ACS APPLIED BIO MATERIALS

www.acsabm.org

Review

Electrospinning Living Bacteria: A Review of Applications from Agriculture to Health Care

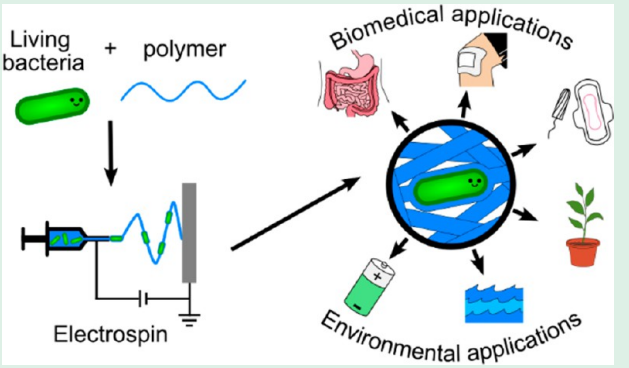
Emily Diep and Jessica D. Schiffman*

Cite This: *ACS Appl. Bio Mater.* 2023, 6, 951−964

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Living bacteria are used in biotechnologies that lead to improvements in health care, agriculture, and energy. Encapsulating bacteria into flexible and modular electrospun polymer fabrics that maintain their viability will further enable their use. This review will first provide a brief overview of electrospinning before examining the impact of electrospinning parameters, such as precursor composition, applied voltage, and environment on the viability of encapsulated bacteria. Currently, the use of nanofiber scaffolds to deliver live probiotics into the gut is the most researched application space; however, several additional applications, including skin probiotics (wound bandages) and menstruation products have also been explored and will be discussed. The use of bacteria-loaded nanofibers as seed coatings that promote plant growth, for the remediation of contaminated wastewaters, and in energy-generating microbial fuel cells are also covered in this review. In summary, electrospinning is an effective method for encapsulating living microorganisms into dry polymer nanofibers. While these living composite scaffolds hold potential for use across many applications, before their use in commercial products can be realized, numerous challenges and further investigations remain.



KEYWORDS: *bacteria, cells, delivery, electrospun, fibers, encapsulation, probiotics*

1. INTRODUCTION

Bacteria, while infamously known for pathogenic infections, play a major positive role in everyday life from helping with digestion to creating electricity.[1] In general, bacteria grow easily in nutrient-rich liquid media or in fermented foods like yogurt, kombucha, sauerkraut, kimchi, etc.[2] While ideal for bacterial growth, liquid cultures are only viable for a few days and tend to be difficult to maintain and transport.[3] Smaller frozen stocks of bacteria are easier to transport than liquid vats, remain viable for up to 3 years, and reduce chances of contamination.[4,5] Unfortunately, ice formation can puncture and kill bacterial cells. Moreover, maintaining low temperatures during transport and long-term storage is a costly challenge.[6] Dried bacteria can be used as powders that facilitates their transport, storage, and processing into various supplements.[4,7] Conventional drying methods lead to bacterial death because of heat and dehydration,[4] whereas freeze drying or spray drying allow the bacteria to go into a dormant state while maintaining higher viability.[8] To overcome these aforementioned obstacles, cell encapsulation technologies have been explored.

Bacterial encapsulation is a technique that protects the bacteria by forming a physical barrier to support the cell structure and reduce contact with damaging agents.[9] For example, during freeze-drying, bacteria that were encapsulated within rice proteins had higher viability after processing, during storage at various temperatures, and in simulated gastrointestinal tract environments. While numerous different approaches—spray drying, extrusion, emulsion, microfluidics, and 3D printing—are effective methods of encapsulating living microbes into polymer materials, additional drying can be required to store the bacteria over long periods of time.[5,10−14]

Alternatively, electrospinning allows live bacteria to be encapsulated within multifunctional, polymer nanofibers at room temperature.[15−19] The electrospinning process does not require the use of heating, freezing, or organic solvents that can harm encapsulant materials. Thus, suspensions of bacteria are blended into various synthetic and naturally derived polymer solutions for electrospinning. Biopolymers dissolved in aqueous solutions are commonly used as they promote the viability of encapsulated cells within the fibers.[20] Thus,

Received: December 20, 2022
Accepted: February 2, 2023
Published: February 15, 2023

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

biologicals like cells,[17,21,22] proteins,[23] antibiotics,[24,25] and drugs[26−29] have been encapsulated via electrospinning; the high surface area of the cargo-loaded nanofiber mats enabled excellent delivery characteristics.[30] In conjunction with their excellent flexibility and porosity, nanofiber mats with encapsulated biologics hold great potential in wound dressing, protective coatings, and biological scaffolds.

In this review, we will first provide an overview of the electrospinning process while highlighting the key parameters involved with the electrospinning of living bacteria, such as polymer selection, solvent choice, bacteria loading, applied voltage, and electrospinning environment. Notably, since several reviews[9,13,31−33] have covered the encapsulation of bacteria for the delivery of probiotics, we will focus on discussing only bacteria encapsulated within nanofibers during electrospinning (as opposed to bacteria inoculated onto electrospun structures after spinning), as well as recent innovations for the delivery of electrospun probiotics and emerging environmental applications that demonstrate the advantages of these bacteria-loaded nanofibers.

2. BRIEF OVERVIEW OF ELECTROSPINNING

The term "electrospinning" comes from a combination of the words "electrostatic" and "spinning" where "electrostatic" refers to the application of an electric field onto a precursor polymer solution to "spin" or form a polymer fiber.[34] As such, the electrospinning setup (Figure 1) often consists of a

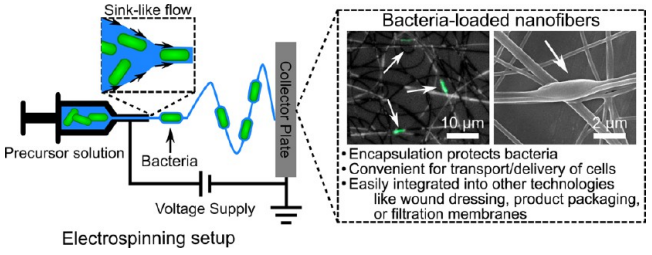


Figure 1. Illustration of the single-nozzle electrospinning setup and the sink-like flow that encapsulates living bacteria cells into the polymer nanofibers. The inset (left) fluorescent and (right) SEM micrographs show the successful encapsulation of live, green-fluorescent protein-producing *Escherichia coli* in electrospun alginate-based nanofibers. The arrows show the location of the cells. Electrospinning safely encapsulates bacteria within a polymer matrix that can deliver the cells to various environments and can easily be incorporated into technologies like wound dressing, product packaging, and filtration membranes. The micrographs are reproduced with permission from ref 22. Copyright 2021 The Royal Society of Chemistry.

polymer solution or melt contained within a syringe with a metallic needle tip, a pump used to extrude the polymer at a constant rate, a metallic collector plate, and a voltage supply connecting the needle tip to the collector plate.[35,36] When the electric field is applied, the precursor solution becomes charged.[36] A buildup of charges on the surface of the polymer solution causes the droplet of the precursor to deform into a Taylor cone and eject a jet of polymer. Stabilizing forces, such as the viscosity of the precursor solution, prevent the electrospinning jet from fracturing into beads. The radial electric forces cause the jet to bend, which evolves into coiling and whipping of the jet that helps to expel solvent from the nanofiber. Eventually, randomly collected nanofibers are deposited onto the collector plate as a nonwoven mat.

Many electrospinning parameters, including precursor solution properties (polymer, solvent, surface tension, etc.) and experimental parameters (needle tip-to-collector distance, needle diameter, humidity, temperature, etc.), affect the diameter and morphology of the electrospun nanofibers, as covered in depth by other publications.[27,35−38] This review will focus on the parameters that potentially influence either the loading or viability of electrospun bacteria, including polymer selection, solvent selection, bacterial precursor solution concentration, applied voltage, and electrospinning environment.

3. EFFECTS OF ELECTROSPINNING PARAMETERS ON THE ENCAPSULATION OF LIVING BACTERIA

The most common way of fabricating nanofibers that contain bacteria is to blend the bacteria into the electrospinning precursor solution. During the formation of the electrospinning jet, the bacteria are pulled into a sink-like flow (Figure 1), which encapsulates them within individual fibers that are collected as a nonwoven mat.[17,21−24] An example of bacteria encapsulated in electrospun nanofibers is shown in the inset of Figure 1. Fluorescence microscopy was used to detect and visualize the live, green fluorescent protein (GFP)-producing bacteria.[22] The bacterial cell presents as a bulge along the nanofiber in the scanning electron microscopy (SEM) image because the diameter of the cell is larger than that of the electrospun nanofiber.

Another popular approach used to encapsulate bacteria cells within fibers is the use of coaxial electrospinning (Figure 2) to

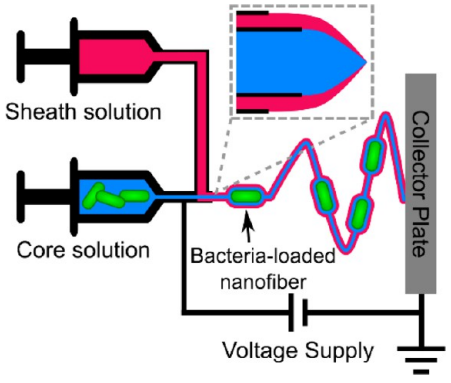


Figure 2. Illustration of the coaxial electrospinning setup for the encapsulation of living bacteria cells within nanofibers. The inset image highlights that the two nozzles become concentrically aligned. The outer precursor solution can entrain and/or protect the bacteria cells, which are typically loaded into the precursor solution that is advanced from the inner nozzle.

simultaneously administer two solutions from concentric needles that are drawn into a singular electrospun nanofiber mat. A carrier precursor solution can be extruded from the outer needle to entrain a potentially "non-electrospinnable" inner solution, or a carrier inner solution can pull along the outer solution because of internal viscous and viscoelastic forces, which is a method typically referred to as liquid-assisted electrospinning.[39,40] Coaxial electrospinning is extremely beneficial for the encapsulation of cargo, like drugs and cells, which cannot be solely electrospun. For example, poorly water-soluble drugs, like flurbiprofen axetil[41] and dexamethasone,[42]

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

have been encapsulated and released by optimizing the inner and/or outer solution flow rates and polymer composite systems.[41−43]

3.1. Polymer and Solvent Selection. For the precursor solution, the use of biocompatible polymers is recommended because they are nontoxic and promote bacteria viability. Poly(ethylene oxide) and poly(vinyl alcohol) are synthetic linear polymers commonly electrospun into fibers because of their ease of electrospinning.[17,35,44,45] When dry, these polymers also have a high oxygen barrier that can protect oxygen-sensitive bacteria after encapsulation in the polymer nanofibers. Natural polymers are also advantageous materials for bacteria electrospinning because of their biocompatibility. In particular, the biopolymer alginate is compatible with bacteria because it is a polysaccharide produced by bacteria to make up their protective biofilm.[46,47] Other natural polymers, like chitosan, have antibacterial properties.[48] Therefore, polymer choice should be carefully considered in terms of compatibility with bacteria cells and the environment of its intended use.[49]

Aqueous solutions are preferred over other solvent systems when electrospinning bacteria to maintain their viability.[31] Though organic solvents can be used to influence the flexibility and electrospinnability of polymers, they are generally avoided in cell electrospinning because of their toxicity.[33] One study encapsulated live bacteria into an organic solvent-based precursor solution using a coaxial electrospinning setup.[50] The bacteria were suspended in an aqueous solution that was extruded into the core needle, whereas a polymer dissolved in organic solvent was extruded from the sheath needle. The researchers incubated the nanofibers in a nutrient-rich broth to regenerate the bacteria activity after contact with the organic solvent.

