plays an important role. Several encapsulation systems have been used to protect and preserve probiotics in response to a range of existential threats during production, storage, and transport, and in the upper gastrointestinal tract environment, including emulsions, gels, powder granules, nanofibers, electrospray capsules, and nanocoatings.[163] Single or complex emulsification systems consisting of different dispersed and continuous phases are capable of protecting bacterial activity to some extent in unfavorable environments. The basic principle is to utilize the barrier effect of the continuous phase to reduce the environmental pressure on the bacteria in the droplets as the dispersed phase.[164] Gel systems include emulsion gels (gelled emulsion systems) and hydrogels. The former can be formed by adding gelling agents to the emulsion system, while the latter can be categorized as simple, core-shell, and biopolymer-complex microgels depending on the components and structure. Some studies have further co-encapsulated antacids with probiotics in microgels to enhance the effect of resistance to gastric acid.[165] Encapsulation systems in solid granular form also facilitate the survival, storage, and transportation of bacteria. After loading the bacteria in a composite agglomerate, the solvent can be removed by spray drying or freeze drying to form powdered granules. For formulation optimization of probiotic powders, researchers have developed high-throughput pipeline methods for identifying materials that confer microbial tolerance to extreme environments. With advances in electrohydrodynamic processes, electrospun nanofibers and electrospray capsules have also been used as delivery vehicles for probiotics. The principle is to make a polymer solution containing probiotics flow out of a syringe needle under the electric field; the solvent evaporates as the jet passes through the air, and finally the nanofibers or microbeads containing probiotics are collected.[166] In this process, factors such as solution concentration, solution flow rate, and voltage can affect the state (beads or fibers) of the product obtained. All of these encapsulation techniques involve embedding multiple probiotics in a single particle. In addition, utilizing surface nanocoatings to protect individual probiotic cells is a viable approach. Another idea is to change the encapsulated substance, such as co-encapsulating the probiotic with other probiotics, prebiotics, and some healthful substances to create a synergistic effect while favoring the increased activity of the probiotic.

While the technologies described above are constantly being optimized, there is still no single system that can completely protect the viability of the encapsulated probiotics. Furthermore, probiotics need to have the ability to adhere to the lining of the intestine in order to colonize rather than rapidly pass through the body. The size of the encapsulation system also needs to be carefully considered, both to accommodate the bacteria effectively and to ensure safety when taken. In addition, there are many obstacles on the path from laboratory preparation to large-scale commercial production, including economic and practical process limitations. As a result, several new attempts have been made to facilitate the activity protection and intestinal colonization of natural and engineered bacteria through more complex, multifunctional, and integrated delivery systems. The benefits of probiotic delivery systems, especially powdered granules, for long-term storage and transportation are very informative. Inspired by this, combining macroencapsulation with microencapsulation technologies has the potential to further extend the shelf life of probiotic products.

Finally, the growing market for probiotic products that need to remain active during production, storage, and distribution, as well as during their passage through the GI tract, is very much informed by food-grade encapsulation systems (Box 1). These forms of encapsulation, including emulsions, gels, and lyophilized powders, can help bacteria to be placed into macrodelivery devices in a safer and more stable state, and the combination of materials and mechanical means has the potential to shape improved bacterial delivery systems.

Implantables: None, and why?

Implantable devices are integral to modern medicine, offering long-term solutions for various medical conditions and improving patient compliance by providing consistent and efficient treatment. Bacteria, when integrated with these devices, offer a promising solution for long-term drug delivery and biosensing. However,

there are currently no academic reports on long-term implantable bacterial devices. In this section, we aim to investigate the challenges associated with implantable bacterial devices and explore potential solutions.