3.2. Bacteria Concentration within the Precursor Solution. The concentration of bacteria loaded in the precursor solution influences the encapsulant concentration in the final fiber. For the encapsulation of *Lactobacillus plantarum* in poly(ethylene oxide),[51] the increase of the bacteria loading in the precursor solution (colony forming units/mL or CFU/mL) by 10-fold led to a 1000-fold increase in the bacteria concentration in the nanofiber mat (CFU/mg). Similarly, Kurečič et al.[52] found that increasing the bacterial loading of *Staphylococcus epidermis* from 1.1×10^9 to 1.6×10^9 CFU/mL led to the bacteria loading increasing from $6.9 \pm 1.8 \times 10^5$ to $2.0 \pm 0.3 \times 10^6$ CFU/g of their carboxymethylcellulose and poly(ethylene oxide) composite nanofiber. They also reported that the higher bacterial suspension led to a change in solution viscosity and conductivity. While solution properties are known to influence the formation and morphology of electrospun fibers,[22,27] more studies should investigate the effects of solution properties on the encapsulation of materials during electrospinning. Additionally, viability likely depends on the strain of bacteria encapsulated because some strains of bacteria could be more resistant to mechanical or oxidative stresses.[17]

3.3. Applied Voltage. The major reasons for cell death during electrospinning are attributed to cell rupture because of electrical forces, mechanical forces, and dehydration.[17,18,53] However, numerous studies have shown that bacteria cells survive the applied voltages used during electrospinning.[17,51,54−56] During electrospinning, the current or flow of electrons traveling through the precursor solution, polymer nanofiber, and encapsulated bacteria remains low because of the high resistivity of the polymer.[57,58] Therefore, the current does not disrupt the cell membrane of the bacteria, which would lead to cell death. Additional concerns surround death caused by the electric field, which applies a radial force that causes the fiber to bend and whip.[36] Reznik et al.[59] determined this whipping force to be 5×10^3 g cm^{-1} s^{-2}, which is smaller than the force a cell of *E. coli* can withstand, determined to be 3×10^6 g cm^{-1} s^{-2}.[17]

A handful of studies attempted to correlate applied voltage with bacteria viability in the encapsulated nanofiber. When Škrlec et al.[51] used an applied voltage of 15 kV (with a separation distance of 15 cm), the cells had a higher viability than when 20 kV was used, which led to a 2-log reduction compared with theoretical loading. However, other studies[55,56] showed that voltage did not significantly affect the viability of *E. coli* Nissle electrospun into polyethylene glycol−polylactide and *Lactobacillus plantarum* in poly(vinyl alcohol). The effect of electrospinning voltage on the viability of bacteria may depend on the strain of bacteria and components of the precursor solution.

3.4. Electrospinning Environment. As the electrospun nanofiber is whipped, excess water is expelled from the nanofiber. For encapsulated bacteria, the expulsion of water can lead to the dehydration of cells.[17,51] Electrospinning typically occurs within a controlled environmental chamber where the relative humidity is ideally controlled. High humidity during electrospinning can hinder the drying process and, in some cases, cause the production of a wet fiber. However, high relative humidity can be favorable to bacteria: in a study that electrospun *Lactobacillus plantarum* cells in poly(ethylene oxide) nanofibers at 20%, 35%, and 55% relative humidity, the highest humidity resulted in the highest viability of encapsulated bacteria.[51]

Aerobic bacteria that prefer oxygen-rich environments and anaerobic bacteria that are sensitive to oxygen have both been successfully electrospun in aerobic environments. Poly(vinyl alcohol) has a high oxygen barrier, thereby making it an ideal material for the encapsulation of anaerobic bacteria.[16,60] In the electrospinning of anaerobic *Lactobacillus acidophilus*, higher viability was demonstrated by using poly(vinyl alcohol) [9.28 log(CFU/mg) or 68% viability] compared with polyvinylpyrrolidone [8.65−8.78 log(CFU/mg) or 34−40% viability], which the authors attributed to the oxygen exclusion properties of poly(vinyl alcohol).[16] Other anaerobes, including *Clostridium butyricum*[61] and *Bifidobacterium animalis*,[21] have also been incorporated into poly(vinyl alcohol) electrospun nanofibers. Pullulan is another polymer with oxygen exclusion properties that has been used to encapsulate oxygen-tolerant, anaerobe *Lactobacillus rhamnosus* into electrospun pullulan nanofibers. Additionally, the layering of the bacterial-loaded pullulan nanofibers between two layers of poly(D,L-lactide-*co*-glycolide) nanofibers increased the viability from 4×10^6 CFU/g in the single-layer system to 2.4×10^9 CFU/g in the multilayer system, which could provide additional support and protection to the bacteria-loaded fibers.[62] Overall, electrospinning in an oxygen-rich environment is an effective method to encapsulate live bacteria in polymer nanofibers, though electrospinning in an inert (nitrogen environment) may provide further opportunities to improve the viability of bacteria that are sensitive to oxygen. The next section will focus on the applications of these bacteria-loaded nanofibers.

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

Table 1. Summary of Electrospun Encapsulated Probiotics for Oral Delivery

probiotic strain	precursor solution(s)	electrospinning conditions	bacterial loading in nanofibers	ref
Bifidobacterium animalis	sheath: polyvinyl alcohol in water core:[a] skim milk	sheath inner needle diameter: 1.5 mm core inner needle diameter: 0.8 mm flow rate: 0.06−0.3 mL/h applied voltage: 11 kV tip-to-collector distance: 13 cm	not quantified	21
Escherichia coli K12	sodium alginate/poly(ethylene oxide)/ polysorbate 80 in water	18-gauge hypodermic needle flow rate: 2 mL/h applied voltage: 17.5 kV tip-to-collector distance: 17 cm temperature: 23 °C relative humidity: 20−30%	2.74×10^5 CFU/g	22
Escherichia coli Nissle	sheath: polyethylene glycol-polylactide in dichloromethane/dimethylformamide core:[a] polyvinylpyrrolidone or glycerol	sheath solution flow rate: 6 mL/h core solution flow rate: 1 mL/h applied voltage: 20 kV tip-to-collector distance: 15 cm	5×10^5 CFU/mg	55
Lactobacillus plantarum	polyvinyl alcohol and fructoolgiosaccharides in water	20-gauge needle flow rate: 0.3−0.6 mL/h applied voltage: 16 kV tip-to-collector distance: 14 cm temperature: 25 ± 1 °C relative humidity: 40−50%	8.88 log(CFU/mL)	56
Clostridium butyricum	hydroxypropyl-β-cyclodextrin	high-speed electrospinning setup using 34 mm diameter spinneret with 330 μm orifices flow rate: 300 mL/h applied voltage: 40 kV airflow: 120 m^3/h room temperature relative humidity: 40−45%	$>10^8$ CFU	61
Lactobacillus acidophilus *Lactobacillus rhamnosus* *Bifidobacterium bifidum* *Bifidobacterium animalis*	corn starch and sodium alginate in water	flow rate: 1.5 mL/h current: 10 μA applied voltage: 24 kV tip-to-collector distance: 12 cm temperature: 25 ± 1 °C	>9 log(CFU/mL) starting loading in gastric fluid	70, 71
Lactobacillus acidophilus	gum arabic and polyvinyl alcohol in water	NS Lab-fluinateke100 nanospider machine carriage rate: 90 mm/s applied voltage: 16.8 kV carriage to collector distance: 15 cm	>9.97 log(CFU/g)	72
Lactobacillus paracasei	sodium alginate and polyvinyl alcohol in water	flow rate: 1.2 mL/h applied voltage: 22 kV current: 10 μA tip-to-collector distance: 10 cm temperature: 25 °C	8.57 log(CFU/g)	73

[a]The probiotic strain was blended into the core precursor solution during coaxial electrospinning.

4. APPLICATIONS OF BACTERIA-LOADED, ELECTROSPUN NANOFIBERS

4.1. Gut Probiotics. The gut is home to a community of bacteria that can influence human health by aiding in digestion, controlling immune responses, and influencing brain function.[63−65] Probiotics, or bacteria that can provide beneficial health effects to a host, can be administered orally to modulate the gut community.[66−69] However, the probiotics must survive the harsh environment of the gastrointestinal tract to reach the gut microbiota. Electrospinning can be used to encapsulate high loadings of bacterial cells such that enough cells are delivered into the gut. Additionally, electrospun nanofibers have a high surface-area-to-volume ratio for the rapid and sustained release of encapsulants.[27] Several probiotic bacteria have been electrospun into a variety of polymer nanofibers, as summarized in Tables 1 and 2.

Few studies have investigated the gut fate of probiotic-loaded nanofibers. The human gastrointestinal tract consisting of the mouth, stomach, and intestinal phases are equipped with chemicals and mechanisms meant to break down food.[74] In particular, the low pH value of the stomach can be detrimental to bacteria.[32] *Lactobacillus acidophilus* encapsulated within a mixture of gum arabic and poly(vinyl alcohol) electrospun nanofibers showed improved survivability after exposure to simulated gastric fluid for 150 min compared with free

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

Table 2. Summary of Electrospun Encapsulated Probiotics for Non-Oral Delivery

application	probiotic strain	precursor solution(s)	electrospinning conditions	bacterial loading in nanofibers	ref
wound dressings	*Staphylococcus epidermidis*	carboxymethylcellulose and poly(ethylene oxide) in water	Nanospider NS500 electrode distance: 170 mm electrode spin velocity: 3.8 rpm applied voltage: 60 kV temperature: 23.9 °C relative humidity: 33%	$6.9 \pm 1.8 \times 10^5$ – $2.0 \pm 0.3 \times 10^5$ CFU/g	52
	Enterococcus mundtii	polyvinyl alcohol/polyvinylpyrrolidone/glycerol in water	25-gauge needle 0.6 mL/h applied voltage: 16 kV tip-to-collector distance: 15 cm	>8 log(CFU/g)	84
vaginal probiotics	*Lactobacillus acidophilus*	polyvinyl alcohol and polyvinylpyrrolidone in water	applied voltage: 35 kV tip-to-collector distance: 15 cm temperature: 25 °C relative humidity: 35%	8.87–9.28 log(CFU) per g of fiber	16
	Lactobacillus acidophilus	poly(ethylene oxide) in water	flow rate: 0.4 mL/h applied voltage: 15 kV tip-to-collector distance: 15 cm	9.18 ± 0.21 log (CFU/mg)	18
	Lactobacillus delbrueckii ssp. Bulgaricus			5.91 ± 0.15 log (CFU/mg)	
	Lactobacillus casei			7.33 ± 0.19 log (CFU/mg)	
	Lactobacillus gasseri			9.20 ± 0.11 log (CFU/mg)	
	Lactobacillus paracasei			8.02 ± 0.18 log (CFU/mg)	
	Lactobacillus plantarum			8.70 ± 0.38 log (CFU/mg)	
	Lactobacillus reuteri			9.32 ± 0.04 log (CFU/mg)	
	Lactobacillus rhamnosus			7.41 ± 0.16 log (CFU/mg)	
	Lactobacillus salivarius			6.75 ± 0.07 log (CFU/mg)	
	Lactococcus lactis			8.19 ± 0.33 log (CFU/mg)	
	Lactobacillus plantarum	poly(ethylene oxide) in water	flow rate: 0.4 or 0.5 mL/h applied voltage: 10, 15, or 20 kV tip-to-collector distance: 15 cm temperature: 24 ± 2 °C relative humidity: 20, 35, or 55%	7.6×10^6 CFU/mg	51
	Lactobacillus gasseri *Lactobacillus rhamnosus*	polyvinyl alcohol in water	22-gauge needle flow rate: 0.4 mL/h tip-to-collector distance: 12 cm applied voltage: 12 kV temperature: 24 ± 2 °C relative humidity: 40%	$>10^7$ CFU/g	85
	Lactobacillus crispatus *Lactobacillus gasseri* *Lactobacillus jensenii*	poly(ethylene oxide) and sucrose in water	flow rate: 150–250 μL applied voltage: 10–13 kV tip-to-collector distance: 15 cm temperature: ~ 20 °C relative humidity: ~ 30%	>10 log(CFU/g)	86

bacterial cells.[72] While typically used as an emulsifier or thickening agent in food products, gum arabic has been used in a variety of encapsulation technologies for delivery into the gut. Other polymers, like alginate, have proven useful for the delayed release of materials into the intestines.[75−77] More specifically, alginate hydrogels contract in acidic environments to protect encapsulants from acid damage in the stomach. In the intestines, swelling of the alginate structure because of a higher pH releases encapsulants.[78−80] Several strains of bacteria have been electrospun into alginate-based nanofibers.[22,70,71,73] Alginate-based encapsulations improved the acid tolerance and viability of probiotics in simulated gastrointestinal systems compared with free cells.[70,73] Furthermore, alginate favorably binds with the intestinal mucosa to prolong the delivery of drugs.[81,82] As gut simulation becomes more complex, the interactions between bacteria-loaded nanofibers and the gut mucosa, the gut microbiota, and the bodily systems that influence human health can be studied.

4.2. Skin Probiotics. The skin is the body's largest organ, which oversees protecting all the other internal organs. Being exposed to the environment, the skin begins to accumulate a microbiome after birth that changes over time.[83] Various injuries like cuts and burns damage the skin, as well as its microbiome, which can hinder healing.[48] Furthermore, without protection from commensal bacteria on the skin, abrasions are inclined toward infections by pathogenic bacteria. A commensal skin bacteria, *Staphococuss epidermis*, was electrospun into carboxymethylcellulose and poly(ethylene oxide) nanofibers for the treatment of diabetic foot ulcers.[52] The fibrous electrospun scaffold could easily be incorporated inside of socks and replaced as needed.

Enterococcus mundtii, a probiotic beneficial for the skin because of its production of the nutrient folate and the antibiotic mundticin, was encapsulated into poly(vinyl alcohol)/polyvinylpyrrolidone/glycerol nanofiber bioscaffolds as a dermal patch to promote wound healing after burn wounds.[84] Wound closure percentage over time was improved

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

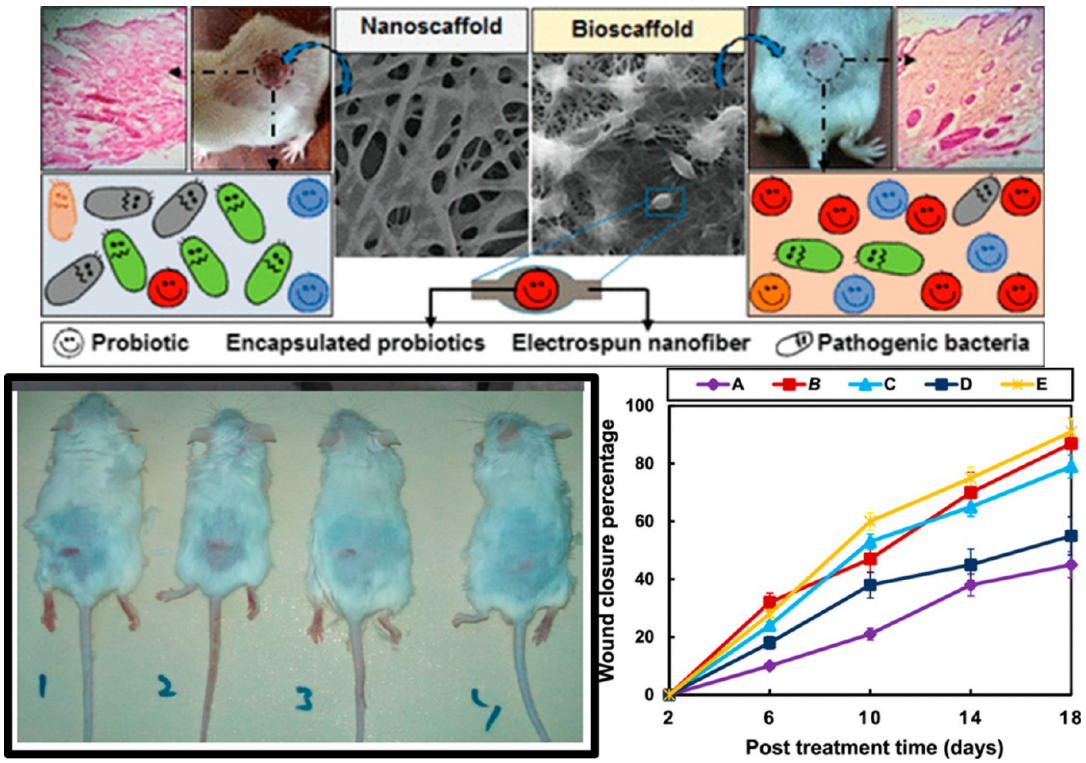


Figure 3. Probiotic *Enterococcus mundtii* was electrospun into poly(vinyl alcohol)/polyvinylpyrrolidone/glycerol bioscaffolds for the treatment of burn wounds on mice models. (Top) The bacteria-loaded bioscaffold showed improved wound closure compared with the electrospun nanoscaffold without bacteria. (Bottom left) The size, shape, and depth of burn wounds (bottom right) were measured over 18 days to determine the wound closure percentage for (A) an untreated wound, (B) a wound treated with a suspension of *Enterococcus mundtii* in biopolymer dispersion, (C) a wound treated with topical antibiotic cream, (D) a wound treated with electrospun scaffold without probiotics, and (E) a wound treated with the bioscaffold containing *Enterococcus mundtii*. Reproduced with permission from ref 84. Copyright 2019 American Chemical Society.

with the addition of the bacteria-loading wound dressing compared with both untreated wounds and wounds treated with a topical antibiotic cream, as seen in Figure 3. Though the nanofiber structure experienced significant swelling and loss of structure in a simulated wound fluid, the moist environment is essential for the reactivation and release of encapsulated bacteria. Additionally, the bioscaffold showed antibacterial activity against *S. aureus* ATCC 25923, a model pathogenic bacterium.

4.3. Vaginal Probiotics. Vaginal probiotics can be administered orally or vaginally. Electrospun vaginal bacteria could be administered similarly to a tampon or hygienic towel to treat bacterial vaginosis or other infections.[16,18,51,85−87] Since the vaginal microbiota is primarily composed of *Lactobacillus* strains, Zupančič et al.[18] evaluated the viability of nine *Lactobacillus* strains and one *Lactococcus* strain when electrospun separately into poly(ethylene oxide) nanofibers (Figure 4). Each strain demonstrated high viability [>5.9 log (CFU/mg)]. Another study[85] encapsulated and tested the antimicrobial activity of a *Lactobacillus* strain in poly(vinyl alcohol) nanofibers. Of the five urogenital pathogens tested, the electrospun *Lactobacillus rhamnosus* strain exhibited significant antimicrobial abilities against *Staphylococcus aureus* and *Escherichia coli* even while encapsulated within the polymer nanofiber for the treatment or prevention of urologic or vaginal-localized ailments.

As far as the authors are aware, the *in vivo* application of probiotic-loaded nanofibers to the vaginal area has not been demonstrated to date. However, electrospun products hold the potential to be an effective and convenient intravaginal drug delivery device.[88,89] A focus-group-based study found that a fabric-like nanofiber mat was perceived favorably over gels or films.[90−92] On top of being easy to transport and store because of the size of the mat, the flexible nature also allows electrospun nanofiber mats to be formed into a capped tube shape (Figure 5) for easy insertion.[91]

4.4. Seed Coatings. In the agricultural industry, electrospun nanofibers have been used to deliver various biocontrol agents to the soil.[93] However, the electrospun bacteria could further promote plant growth by enriching the rhizopheres, that is, the area around the plant root associated with microorganisms, as well as providing antifungal protection. Encapsulated bacteria are easily electrospun onto seeds without harming the seed or the bacteria; after planting, the bacteria will be released into the soil environment. *Methylorubrum aminovorans* was electrospun into poly(vinyl alcohol) nanofibers and onto groundnut (*Arachis hypogaea*) seeds, as seen in Figure 6.[94] The biocompatibility of poly(vinyl alcohol) is especially important for *Arachis hypogaea*, commonly known as peanuts, as the fruit is grown under the soil. As a hydrophilic polymer, poly(vinyl alcohol) aids in germination and plant growth by uptaking water and retaining high moisture levels. Table 3, which summarizes all electrospun bacteria for environmental uses, lists other strains of bacteria that have been incorporated in poly(vinyl alcohol) for seeding coating and plant-growth-promoting applications.[95,96]

Other than promoting growth, bacteria can exhibit antifungal activity to protect the seed. *Bacillus subtilis* and *Seratia marcescens* were electrospun together into poly(vinyl alcohol) and polyvinylpyrrolidone. In a disk diffusion assay,