Among all concerns, *in vivo* safety is regarded as the most significant challenge. Implantation processes generally elicit host responses that include tissue injury, inflammation, proliferation, and tissue remodeling.[118] Upon implantation, the device rapidly becomes coated with proteins and danger-associated molecular patterns from the surrounding tissue.[119] These can lead to the infiltration, adherence, and activation of immune cells, ultimately resulting in the fibrous encapsulation of the implant—a phenomenon known as the foreign-body reaction (FBR).[120] This encapsulation forms a barrier around the implant, limiting access to interstitial fluid, which in turn impairs the function of implantable bacterial devices, whether they serve as sensors or therapeutic delivery platforms. Furthermore, this process can induce excessive inflammation, chronic antigenic stimulation, and even risk of lymphoma.[121]

Common solutions involve using biodegradable materials to mitigate potential long-term health risks associated with non-biodegradable ones.[122,123] Additionally, adjustments to the material's elastic modulus and porosity can be considered.[124] However, implementing these solutions for implantable bacterial devices can be challenging due to new biocontainment requirements. Ideally, implantable bacterial devices should possess permanent encapsulation capability to prevent bacteria from entering the bloodstream and the internal environment, which conflicts with the use of biodegradable materials. Furthermore, research has indicated that specific porosity characteristics, such as those found in crosslinked poly(2-hydroxyethyl methacrylate) with pores in the 30- to 40-μm range, can lead to non-fibrotic, vascularized tissue integration in the host tissue.[125,126] However, it is worth noting that bacteria are typically much smaller in size, ranging from 1 to 2 μm. Besides, there is a risk of smaller cellular contents such as lipopolysaccharides leaking into the surrounding environment after bacterial death. Further research is therefore warranted to explore the relationship between materials with smaller porosity and the body's immune response. Additionally, it is necessary to develop non-degradable materials that strike a balance between effective biocontainment and excellent biocompatibility.

Infection poses another significant and complex challenge for implantable bacterial devices. The FBR and subsequent fibrous encapsulation can create an immune-depressed niche, rendering the implant more vulnerable to microbial colonization and infection.[127] Moreover, implants provide surfaces that facilitate bacterial attachment and biofilm formation, further enhancing their ability to flourish in the hostile host environment.[128] While infections usually originate from microbial contamination during surgical procedures, achieving effective sterilization becomes a crucial yet intricate endeavor. In the context of implantable bacterial devices, there is an urgent need to prioritize sterilization methods that ensure the implant's sterility without compromising the viability of the enclosed bacteria. Besides this, the utilization of genetically engineered microorganisms carries inherent risks. Encapsulation failure may lead to the invasion of engineered bacteria into the bloodstream, potentially causing bacteremia or sepsis.[129] Through horizontal gene transfer, engineered bacteria may engage in unpredictable interactions with the resident microbiota, including the transfer of antibiotic resistance cassettes, posing serious risks and potential harm to patients.[130]

Apart from safety concerns, there are additional challenges on the application side. The preservation of bacterial viability and stability are critical factors that

must be carefully considered to maximize the effectiveness of long-term implant delivery. Ensuring the continued viability and functionality of engineered bacteria within the host over an extended duration while safeguarding against genetic mutations and losses poses another significant challenge.[79] Managing the bacterial population within the devices is crucial, with total cell count needing to meet functional requirements while remaining controllable. Unchecked bacterial proliferation can lead to nutrient depletion, waste accumulation, increased survival pressures, and reduced metabolic activity.[131] Implementing a quorum-sensing system, such as the LuxI/LuxR system, is advisable to autonomously regulate the population density of probiotics such as *E. coli*.[132] Additionally, aerobic organisms and facultative anaerobes, like *E. coli*, thrive in the presence of oxygen. Reduced oxygen levels in the body can pose challenges. The approach reported by Krishnan et al. presents a potential solution by oxygenating cells through electrolytic water vapor splitting within the implanted cell chambers.[57] Furthermore, looking ahead to extensive use of implantable bacterial devices as sensors, integrating the system with sensing and communication components, such as signal transceivers and processing units, is anticipated. Concerted efforts are needed to develop high-efficiency wireless battery-charging systems for extended operational duration through the body.[123]

In summary, advancing bacteria-encapsulated implants requires further development of biomaterials with long-term biocompatibility, stability, and biocontainment. Simultaneously, it is crucial to explore methods for safeguarding against genetic mutations and losses as well as for on-demand bacterial population management. Additionally, special attention needs to be devoted to the improvement of large-scale sterilization and preparation processes to facilitate clinical translation.