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

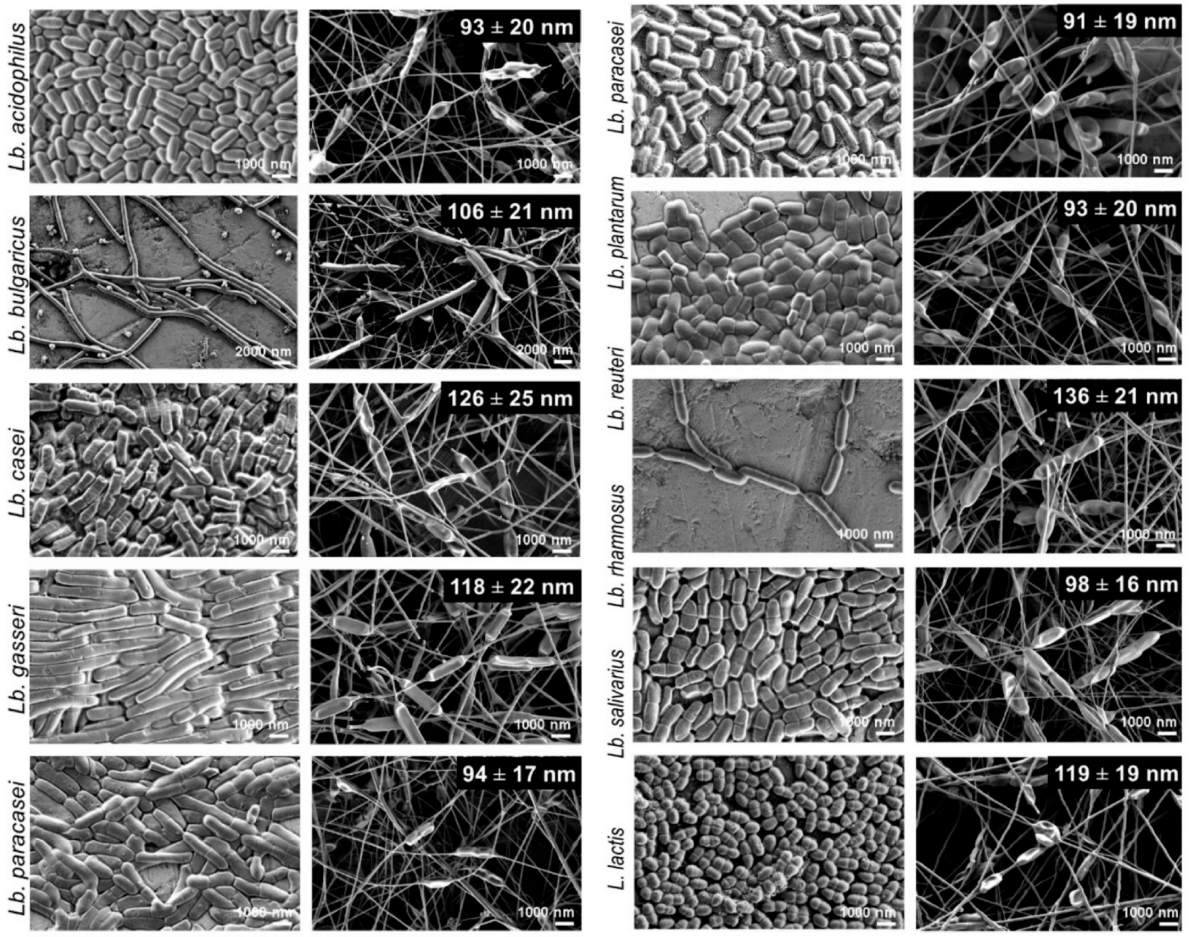


Figure 4. Several strains of vaginal probiotics electrospun into poly(ethylene oxide). These nanofibers can be delivered orally or vaginally. Reproduced with permission from ref 18. Copyright 2019 Zupančič et al.

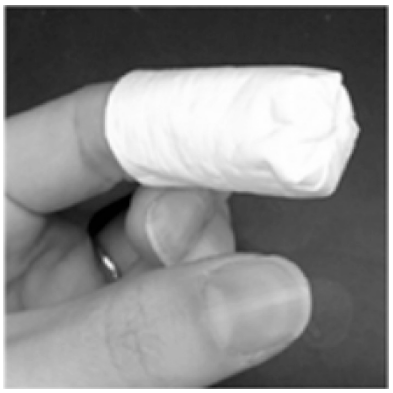


Figure 5. A focus-group-based study found that electrospun fabric shaped into a capped tube are promising materials because of its ease of use and insertion for vaginal delivery. Ideally, a mat of electrospun bacteria could be inserted as easily as a tampon for the regulation of the vaginal microbiome. Reproduced with permission from ref 91. Copyright 2018 Laborde et al.

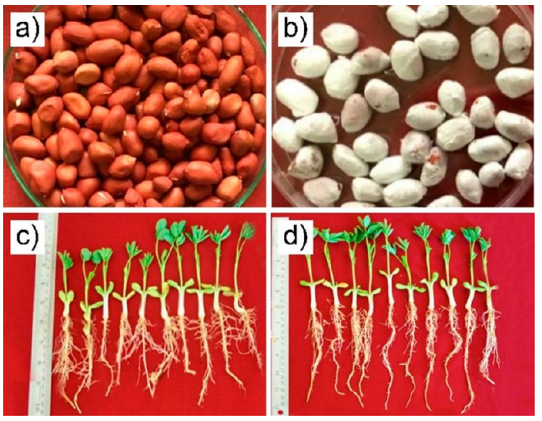


Figure 6. (a) *Arachis hypogaea* seeds were (b) coated with electrospun nanofibers containing *Methylorubrum aminovorans* bacteria cells. Through comparison of the seedlings grown from (c) uncoated and (d) coated seeds, coating the seeds leads to a longer root and shoot lengths. Reproduced with permission from ref 94. Copyright 2022 Mukiri et al., Springer Nature.

their antifungal properties were determined against *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium oxysporum*, as shown in Figure 7.[97] Indeed, the bacteria-loaded fibers inhibited the growth of the fungus (dark, opaque portions of the agar plate), as evidenced by the zones of inhibition (transparent zones) around the electrospun mats (white disks). Coating canola seeds with antifungal, bacteria-loaded nanofibers can help to meet the high demand for canola, which is harvested and processed into food grade oil, feed for livestock, and biofuels, while also reducing the use of environmentally hazardous fungicides.

4.5. Water Remediation. Pollutants, like dyes, herbicides, and heavy metals, are harmful to the environment and its

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

Table 3. Summary of Electrospun Encapsulated Probiotics for Environmental Applications

application	bacterial strain	precursor solutions[a]	electrospinning conditions	bacterial loading in nanofibers	ref
seed coatings	*Methylobacterim aminovorans*	polyvinyl alcohol in water	flow rate: 0.6 mL/h applied voltage: 15 kV	6.6×10^5 CFU/g	94
	Pantoea agglomerans ISIB55 *Burkholderia caribensis ISIB40*	polyvinyl alcohol in water with and without glycerol	22-gauge needle 3 mL syringe flow rate: 0.01 mm/s applied voltage: 12 or 22 kV tip-to-collector distance: 12 cm	>7.42 ± 0.02 log (CFU/g) >7.23 ± 0.02 log (CFU/g)	95
	Bradyrhizobium japonicum SEMIA 5079 and *Bradyrhizobium elkanii SEMIA 587*	polyvinyl alcohol in water	5 mL syringe flow rate: 0.01 mm/s applied voltage: 22 kV tip-to-collector distance: 14 cm	4.29×10^5 CFU/seed	96
	Bifidobacterium subtilis and *Serratia marcescens*	polyvinyl alcohol, polyvinylpyrrolidone, and glycerol in water	24-gauge needle flow rate: 0.6 mL/h applied voltage: 17 ± 1 kV tip-to-collector distance: 13 cm temperature: ∼20 °C	5.7 ± 0.48 log (CFU/seed)	97
bioremediation	*Pseudomonas aeruginosa*	sheath: polyvinylpyrrolidone and polyvinylidene fluoride-*co*-hexafluoropropylene in tetrahydrofuran and dimethylformamide core:[a] water	sheath flow rate: 5 mL/h core flow rate: 0.5 mL/h applied electric field: 11 kV/cm tip-to-collector distance: 11 cm temperature: 27 °C relative humidity: 54−64%	not quantified	50
	Pseudomonas aeruginosa	polyvinyl alcohol or poly(ethylene oxide) in water	0.6 mm inner diameter needle flow rate: 1 mL/h applied voltage: 10−15 kV tip-to-collector distance: 10−12 cm temperature: 24 ± 1 °C relative humidity: ∼20%	10^7−10^8 CFU/mL per 10 mg of polyvinyl alcohol web 10^8 CFU/mL per 10 mg of poly (ethylene oxide) web	98
	Lysinibacillus sphaericus	cyclodextrin in water and skim milk	flow rate: 0.5 mL/h applied voltage: 15 kV tip-to-collector distance: 10 cm temperature: ∼24 °C relative humidity: 25%	6.0×10^2 CFU/mL after storage at 4 °C for 24 h	99
microbial fuel cells	*Electroactive consortia*	poly(ethylene oxide) in water	flow rate: 0.3 mL/h applied voltage: 10 kV tip-to-collector distance: 15 cm	not quantified	100
	Shewanella oneidensis	sheath: poly(ε-caprolactone) in chloroform/dimethylformamide core:[a] poly(ethylene oxide) in phosphate buffer saline	sheath flow rate: 3.5 mL/h core flow rate: 0.5 mL/h electric field: 1 kV/cm applied voltage: 13 kV temperature: 21 °C relative humidity: 30 or 60%	not quantified	101

[a]The probiotic strain was blended into the core precursor solution during coaxial electrospinning.

[a]The probiotic strain was blended into the core precursor solution during coaxial electrospinning.

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

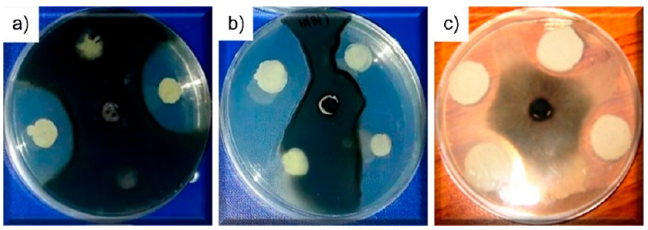


Figure 7. Zones of inhibition produced by a mixture of *Bacillus subtilis* and *Seratia marcescens* cells electrospun into poly(vinyl alcohol) and polyvinylpyrrolidone nanofibers (white disks) proved the antifungal properties of the mats against (a) *Macrophomina phaseolina*, (b) *Rhizoctonia solani*, and (c) *Fusarium oxysporum*. As a seed coating, this mat can protect the seed against fungal infestation. Reproduced with permission from ref 97. Copyright 2019 American Chemical Society.

inhabitants, even causing mutations in some cases.[50,99,102] While the removal of these chemicals can be challenging or costly, bacteria are able to degrade certain pollutants. For example, *Pseudomonas aeruginosa* showed a methylene blue (MB) removal of 57% when electrospun into poly(vinyl alcohol) or 76% when electrospun in poly(ethylene oxide) (Figure 8a).[98] Since the immobilization of bacteria poses a concern for the availability of nutrients for the bacteria, San Keskin et al.[99] electrospun *Lysinibacillus sphaericus* in cyclodextrin, an oligosaccharide that can be used by bacteria as a carbon source. The encapsulation of bacteria in this nutrient-rich nanofiber improved the removal efficiency of nickel ions by 70% compared with free bacteria (removal efficiency 25.5%). The nanofibers from the previously mentioned studies[98,99] were successful in delivering live bacterial cells to pollutant-containing solutions, though the fibers dissolved upon being added to the solution.