SYNTHETIC ENGRAFTMENT OF ORALLY ADMINISTERED BACTERIA

Introduction of bacteria into the human body is the very first step, while the realization of their sensing or therapeutic role requires the effective completion of *in vivo* engraftment. Successful bacterial engraftment is demonstrated by the proper functioning of response and secretion, which depends on bacterial activity, colonization ability, and residence time. Although many reported engineered bacteria are administered orally, they are subject to attacks from digestive enzymes, stomach acids, and the host immune system before reaching the small intestines—the most common engraftment site for GI microbiota—which significantly reduce their vitality and chances for successful engraftment.[133] To address these issues, various material-based approaches have been developed to protect active substances and macromolecules, including micromotors,[134] microcapsules,[135] and surface coating.[136] However, they still face multiple challenges after reaching the intended locations in the GI tract, such as resistance from host microbiota and rapid shedding time of intestinal epithelial cells, which further impede long-term engraftment. As such, in this section we discuss and compare various strategies to enhance the engraftment of orally administered bacteria (Figure 4A) and their performance metrics (engraftment time versus total bacterial dosage, Figure 4B).

Multicellular encapsulation

Monolithic encapsulation of multiple bacteria can be done in the form of emulsions or gels. The surface hydrophilicity of most bacteria allows them to be loaded in the inner water phase of water-in-oil or water-in-oil-in-water emulsions, whereas the barrier effect of the outer phase gives the bacteria better stability in the digestive environment.[137] Other material systems such as semi-solid emulsion gels[116] and

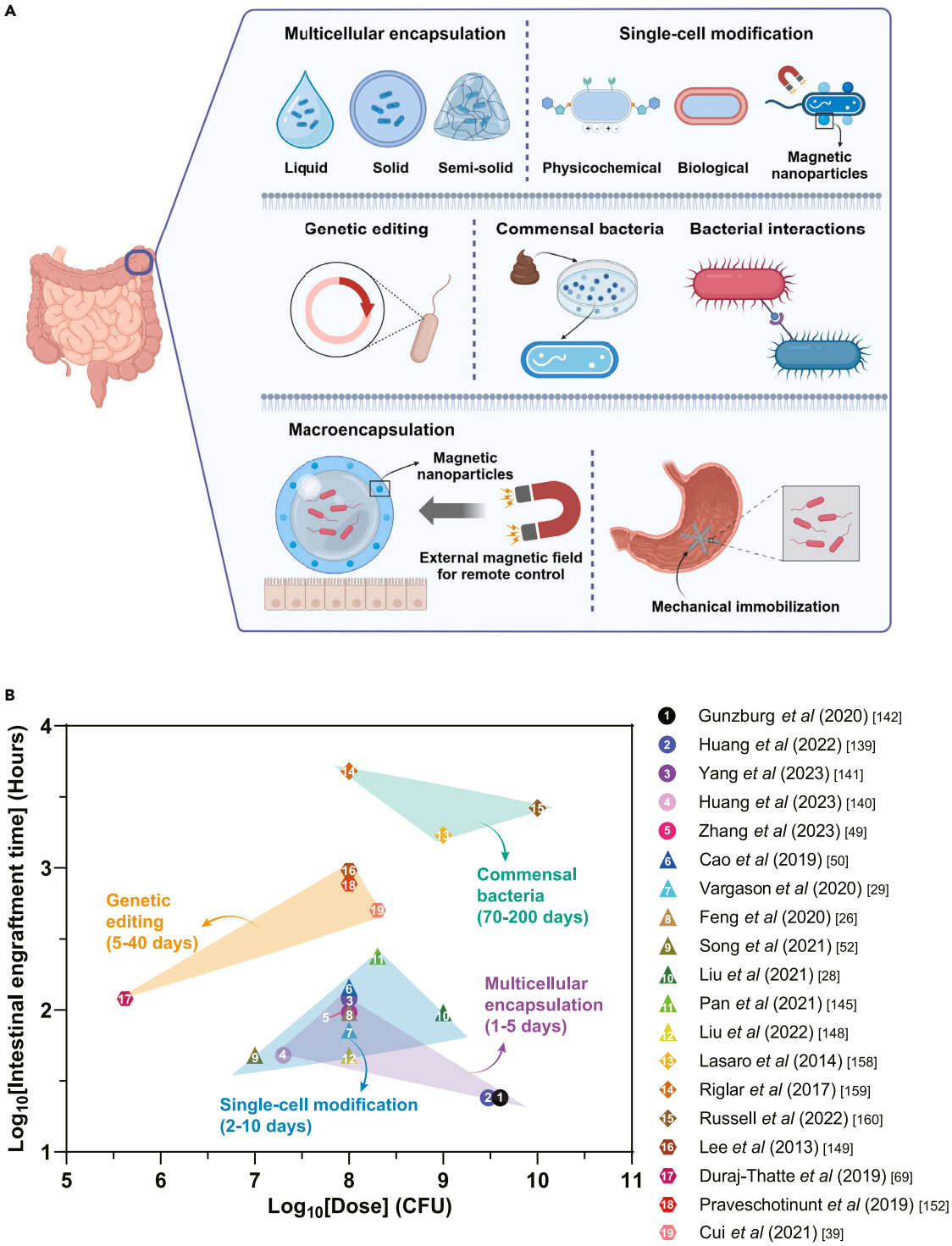

Figure 4. Various approaches to oral bacterial engraftment and comparison of their retention effectiveness

(A) Different approaches to assist engraftment of orally administered bacteria.

(B) Comparison of bacterial dose and engraftment time for different approaches.

hydrogel-based microcapsules or beads[117] have been applied to encapsulate hydrophobic or hydrophilic active agents for oral bacterial delivery. The above methods have been shown to increase bacterial activity in simulated gastric or intestinal fluids, but there is little validation of *in vivo* engraftment. Recent studies have showed that bulk hydrogels can serve as protective carriers of larger numbers of bacteria, but only 24–48 h of bacterial residency was verified in mice.[138–140] Yang et al. utilized calcium tungstate microgel as an effective oral delivery system that not only resisted the harsh environment of the GI tract but also achieved bacterial colonization of the intestines for more than 96 h.[141]

On the other hand, polymeric microspheres can exhibit both resistance to gastric acid and enhancement of intestinal residence; for example, cellulose sulfate microspheres[142] helped bacteria to achieve 24-h engraftment in mouse intestines. It is noteworthy that mucosal adhesion of bacteria can be further enhanced by chemical bonding connections on top of the protective encapsulation of microspheres. For instance, thiolated oxidized konjac glucomannan (sOKGM) microspheres[143] could rupture responsively at intestinal pH after protecting bacteria through the stomach. The thiolated sOKGM polymers were then released to interact with the bacteria and mucus layer via disulfide bonding, which allowed for up to 1 month of long-term bacterial adhesion and proliferation in the gut.

Overall, this type of multi-bacterial amorphous encapsulation serves multiple purposes: it protects the bacteria from the external environment, facilitates dispensing and storage, and enables an extended *in vivo* residence time. However, the uncontrolled swelling and chemical instability of beads and gels may lead to leakage of the encapsulated bacteria and may require additional surface modification treatments such as coating.[144] This approach also prevents direct contact of individual bacteria with the intestinal surface, thus reducing their adhesion and engraftment at specific sites and therefore limiting their residence time in the body.