To produce water-insoluble "microtubes" containing bacteria, a core solution of *Pseudomonas aeruginosa* suspension in an aqueous solution of polyvinylpyrrolidone was coaxially electrospun with a sheath solution of polyvinylidene fluoride-*co*-hexafluoro-propylene dissolved in tetrahydrofuran and dimethylformamide.[50] Though live, fluorescent bacteria (Figure 8b) were detected in the electrospun microtubes, the biodegradation activity of the bacteria was reduced by contact with organic solvents. Submerging the water-insoluble structure in growth media regenerates atrazine degradation activity. Furthermore, the degradation of atrazine occurred more rapidly after regeneration than without regeneration. Effective bioremediation of atrazine (0.29 mg/day) was carried out over 50 days in a continuous reactor.

4.6. Fuel Cells. Microbial fuel cells utilize the energy producing capabilities of bacteria as a sustainable energy source. Figure 9a depicts a single chamber microbial fuel cell where bacteria are loading into the chamber containing the anode.[103] While suspended bacteria can naturally adhere to the surface of the anode, the encapsulation of bacteria onto the anode would allow for more direct interaction between the electrons produced by the bacteria and the fuel cell. Massaglia et al.[100] electrospun an electroactive consortia of bacteria derived from seawater sediment within poly(ethylene oxide) nanofibers (bio-NFs) that were directly collected on carbon paper to be used as the anode. The bio-NF anode produced double the maximum power density (~7 mW/m^2), a higher short circuit current (~33.75 mA/m^2), and comparable current density (250 mA/m^2 after 4 days) compared with the carbon paper anode used as a control, as shown in Figure

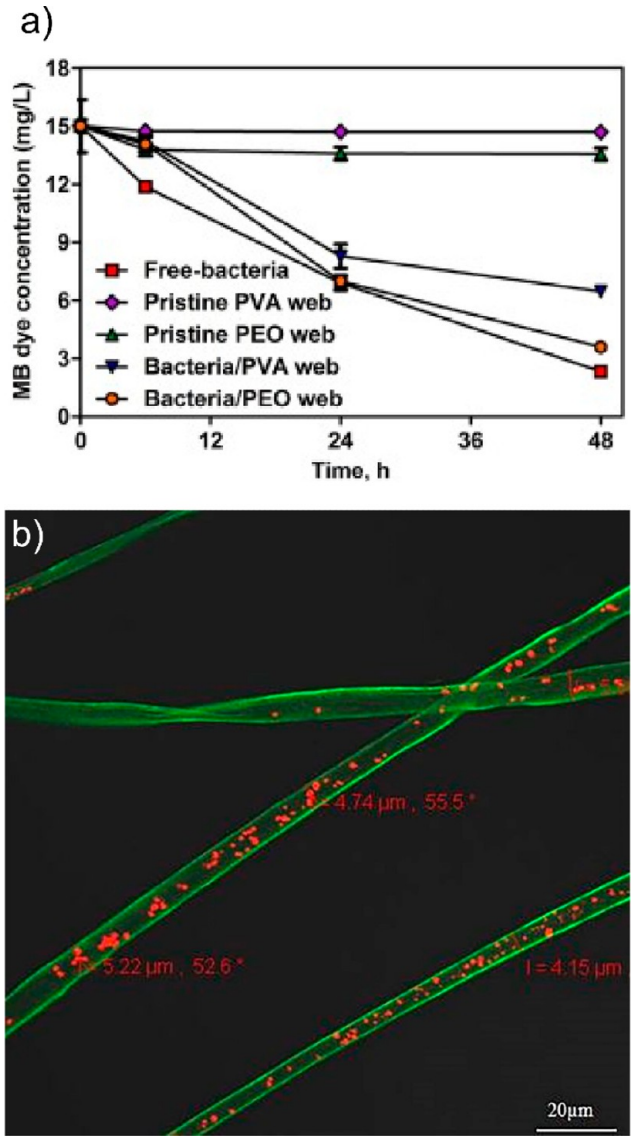


Figure 8. (a) Methylene blue (MB) dye removal by free *Pseudomonas aeruginosa* (free bacteria), control electrospun poly(vinyl alcohol) mats (Pristine PVA web), control electrospun poly(ethylene oxide) web (Pristine PEO web), electrospun *Pseudomonas aeruginosa* in poly(vinyl alcohol) web (bacteria/PVA web), and electrospun *Pseudomonas aeruginosa* in poly(ethylene oxide) web (bacteria/PEO web). Reproduced with permission from ref 98. Copyright 2017 Elsevier. (b) Live, CTC-stained (red) *Pseudomonas aeruginosa* were immobilized in water-insoluble electrospun microtubes stained with STYO-9 (green) such that the bacteria could easily be delivered to wastewaters and degrade pollutants. Reproduced with permission from ref 50. Copyright 2017 Elsevier.

9b,c. Sanchez and Laberty-Robert[101] (Figure 9d,e) were able to increase current density by coaxially electrospinning *Shewanella oneidensis* into an "integrated bioanode." The bacteria were suspended in poly(ethylene oxide) as the core solution, and carbon black was mixed into a poly(ε-caprolactone) solution in chloroform/dimethylformamide as a shell solution. Additionally, a conductive scaffold consisting of polyacrylonitrile and carbon black dissolved in dimethylformamide were simultaneously electrospun onto the same collector. As a conductive material, carbon black promotes electron percolation to enable extracellular electron transfer

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

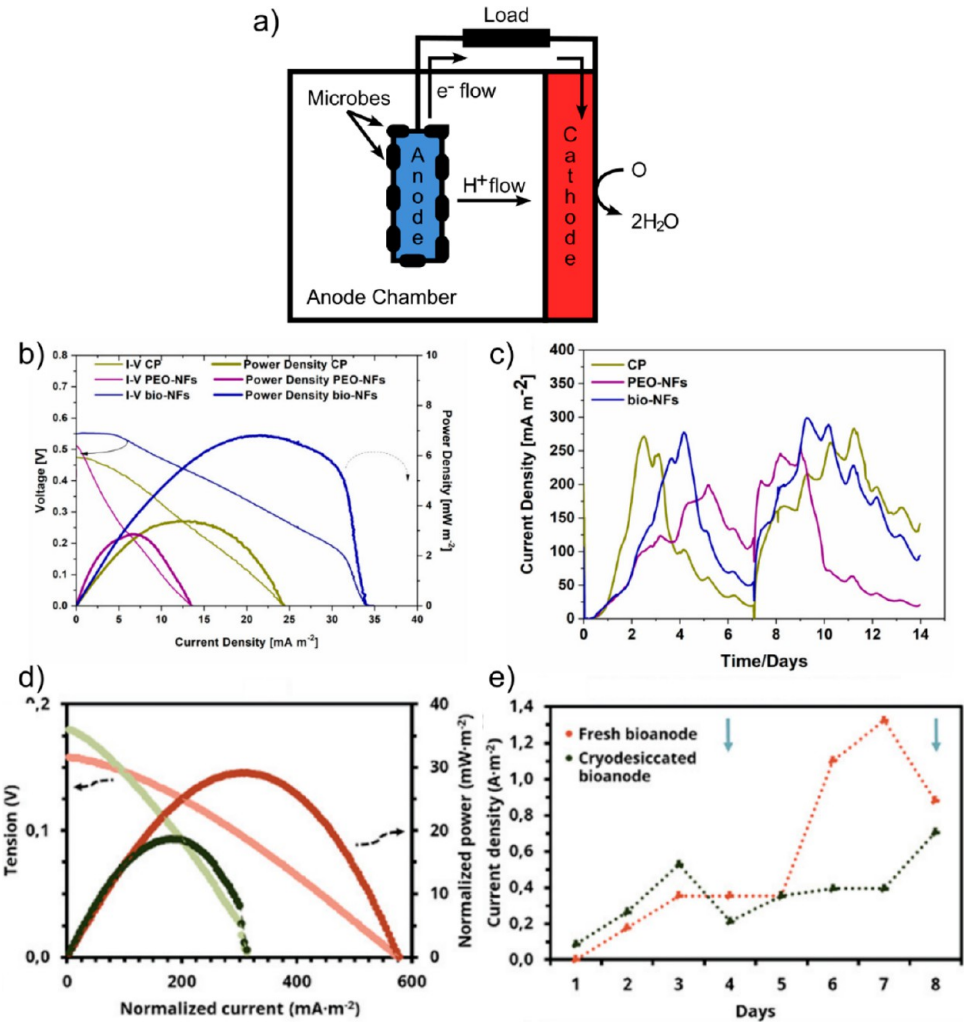


Figure 9. (a) Diagram of microbial attachment to the anode of a single chamber microbial fuel cell. Redrawn with permission from ref 103. Copyright 2018 Society of Chemical Industry. Electrical properties of microbial fuel cells developed by (b,c) Massaglia et al.[100] and (d,e) Sanchez and Laberty-Robert.[101] (b,d) Polarization curves showing potential (V) and powder density vs current density. (c,e) Current density of respective microbial fuel cells over time. Panels (b) and (c) are reproduced with permission from ref 100. Copyright 2021 Massaglia et al. Panels (d) and (e) are reproduced with permission from ref 101. Copyright 2021 The Royal Society of Chemistry.

between the bacteria and the anode. The integrated bioanode had a maximum power density of 30 mW/m^2 and a maximum current density of 1350 mA/m^2 after 8 days. Additionally, a maximum current density of 700 mA/m^2 was observed after cryodesiccation of the bacteria-loaded nanofibers for long-term storage. Microbe-powered fuel cells can be combined with environmental remediation, water remediation, and environmental sensing technologies.[100−102]

5. PERSPECTIVE

Bacteria offer a wide range of beneficial functions, from producing chemicals like various nutrients to degrading harmful agents like toxins found in waste waters. Commercially, encapsulation technologies are often used to protect probiotic bacteria during their processing, storage, and delivery to various environments where the bacteria will be useful. As discussed in this review, electrospinning has proven to be an effective method for encapsulating high loadings of bacteria in the laboratory setting. The nanofiber structure formed by electrospinning protects the microbes and is convenient for probiotic delivery, wound dressings, feminine hygiene products, water filtration membranes, coatings, and even microbial fuel cells.

While promising, bacterial electrospinning still faces a number of challenges. Several strains of bacteria exhibit varying tolerance for electrospinning. Though Gram-negative, Gram-positive, aerobic, and anaerobic bacteria of various shapes and sizes have been successfully electrospun, it is unclear how and by what other properties, like electroactivity, one strain of bacteria can be more robust toward electrospinning than another strain. However, it is known that biocompatible polymers and aqueous solvents are generally safe for the electrospinning of the cells. Much research has been conducted encapsulating bacteria into poly(vinyl alcohol) and poly(ethylene oxide) nanofibers. Bacteria encapsulated within water-soluble, biocompatible polymers are quickly released upon contact with moisture for the treatment of an open wound or to rebalance the vaginal microbiome. However, research studies are also shifting toward other polysaccharide polymers, like gum arabic and alginate, which can provide controlled delivery of the bacteria.