Single-cell modification

Unlike multicellular encapsulation, modifying the surface of individual bacteria results in less disruption to bacterial growth and better interaction with the GI mucosa. These microencapsulation strategies also reduce the immunogenicity and pathogenicity of bacteria by camouflaging them[38] or achieve more precise control of bacterial activities by conferring specific exogenous functions.[21]

Surface decoration of individual bacteria can be categorized into physicochemical and biological methods. The former may include covalent coupling reactions and electrostatic and/or hydrophobic interactions.[21] For covalent conjugation, Pan et al. reported co-deposition of polydopamine onto bacterial surfaces to provide better versatility for adding different modifiers. The method improves bacterial retention in the mouse intestine by more than 30-fold compared to unmodified bacteria.[145] This type of modification has drawbacks, however, such as the creation of thick shells that may compromise bacterial activity. Another widely used approach exploits electrostatic interactions, as the outer membrane of most bacteria is negatively charged.[26] This modification can be carried out by layer-by-layer self-assembly to enhance bacterial resistance and adhesion, thus favoring rapid multiplication of bacteria within the first few hours of their entry into the GI tract.[25]

Whereas chemical modifications may cause damage to the bacteria, biological modifications offer better biocompatibility and safety. For example, natural cell membranes have been applied to enhance biocompatibility and targeting properties

of the bacteria while protecting the bacteria from host immune systems through immune camouflage.[38] Modifications can also be made through ligand-receptor interactions, which do not encapsulate the entire bacteria but occur only at specific sites, thus allowing for site-specific modifications while minimizing the impact on its interaction with the environment. Inspired by bacterial adhesins, Vargason et al. developed synthetic adhesins to bind to bacterial surfaces in the form of antibodies, resulting in colonization for approximately 3 days.[29] The modification effect dwindles over time, however, as affixed ligand is diluted due to bacterial growth.

There are various pathways to improve bacterial engraftment via single-cell surface modifications. Most surface modifications improve bacterial survival *in vivo*; for example, the protective effect of lipid membranes can help achieve a 6-day intestinal colonization of bacteria.[50] Therapeutic nanocoatings can also be used to exert drug activity while improving *in vivo* bacterial vitality.[146,147] In addition, many approaches have been developed to enhance bacterial adhesion to the intestinal surface, thereby prolonging *in vivo* bacterial retention. Coatings of phenols,[70] polynorepinephrine,[148] tannic acid,[28] and self-produced biofilms of bacteria[71] can increase the mucosal adhesion of bacteria by up to 11 times. *In situ* chemical reactions can also be utilized to increase the adhesion of bacteria to intestinal surfaces by as much as 170-fold.[27]

Overall, single-cell surface modifications offer a promising and effective approach to extend bacterial engraftment.[29,52] However, most of these approaches are cumbersome and require genetically tractable strains in conjunction with strain-specific modifications. The thickness of the microencapsulation also needs to be well controlled so as not to impede normal activities of the bacteria.

Genetic editing

Genetic editing, a means of integrating foreign genes and modifying specific endogenous genes on demand, has been widely adopted for enhancing the *in vivo* vitality and adhesion of engineered bacteria. For example, it has been shown that specific genes such as the commensal colonization factors (*ccf*), which are conserved among intestinal *Bacteroides*, can profoundly affect the intestinal colonization of bacteria.[149] A deeper understanding of these specific genes or domains could facilitate the regulation of bacterial colonization and adaptation in the mammalian gut.[150] Mays et al. demonstrated control of the mucus-binding properties of probiotics by heterologously expressing or altering surface proteins and cellular components.[151] In addition, by using optogenetics, recombinant light-responsive bacteria can secrete substances that enhance intestinal adhesion for up to 21 days in response to specific light stimuli.[39]

Extracellular appendages are closely related to bacterial adhesion and can be genetically engineered.[152] For example, the biofilm-integrated nanofiber display system designed by Nguyen et al.[153] made it possible to endow *E. coli* biofilms with a variety of artificial functions, such as substrate adhesion and covalent immobilization of proteins, through genetic engineering. Additionally, Duraj-Thatte et al. described an engineered living material using *E. coli* as its cellular chassis and engineered curli nanofibers as its extracellular matrix component.[69] The material exhibited *in situ* regeneration properties and could remain in the mammalian GI tract for more than 5 days. Their team also demonstrated the use of engineered curli fibers to rationally program interactions between bacteria and mucosal epithelial components.[154]

While improving GI residency, genetically engineered bacteria may induce transgenation to the commensal bacteria and potentially threaten the native microbiome. Also, accurate strain selection and genetic modification are critical to achieving targeted outcomes, which increase design complexity and limit the scaling up of production.

Commensal bacteria and bacterial interactions

An intuitive yet highly effective approach to enhance engraftment of engineered bacteria is to choose a starting strain that already lives in the host. Introducing foreign bacteria into the host microbiota can be challenging and often necessitates pretreatment with antibiotics, whereas using native bacteria that already flourish in the host microbiota avoids the pre-emptive use of antibiotics and thus can survive much better.[155] For probiotic supplementation, probiotics of native origin, low immunogenicity, high bacterial content, and with colonizing sites are often selected.[156] These probiotics can act as the dominant intestinal bacteria, performing beneficial functions such as nourishing the intestinal tract, regulating immunity, balancing the bacterial flora, and repairing the damaged intestinal barrier.[157] Based on the growth advantages of commensal bacteria, their diagnostic and therapeutic functions continue to be exploited.[158] Riglar et al. designed a commensal murine *E. coli* strain that could maintain the ability to respond to inflammation in the mouse gut for at least 6 months, enabling prolonged disease monitoring.[159] Similarly, *E. coli* isolated from fecal cultures of conventionally raised mice by Russell et al. could be reintroduced into mice after modification to achieve engraftment for a record-holding duration of 110 days.[160]

Studies have also explored the optimization of residency and therapeutic efficacy through mutually beneficial or foreign-native bacterial interactions. Diverse bacterial strains engage in various interactions including mutualism, aggression, and competition. Utilization of these interactions fosters the desired colonization effect. In the gut microbiota, metabolic exchange between microorganisms can enhance colonization and influence host physiology. Yahav et al. proposed utilization of the extracellular matrix produced by *Bacillus subtilis* to protect other bacteria, and the resulting co-culture system contributes to superior survival under simulated intestinal conditions.[161] Song et al. employed bio-orthogonal techniques to achieve probiotic colonization for at least 48 h by modulating bacterial adhesion between probiotics and gut inhabitants.[72]

Nonetheless, challenges to these approaches remain. First, there are barriers to culturing and modifying native bacteria. For instance, isolated human *E. coli* are highly resistant to genetic manipulation.[158] Second, commensal bacteria often exclude similar invasive strains and may require targeted dietary support to achieve strain substitution. Third, any functionality engineered into natural bacteria will likely reduce bacterial fitness.[160] Therefore, how natural bacteria maintain engineered functions over time without selective pressure needs to be explored.

Synthetic engraftment by macroencapsulation

The above techniques enhance bacterial engraftment in various ways, but they are mutually impacted by the fast shedding time of surface epithelia, as they all rely on microscopic interactions with the mucosal surface and are unable to precisely control the location of bacterial engraftment in the GI tract. Alternatively, macroencapsulation systems may provide a perpetual and localizable platform to achieve "synthetic engraftment" of engineered bacteria in the GI tract through mechanical immobilization,[78] magnetic field control,[32,65] and other diversified approaches.[74]

For example, magnetic hydrogels loaded with bacteria are capable of *in vivo* localization under the control of wearable magnets.[32] Advances in various forms of GI residence devices and ingestible robots may also serve as hosts for engineered bacteria.[162]

Overall, what can be envisioned is that macroencapsulation has the potential to greatly extend the time of synthetic engraftment *in vivo* applied to a wide range of bacterial strains regardless of its origin. The integration of bacteria and devices is poised to facilitate the realization of telematic messaging and externally controllable precision therapy, and potentially form an intelligent closed-loop system from sensing to therapeutics.