Coaxial electrospinning is also very promising for creating water-stable, bacteria-loaded nanofibers; the bacteria sus-

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

pended in biocompatible solvents in the inner needle is simultaneously extruded with a spinnable precursor solution in the outer needle. The bacterial suspension is then "pulled along" by the spinnable precursor, thereby allowing for the formation of the electrospun fiber. Notably, a polymer dissolved in an organic solvent has been used to create a water-insoluble fiber containing living bacteria cells. For significant advances toward "real-world" applications, the performance of the bacteria or the physical properties of the fibers might need to be improved by the incorporation of additives, such as pharmaceuticals or other functional agents.

Despite the availability of commercial probiotics for gut and even plant health, the translation of electrospun bacteria into commercial products has yet to be realized. The gastrointestinal tract models and mice models are often used for the delivery of gut and skin probiotics, respectively, and have not moved on to clinical trials, much less commercial production. Electrospun vaginal probiotics have not yet been demonstrated in any animal models or simulated vaginal environments. Fewer researchers have focused on environmental applications, such as plant growth. Antifungal, plant-growth-promoting technology is encouraging. It will be interesting to see how electrospun bacteria can evolve for the coating of grown plants or even food products. Perhaps, future fibers engineered for environmental applications will explore multishell fibers that can uptake toxins while releasing nutrients that will repair the soil. Barely any attention has explored bioremediation and microbial fuel cell applications. Additionally, in the future, a life-cycle analysis of these materials will be needed to determine the influence of the encapsulated bacteria on the soil microbial communities and the reusability of bacteria-loaded fibers in bioremediation and microbial fuel cell applications. In tandem with the enormous efforts being made to develop permeable, porous, and stable polymer nanofiber scaffolds that provide an optimized environment for microorganisms, exciting developments in synthetic biology have been emerging; we are excited to learn about future bacteria-loaded polymer fibers biotechnologies.

AUTHOR INFORMATION

Corresponding Author

Jessica D. Schiffman − *Department of Chemical Engineering, University of Massachusetts Amherst, Amherst, Massachusetts 01003-9303, United States;* orcid.org/0000-0002-1265-5392; Email: schiffman@umass.edu

Author

Emily Diep − *Department of Chemical Engineering, University of Massachusetts Amherst, Amherst, Massachusetts 01003-9303, United States;* orcid.org/0000-0001-8742-3249

Complete contact information is available at: https://pubs.acs.org/10.1021/acsabm.2c01055

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

E.D. acknowledges support from the Spaulding Smith Fellowship. The authors acknowledge the support from the National Science Foundation (Award #1921839).

REFERENCES

(1) Singleton, P. *Bacteria in Biology, Biotechnology, and Medicine.*, Fourth ed..; J. Wiley & Sons, 1997.

(2) Asaithambi, N.; Singh, S. K.; Singha, P. Current Status of Non-Thermal Processing of Probiotic Foods: A Review. *J. Food Eng.* **2021**, *303*, 110567.

(3) Finkel, S. E. Long-Term Survival during Stationary Phase: Evolution and the GASP Phenotype. *Nat. Rev. Microbiol.* **2006**, *4* (2), 113−120.

(4) Sehrawat, R.; Abdullah, S.; Khatri, P.; Kumar, L.; Kumar, A.; Mujumdar, A. S. Role of Drying Technology in Probiotic Encapsulation and Impact on Food Safety. *Dry. Technol.* **2022**, *40* (8), 1562−1581.

(5) Goderska, K. *Different Methods of Probiotics Stabilization*; IntechOpen, 2012. DOI: 10.5772/50313.

(6) Hubálek, Z. Protectants Used in the Cryopreservation of Microorganisms. *Cryobiology* **2003**, *46* (3), 205−229.

(7) Cook, M. T.; Tzortzis, G.; Charalampopoulos, D.; Khutoryanskiy, V. V. Microencapsulation of Probiotics for Gastrointestinal Delivery. *J. Controlled Release* **2012**, *162* (1), 56−67.

(8) Lahtinen, S. J.; Ouwehand, A. C.; Salminen, S. J.; Forssell, P.; Myllärinen, P. Effect of Starch- and Lipid-based Encapsulation on the Culturability of Two Bifidobacterium Longum Strains. *Lett. Appl. Microbiol.* **2007**, *44* (5), 500−505.

(9) Vidyalakshmi, R.; Bhakyaraj, R.; Subhasree, R. S. Encapsulation "The Future of Probiotics"-A Review. *Adv. Biol. Res.* **2009**, *3*, 96−103.

(10) Huq, T.; Khan, A.; Khan, R. A.; Riedl, B.; Lacroix, M. Encapsulation of Probiotic Bacteria in Biopolymeric System. *Crit. Rev. Food Sci. Nutr.* **2013**, *53* (9), 909−916.

(11) Moumita, S.; Goderska, K.; Johnson, E. M.; Das, B.; Indira, D.; Yadav, R.; Kumari, S.; Jayabalan, R. Evaluation of the Viability of Free and Encapsulated Lactic Acid Bacteria Using In-Vitro Gastro Intestinal Model and Survivability Studies of Synbiotic Microcapsules in Dry Food Matrix during Storage. *LWT* **2017**, *77*, 460−467.

(12) Butorac, K.; Novak, J.; Bellich, B.; Terán, L. C.; Banić, M.; Leboš Pavunc, A.; Zjalić, S.; Cescutti, P.; Šušković, J.; Kos, B. Lyophilized Alginate-Based Microspheres Containing Lactobacillus Fermentum D12, an Exopolysaccharides Producer, Contribute to the Strain's Functionality in Vitro. *Microb. Cell Factories* **2021**, *20*, 85.

(13) Alkayyali, T.; Cameron, T.; Haltli, B.; Kerr, R. G.; Ahmadi, A. Microfluidic and Cross-Linking Methods for Encapsulation of Living Cells and Bacteria - A Review. *Anal. Chim. Acta* **2019**, *1053*, 1−21.

(14) Rodrigo-Navarro, A.; Sankaran, S.; Dalby, M. J.; del Campo, A.; Salmeron-Sanchez, M. Engineered Living Biomaterials. *Nat. Rev. Mater.* **2021**, *6* (12), 1175−1190.

(15) Tromp, R. H.; Vink, C.; Stijnman, A. C. Encapsulation by Electrospinning of Live Bacteria Used in the Food Industry. In *Gums and Stabilisers for the Food Industry 16*, Vol. *335*; Williams, P. A., Phillips, G. O., Eds.; The Royal Society of Chemistry: Cambridge, UK, 2012; pp 247−255.

(16) Nagy, Zs. K.; Wagner, I.; Suhajda, A.; Tobak, T.; Harasztos, A. H.; Vigh, T.; Soti, P. L.; Pataki, H.; Molnar, K.; Marosi, Gy Nanofibrous Solid Dosage Form of Living Bacteria Prepared by Electrospinning. *Express Polym. Lett.* **2014**, *8* (5), 352−361.

(17) Salalha, W.; Kuhn, J.; Dror, Y.; Zussman, E. Encapsulation of Bacteria and Viruses in Electrospun Nanofibres. *Nanotechnology* **2006**, *17* (18), 4675−4681.

(18) Zupančič, Š.; Škrlec, K.; Kocbek, P.; Kristl, J.; Berlec, A. Effects of Electrospinning on the Viability of Ten Species of Lactic Acid Bacteria in Poly(Ethylene Oxide) Nanofibers. *Pharmaceutics* **2019**, *11* (9), 483.

(19) Weinbreck, F.; Bodnár, I.; Marco, M. L. Can Encapsulation Lengthen the Shelf-Life of Probiotic Bacteria in Dry Products? *Int. J. Food Microbiol.* **2010**, *136* (3), 364−367.

(20) Bhattarai, D. P.; Aguilar, L. E.; Park, C. H.; Kim, C. S. A Review on Properties of Natural and Synthetic Based Electrospun Fibrous Materials for Bone Tissue Engineering. *Membranes* **2018**, *8* (3), 62.