OUTLOOK

Bacterial therapies have exhibited encouraging outcomes in preclinical studies, motivating more and more efforts toward commercialization. Nonetheless, the transition from these achievements to universally accepted clinical products represents a daunting endeavor, subject to the resolution of critical challenges such as *in vivo* safety, efficacy, precision, and visualization of bacterial therapeutics.

As such, the macroencapsulation of bacteria offers a generic and controlled solution for isolating bacterial cells to mitigate immunogenicity and toxicity. However, the exploration of this pathway introduces new challenges, including the safety concerns of the macroencapsulation systems, restrictions on the quantity of loaded bacteria, and the need to reduce the dimensions of the overall devices.

Addressing these issues requires interdisciplinary collaboration. The continued development of highly biocompatible biocontainment materials holds the promise of offering a fresh perspective in this direction. Further discoveries in genetic editing of engineered bacteria can provide insights into extending the stability and viability of these bacteria. Additionally, leveraging the design freedom in geometries and materials offered by microencapsulation approaches can facilitate the creation of tailored delivery systems for specific sites, including tumors, skin, and various segments of the GI tract.

The GI tract hosts the most densely populated microbiota in the human body and is easily accessible via the oral route. However, it also presents one of the most challenging environments due to harsh pH conditions, constant peristalsis, epithelial shedding, and resistance to colonization by the native GI microbiota. Integrating engineered bacteria with GI retentive systems such as star-shaped ingestible devices offers a potential solution to overcome the aforementioned challenges in the GI tract, enabling continuous, *in situ* production of therapeutic small molecules or long-term biosensing in the GI tract for months. Moreover, through genetic engineering, surface modification, and the use of acidic buffer materials, it is believed that bacterial survival in the harsh gastric environment can be further enhanced. For example, the method described by Liu et al. involves the development of a hydrogel based on 4-arm poly(ethylene glycol) maleimide. When co-encapsulating with calcium carbonate, this system demonstrates enhanced capability in protecting bacterial viability in low-pH gastric fluid.[54]

For future applications, engineered bacteria have the potential to provide a suitable chassis for integrating sensing and therapeutic capabilities through genetic engineering, allowing for closed-loop treatment. Consequently, electronic components integrated into macroencapsulated systems can collaborate with these bacteria to

offer additional sensing and control functions.[47] Furthermore, the development of externally responsive macroencapsulated systems could enable remote manipulation of bacteria for controlled drug release and therapeutic interventions. These synergic integrations represent promising future research directions toward constructing a dynamic framework for detecting physiological signals, tailoring treatments to individual needs, and remotely and systematically guiding therapeutics.

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AUTHOR CONTRIBUTIONS

Conceptualization, Y.L., H.H., and K.N.; investigation, Y.L., H.H., and Y.S.; writing – original draft, Y.L., H.H., and Y.S.; writing – review & editing, Y.L., H.H., Y.S., B.Y., W.-C.L., K.D., N.D., R.S.L., Z.G., and K.N.; funding acquisition, B.Y., Z.G., and K.N.; resources, R.S.L., Z.G., and K.N.; supervision, R.S.L., Z.G., and K.N.

DECLARATION OF INTERESTS

For a list of entities with which R.L. is or has been recently involved, compensated or uncompensated, see https://www.dropbox.com/s/yc3xqb5s8s94v7x/Rev%20Langer%20COI.pdf?dl=0.

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