(21) López-Rubio, A.; Sanchez, E.; Sanz, Y.; Lagaron, J. M. Encapsulation of Living Bifidobacteria in Ultrathin PVOH Electrospun Fibers. *Biomacromolecules* **2009**, *10* (10), 2823−2829.
(22) Diep, E.; Schiffman, J. D. Encapsulating Bacteria in Alginate-Based Electrospun Nanofibers. *Biomater. Sci.* **2021**, *9* (12), 4364−4373.
(23) Li, T. X.; Ding, X.; Sui, X.; Tian, L. L.; Zhang, Y.; Hu, J. Y.; Yang, X. D. Sustained Release of Protein Particle Encapsulated in Bead-on-String Electrospun Nanofibers. *J. Macromol. Sci. Part B* **2015**, *54* (8), 887−896.
(24) Castellano, M.; Alloisio, M.; Darawish, R.; Dodero, A.; Vicini, S. Electrospun Composite Mats of Alginate with Embedded Silver Nanoparticles. *J. Therm. Anal. Calorim.* **2019**, *137* (3), 767−778.
(25) Bölgen, N.; Vargel, İ.; Korkusuz, P.; Menceloğlu, Y. Z.; Pişkin, E. In Vivo Performance of Antibiotic Embedded Electrospun PCL Membranes for Prevention of Abdominal Adhesions. *J. Biomed. Mater. Res. B Appl. Biomater.* **2007**, *81B* (2), 530−543.
(26) De Silva, R. T.; Dissanayake, R. K.; Mantilaka, M. M. M. G. P. G.; Wijesinghe, W. P. S. L.; Kaleel, S. S.; Premachandra, T. N.; Weerasinghe, L.; Amaratunga, G. A. J.; de Silva, K. M. N. Drug-Loaded Halloysite Nanotube-Reinforced Electrospun Alginate-Based Nanofibrous Scaffolds with Sustained Antimicrobial Protection. *ACS Appl. Mater. Interfaces* **2018**, *10* (40), 33913−33922.
(27) Pillay, V.; Dott, C.; Choonara, Y. E.; Tyagi, C.; Tomar, L.; Kumar, P.; du Toit, L. C.; Ndesendo, V. M. K. A Review of the Effect of Processing Variables on the Fabrication of Electrospun Nanofibers for Drug Delivery Applications. *J. Nanomater.* **2013**, *2013*, 1−22.
(28) Kataria, K.; Gupta, A.; Rath, G.; Mathur, R. B.; Dhakate, S. R. In Vivo Wound Healing Performance of Drug Loaded Electrospun Composite Nanofibers Transdermal Patch. *Int. J. Pharm.* **2014**, *469* (1), 102−110.
(29) Sill, T. J.; von Recum, H. A. Electrospinning: Applications in Drug Delivery and Tissue Engineering. *Biomaterials* **2008**, *29* (13), 1989−2006.
(30) Ghorani, B.; Tucker, N. Fundamentals of Electrospinning as a Novel Delivery Vehicle for Bioactive Compounds in Food Nanotechnology. *Food Hydrocoll* **2015**, *51*, 227−240.
(31) Deng, L.; Zhang, H. Recent Advances in Probiotics Encapsulation by Electrospinning. *ES Food Agrofor*. **2020**. DOI: 10.30919/esfaf1120
(32) Anal, A. K.; Singh, H. Recent Advances in Microencapsulation of Probiotics for Industrial Applications and Targeted Delivery. *Trends Food Sci. Technol.* **2007**, *18* (5), 240−251.
(33) Mendes, A. C.; Chronakis, I. S. Electrohydrodynamic Encapsulation of Probiotics: A Review. *Food Hydrocoll* **2021**, *117*, 106688.
(34) Zheng, Y. 3 - Fabrication on Bioinspired Surfaces. In *Bioinspired Design of Materials Surfaces*; Zheng, Y., Ed.; Materials Today; Elsevier, 2019; pp 99−146.
(35) Haider, A.; Haider, S.; Kang, I.-K. A Comprehensive Review Summarizing the Effect of Electrospinning Parameters and Potential Applications of Nanofibers in Biomedical and Biotechnology. *Arab. J. Chem.* **2018**, *11* (8), 1165−1188.
(36) Reneker, D. H.; Yarin, A. L. Electrospinning Jets and Polymer Nanofibers. *Polymer* **2008**, *49* (10), 2387−2425.
(37) Ramakrishna, S.; Fujihara, K.; Teo, W.-E.; Lim, T.-C.; Ma, Z. *An Introduction to Electrospinning and Nanofibers*; World Scientific Publishing Co. Pte. Ltd., 2005.
(38) Bhardwaj, N.; Kundu, S. C. Electrospinning: A Fascinating Fiber Fabrication Technique. *Biotechnol. Adv.* **2010**, *28* (3), 325−347.
(39) Sun, Z.; Zussman, E.; Yarin, A. l.; Wendorff, J. h.; Greiner, A. Compound Core−Shell Polymer Nanofibers by Co-Electrospinning. *Adv. Mater.* **2003**, *15* (22), 1929−1932.
(40) Rathore, P.; Schiffman, J. D. Beyond the Single-Nozzle: Coaxial Electrospinning Enables Innovative Nanofiber Chemistries, Geometries, and Applications. *ACS Appl. Mater. Interfaces* **2021**, *13* (1), 48−66.
(41) Zhu, T.; Chen, S.; Li, W.; Lou, J.; Wang, J. Flurbiprofen Axetil Loaded Coaxial Electrospun Poly(Vinyl Pyrrolidone)−Nanopoly-(Lactic-Co-Glycolic Acid) Core−Shell Composite Nanofibers: Preparation, Characterization, and Anti-Adhesion Activity. *J. Appl. Polym. Sci.* **2015**, *132* (22), 41982.
(42) Su, Y.; Su, Q.; Liu, W.; Lim, M.; Venugopal, J. R.; Mo, X.; Ramakrishna, S.; Al-Deyab, S. S.; El-Newehy, M. Controlled Release of Bone Morphogenetic Protein 2 and Dexamethasone Loaded in Core−Shell PLLACL−Collagen Fibers for Use in Bone Tissue Engineering. *Acta Biomater* **2012**, *8* (2), 763−771.
(43) Jiang, H.; Hu, Y.; Li, Y.; Zhao, P.; Zhu, K.; Chen, W. A Facile Technique to Prepare Biodegradable Coaxial Electrospun Nanofibers for Controlled Release of Bioactive Agents. *J. Controlled Release* **2005**, *108* (2), 237−243.
(44) Sethupathy, M.; Sethuraman, V.; Jeyaraman, A. R.; Muthuraja, P.; Manisankar, P. Electrospun Polyethylene Oxide (PEO) Nanofiber Membranes Based Polymer Electrolyte for Dye Sensitized Solar Cell. *AIP Conf. Proc.* **2014**, *1620*, 253−257.
(45) Park, J.-C.; Ito, T.; Kim, K.-O.; Kim, K.-W.; Kim, B.-S.; Khil, M.-S.; Kim, H.-Y.; Kim, I.-S. Electrospun Poly(Vinyl Alcohol) Nanofibers: Effects of Degree of Hydrolysis and Enhanced Water Stability. *Polym. J.* **2010**, *42* (3), 273−276.
(46) Ning, E.; Turnbull, G.; Clarke, J.; Picard, F.; Riches, P.; Vendrell, M.; Graham, D.; Wark, A. W.; Faulds, K.; Shu, W. 3D Bioprinting of Mature Bacterial Biofilms for Antimicrobial Resistance Drug Testing. *Biofabrication* **2019**, *11* (4), 045018.
(47) Li, Z.; Behrens, A. M.; Ginat, N.; Tzeng, S. Y.; Lu, X.; Sivan, S.; Langer, R.; Jaklenec, A. Biofilm-Inspired Encapsulation of Probiotics for the Treatment of Complex Infections. *Adv. Mater.* **2018**, *30* (51), 1803925.
(48) Rieger, K. A.; Birch, N. P.; Schiffman, J. D. Designing Electrospun Nanofiber Mats to Promote Wound Healing − a Review. *J. Mater. Chem. B* **2013**, *1* (36), 4531−4541.
(49) Gbassi, G. K.; Vandamme, T. Probiotic Encapsulation Technology: From Microencapsulation to Release into the Gut. *Pharmaceutics* **2012**, *4* (1), 149−163.
(50) Desitti, C.; Beliavski, M.; Tarre, S.; Avrahami, R.; Zussman, E.; Green, M. Durable Electrospun Microtubes for Encapsulation of Bacteria in Atrazine Bioremediation. *J. Water Process Eng.* **2017**, *19*, 205−211.
(51) Škrlec, K.; Zupančič, Š.; Prpar Mihevc, S.; Kocbek, P.; Kristl, J.; Berlec, A. Development of Electrospun Nanofibers That Enable High Loading and Long-Term Viability of Probiotics. *Eur. J. Pharm. Biopharm.* **2019**, *136*, 108−119.
(52) Kurečič, M.; Rijavec, T.; Hribernik, S.; Lapanje, A.; Kleinschek, K. S.; Maver, U. Novel Electrospun Fibers with Incorporated Commensal Bacteria for Potential Preventive Treatment of the Diabetic Foot. *Nanomed.* **2018**, *13* (13), 1583−1594.
(53) Canbolat, M. F.; Tang, C.; Bernacki, S. H.; Pourdeyhimi, B.; Khan, S. Mammalian Cell Viability in Electrospun Composite Nanofiber Structures. *Macromol. Biosci.* **2011**, *11* (10), 1346−1356.
(54) Deng, L.; Zhang, H. Recent Advances in Probiotics Encapsulation by Electrospinning. *ES Food Agrofor* **2020**, DOI: 10.30919/esfaf1120.
(55) Xie, S.; Tai, S.; Song, H.; Luo, X.; Zhang, H.; Li, X. Genetically Engineering of Escherichia Coli and Immobilization on Electrospun Fibers for Drug Delivery Purposes. *J. Mater. Chem. B* **2016**, *4*, 6820−6829.
(56) Feng, K.; Zhai, M.-Y.; Zhang, Y.; Linhardt, R. J.; Zong, M.-H.; Li, L.; Wu, H. Improved Viability and Thermal Stability of the Probiotics Encapsulated in a Novel Electrospun Fiber Mat. *J. Agric. Food Chem.* **2018**, *66* (41), 10890−10897.
(57) Deitzel, J. M.; Kleinmeyer, J.; Harris, D.; Beck Tan, N. C. The Effect of Processing Variables on the Morphology of Electrospun Nanofibers and Textiles. *Polymer* **2001**, *42* (1), 261−272.
(58) Baumgarten, P. K. Electrostatic Spinning of Acrylic Microfibers. *J. Colloid Interface Sci.* **1971**, *36* (1), 71−79.
(59) Reznik, S. N.; Yarin, A. L.; Zussman, E.; Bercovici, L. Evolution of a Compound Droplet Attached to a Core-Shell Nozzle under the Action of a Strong Electric Field. *Phys. Fluids* **2006**, *18* (6), 062101.

(60) Miller, K. S.; Krochta, J. M. Oxygen and Aroma Barrier Properties of Edible Films: A Review. *Trends Food Sci. Technol.* **1997**, *8* (7), 228−237.
(61) Vass, P.; Pantea, E.; Domokos, A.; Hirsch, E.; Domján, J.; Németh, Á.; Molnár, M.; Fehér, C.; Andersen, S. K.; Vigh, T.; Verreck, G.; Csontos, I.; Marosi, G.; Nagy, Z. K. Electrospun Solid Formulation of Anaerobic Gut Microbiome Bacteria. *AAPS PharmSciTech* **2020**, *21* (6), 214.
(62) Ajalloueian, F.; Guerra, P. R.; Bahl, M. I.; Torp, A. M.; Hwu, E. T.; Licht, T. R.; Boisen, A. Multi-Layer PLGA-Pullulan-PLGA Electrospun Nanofibers for Probiotic Delivery. *Food Hydrocoll* **2022**, *123*, 107112.
(63) Scotti, E.; Boué, S.; Sasso, G. L.; Zanetti, F.; Belcastro, V.; Poussin, C.; Sierro, N.; Battey, J.; Gimalac, A.; Ivanov, N. V.; Hoeng, J. Exploring the Microbiome in Health and Disease: Implications for Toxicology. *Toxicol. Res. Appl.* **2017**, *1*, 239784731774188.
(64) Round, J. L.; Mazmanian, S. K. The Gut Microbiota Shapes Intestinal Immune Responses during Health and Disease. *Nat. Rev. Immunol.* **2009**, *9* (5), 313−323.
(65) Lyte, M. Microbial Endocrinology in the Microbiome-Gut-Brain Axis: How Bacterial Production and Utilization of Neurochemicals Influence Behavior. *PLOS Pathog.* **2013**, *9* (11), e1003726.
(66) Dash, S. K.; Spreen, A. N.; Ley, B. M. *Health Benefits of Probiotics*; BL Publications, 2000.
(67) FAO/WHO. *Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria*; World Health Organization: Cordoba, Argentina, 2001; p 34.
(68) Riezzo, G.; Orlando, A.; D'Attoma, B.; Guerra, V.; Valerio, F.; Lavermicocca, P.; De Candia, S.; Russo, F. Randomised Clinical Trial: Efficacy of Lactobacillus Paracasei-Enriched Artichokes in the Treatment of Patients with Functional Constipation–a Double-Blind, Controlled, Crossover Study. *Aliment. Pharmacol. Ther.* **2012**, *35* (4), 441−450.
(69) Suez, J.; Zmora, N.; Zilberman-Schapira, G.; Mor, U.; Dori-Bachash, M.; Bashiardes, S.; Zur, M.; Regev-Lehavi, D.; Ben-Zeev Brik, R.; Federici, S.; Horn, M.; Cohen, Y.; Moor, A. E.; Zeevi, D.; Korem, T.; Kotler, E.; Harmelin, A.; Itzkovitz, S.; Maharshak, N.; Shibolet, O.; Pevsner-Fischer, M.; Shapiro, H.; Sharon, I.; Halpern, Z.; Segal, E.; Elinav, E. Post-Antibiotic Gut Mucosal Microbiome Reconstitution Is Impaired by Probiotics and Improved by Autologous FMT. *Cell* **2018**, *174* (6), 1406−1423.e16.
(70) Atraki, R.; Azizkhani, M. Survival of Probiotic Bacteria Nanoencapsulated within Biopolymers in a Simulated Gastrointestinal Model. *Innov. Food Sci. Emerg. Technol.* **2021**, *72*, 102750.
(71) Ghorbani, S.; Azizkhani, M. Encapsulation of Lactic Acid Bacteria and Bifidobacteria Using Starch-Sodium Alginate Nanofibers to Enhance Viability in Food Model. *J. Food Process. Preserv.* **2021**, *45* (12), e16048.
(72) Fareed, F.; Saeed, F.; Afzaal, M.; Imran, A.; Ahmad, A.; Mahmood, K.; Shah, Y. A.; Hussain, M.; Ateeq, H. Fabrication of Electrospun Gum Arabic−Polyvinyl Alcohol Blend Nanofibers for Improved Viability of the Probiotic. *J. Food Sci. Technol.* **2022**, *59* (12), 4812−4821.
(73) Yilmaz, M. T.; Taylan, O.; Karakas, C. Y.; Dertli, E. An Alternative Way to Encapsulate Probiotics within Electrospun Alginate Nanofibers as Monitored under Simulated Gastrointestinal Conditions and in Kefir. *Carbohydr. Polym.* **2020**, *244*, 116447.
(74) Zhang, Z.; Zhang, R.; Xiao, H.; Bhattacharya, K.; Bitounis, D.; Demokritou, P.; McClements, D. J. Development of a Standardized Food Model for Studying the Impact of Food Matrix Effects on the Gastrointestinal Fate and Toxicity of Ingested Nanomaterials. *NanoImpact* **2019**, *13*, 13−25.
(75) Lee, K. Y.; Mooney, D. J. Alginate: Properties and Biomedical Applications. *Prog. Polym. Sci.* **2012**, *37* (1), 106−126.
(76) Jacobsen, N. M. Y.; Caglayan, I.; Caglayan, A.; Bar-Shalom, D.; Müllertz, A. Achieving Delayed Release of Freeze-Dried Probiotic Strains by Extrusion, Spheronization and Fluid Bed Coating - Evaluated Using a Three-Step in Vitro Model. *Int. J. Pharm.* **2020**, *591*, 120022.
(77) Cheow, W. S.; Kiew, T. Y.; Hadinoto, K. Controlled Release of Lactobacillus Rhamnosus Biofilm Probiotics from Alginate-Locust Bean Gum Microcapsules. *Carbohydr. Polym.* **2014**, *103*, 587−595.
(78) Gu, M.; Zhang, Z.; Pan, C.; Goulette, T. R.; Zhang, R.; Hendricks, G.; McClements, D. J.; Xiao, H. Encapsulation of Bifidobacterium Pseudocatenulatum G7 in Gastroprotective Microgels: Improvement of the Bacterial Viability under Simulated Gastrointestinal Conditions. *Food Hydrocoll* **2019**, *91*, 283−289.
(79) Mei, L.; He, F.; Zhou, R.-Q.; Wu, C.-D.; Liang, R.; Xie, R.; Ju, X.-J.; Wang, W.; Chu, L.-Y. Novel Intestinal-Targeted Ca-Alginate-Based Carrier for PH-Responsive Protection and Release of Lactic Acid Bacteria. *ACS Appl. Mater. Interfaces* **2014**, *6* (8), 5962−5970.
(80) Su, Y.; Gu, L.; Zhang, Z.; Chang, C.; Li, J.; McClements, D. J.; Yang, Y. Encapsulation and Release of Egg White Protein in Alginate Microgels: Impact of PH and Thermal Treatment. *Food Res. Int.* **2019**, *120*, 305−311.
(81) Liu, Y.; Liu, B.; Li, D.; Hu, Y.; Zhao, L.; Zhang, M.; Ge, S.; Pang, J.; Li, Y.; Wang, R.; Wang, P.; Huang, Y.; Huang, J.; Bai, J.; Ren, F.; Li, Y. Improved Gastric Acid Resistance and Adhesive Colonization of Probiotics by Mucoadhesive and Intestinal Targeted Konjac Glucomannan Microspheres. *Adv. Funct. Mater.* **2020**, *30* (35), 2001157.
(82) Varum, F. J. O.; Veiga, F.; Sousa, J. S.; Basit, A. W. Mucoadhesive Platforms for Targeted Delivery to the Colon. *Int. J. Pharm.* **2011**, *420* (1), 11−19.
(83) Grice, E. A.; Segre, J. A. The Skin Microbiome. *Nat. Rev. Microbiol.* **2011**, *9* (4), 244−253.
(84) Khan, M. A.; Hussain, Z.; Ali, S.; Qamar, Z.; Imran, M.; Hafeez, F. Y. Fabrication of Electrospun Probiotic Functionalized Nanocomposite Scaffolds for Infection Control and Dermal Burn Healing in a Mice Model. *ACS Biomater. Sci. Eng.* **2019**, *5* (11), 6109−6116.
(85) Silva, J. A.; De Gregorio, P. R.; Rivero, G.; Abraham, G. A.; Nader-Macías, M. E. F. Immobilization of Vaginal Lactobacillus in Polymeric Nanofibers for Its Incorporation in Vaginal Probiotic Products. *Eur. J. Pharm. Sci.* **2021**, *156*, 105563.
(86) Stojanov, S.; Kristl, J.; Zupančič, Š.; Berlec, A. Influence of Excipient Composition on Survival of Vaginal Lactobacilli in Electrospun Nanofibers. *Pharmaceutics* **2022**, *14* (6), 1155.
(87) Zárate, G.; Nader-Macias, M. E. Viability and Biological Properties of Probiotic Vaginal Lactobacilli after Lyophilization and Refrigerated Storage into Gelatin Capsules. *Process Biochem.* **2006**, *41* (8), 1779−1785.
(88) Cazorla-Luna, R.; Ruiz-Caro, R.; Veiga, M.-D.; Malcolm, R. K.; Lamprou, D. A. Recent Advances in Electrospun Nanofiber Vaginal Formulations for Women's Sexual and Reproductive Health. *Int. J. Pharm.* **2021**, *607*, 121040.
(89) das Neves, J.; Notario-Pérez, F.; Sarmento, B. Women-Specific Routes of Administration for Drugs: A Critical Overview. *Adv. Drug Deliv. Rev.* **2021**, *176*, 113865.
(90) Palmeira-de-Oliveira, R.; Oliveira, A. S.; Rolo, J.; Tomás, M.; Palmeira-de-Oliveira, A.; Simões, S.; Martinez-de-Oliveira, J. Women's Preferences and Acceptance for Different Drug Delivery Routes and Products. *Adv. Drug Deliv. Rev.* **2022**, *182*, 114133.
(91) Laborde, N. D.; Leslie, J.; Krogstad, E.; Morar, N.; Mutero, P.; Etima, J.; Woodrow, K.; van der Straten, A. Perceptions of the "Fabric" − An Exploratory Study of a Novel Multi-Purpose Technology among Women in Sub Saharan Africa. *PLoS One* **2018**, *13* (10), e0204821.
(92) Krogstad, E. A.; Woodrow, K. A. Manufacturing Scale-up of Electrospun Poly(Vinyl Alcohol) Fibers Containing Tenofovir for Vaginal Drug Delivery. *Int. J. Pharm.* **2014**, *475* (1), 282−291.
(93) Noruzi, M. Electrospun Nanofibres in Agriculture and the Food Industry: A Review. *J. Sci. Food Agric.* **2016**, *96* (14), 4663−4678.
(94) Mukiri, C.; Raja, K.; Senthilkumar, M.; Subramanian, K. S.; Govindaraju, K.; Pradeep, D.; Ranjan, S. Immobilization of Beneficial Microbe Methylobacterium Aminovorans in Electrospun Nanofibre as Potential Seed Coatings for Improving Germination and Growth of Groundnut Arachis Hypogaea. *Plant Growth Regul.* **2022**, *97* (2), 419−427.

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964

(95) De Gregorio, P. R.; Michavila, G.; Muller, L. R.; de Souza Borges, C.; Pomares, M. F.; de Sá, E. L. S.; de Pereira, C.; Vincent, P. A. Beneficial Rhizobacteria Immobilized in Nanofibers for Potential Application as Soybean Seed Bioinoculants. *PLoS One* **2017**, *12* (5), e0176930.
(96) Damasceno, R.; Roggia, I.; Pereira, C.; de Sá, E. Rhizobia Survival in Seeds Coated with Polyvinyl Alcohol (PVA) Electrospun Nanofibres. *Can. J. Microbiol.* **2013**, *59* (11), 716−719.
(97) Hussain, Z.; Khan, M. A.; Iqbal, F.; Raffi, M.; Hafeez, F. Y. Electrospun Microbial-Encapsulated Composite-Based Plasticized Seed Coat for Rhizosphere Stabilization and Sustainable Production of Canola (Brassica Napus L.). *J. Agric. Food Chem.* **2019**, *67* (18), 5085−5095.
(98) Sarioglu, O. F.; Keskin, N. O. S.; Celebioglu, A.; Tekinay, T.; Uyar, T. Bacteria Encapsulated Electrospun Nanofibrous Webs for Remediation of Methylene Blue Dye in Water. *Colloids Surf. B Biointerfaces* **2017**, *152*, 245−251.
(99) San Keskin, N. O.; Celebioglu, A.; Sarioglu, O. F.; Uyar, T.; Tekinay, T. Encapsulation of Living Bacteria in Electrospun Cyclodextrin Ultrathin Fibers for Bioremediation of Heavy Metals and Reactive Dye from Wastewater. *Colloids Surf. B Biointerfaces* **2018**, *161*, 169−176.
(100) Massaglia, G.; Sacco, A.; Chiodoni, A.; Pirri, C. F.; Quaglio, M. Living Bacteria Directly Embedded into Electrospun Nanofibers: Design of New Anode for Bio-Electrochemical Systems. *Nanomaterials* **2021**, *11* (11), 3088.
(101) Sanchez, J.-L.; Laberty-Robert, C. A Novel Microbial Fuel Cell Electrode Design: Prototyping a Self-Standing One-Step Bacteria-Encapsulating Bioanode with Electrospinning. *J. Mater. Chem. B* **2021**, *9* (21), 4309−4318.
(102) Karbelkar, A. A.; Reynolds, E. E.; Ahlmark, R.; Furst, A. L. A Microbial Electrochemical Technology to Detect and Degrade Organophosphate Pesticides. *ACS Cent. Sci.* **2021**, *7* (10), 1718−1727.
(103) Breheny, M.; Bowman, K.; Farahmand, N.; Gomaa, O.; Keshavarz, T.; Kyazze, G. Biocatalytic Electrode Improvement Strategies in Microbial Fuel Cell Systems. *J. Chem. Technol. Biotechnol.* **2019**, *94*, 2081−2091.

https://doi.org/10.1021/acsabm.2c01055
ACS Appl. Bio Mater. 2023, 6, 951−964