SCIENCE CHINA
Chemistry

•ARTICLES•
SPECIAL ISSUE: 2023 Emerging Investigator Issue

December 2023 Vol.66 No.12: 3594–3601
https://doi.org/10.1007/s11426-023-1880-8

Chiral coating-mediated interactions of bacteria with diverse biointerfaces

Hejin Jiang, Rui Liu, Ying Liu, Huilong Luo*, Zhenping Cao* & Jinyao Liu*

Shanghai Key Laboratory for Nucleic Acid Chemistry and Nanomedicine, Institute of Molecular Medicine, State Key Laboratory of Systems Medicine for Cancer, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China

Received September 20, 2023; accepted November 16, 2023; published online November 22, 2023

Bacteria, as living agents, have been widely used for microbial therapy due to their inherent ability to colonize different *in vivo* microenvironments, particularly in the gut and tumor. The interaction with diverse biointerfaces plays a critical role in bacterial colonization, and thereby determine the ultimate efficacy of microbial therapy. Although surface modification of bacteria with exogenous functional motifs can vary their interaction with surroundings, the effects of surface chirality of modified bacteria on mucous adhesion, tumor cell binding, and bacterial competition remain unknown. Here, we describe surface chirality-dependent selective interactions of bacteria to mucins, tumor cells, and pathogens. By coating bacteria with cationic polyethyleneimine modified with different chiral amino acids through electrostatic interaction, we find that bacteria coated with a D-chiral surface structure exhibit greatly increased adhesion to both mucins and tumor cells compared with those of L- and DL-structures. In addition, by adjusting the chirality of the coating, wrapped probiotic bacteria can selectively resist pathogenic bacteria, showing great potential to enhance colonization and positively modulate the gut and tumor microbiota. This work discloses surface chirality-dependent interaction of bacteria with different biointerfaces, showing a potential to tune the colonization and therapeutic effect of living bacterial agents for disease treatment.

bacteria, coating, chiral, adhesion, living agents

Citation: Jiang H, Liu R, Liu Y, Luo H, Cao Z, Liu J. Chiral coating-mediated interactions of bacteria with diverse biointerfaces. *Sci China Chem*, 2023, 66: 3594–3601, https://doi.org/10.1007/s11426-023-1880-8

1 Introduction

Microorganisms exist widely in human oral cavity, nasal cavity, gastrointestinal tract, skin, and other tissues and organs and play a vital role in maintaining human health [1,2]. With gradual deepening of the study of the gut microbiome, it has been recognized that imbalance of the gut microbiome can negatively affect the metabolic and immune homeostasis of the host [3–6]. For example, disruption of the gut microbiota may contribute to the development of a broad variety of diseases, such as inflammatory bowel disease, obesity, diabetes, and cancer [7,8]. On the other hand, recent studies have found that various types of solid tumors are colonized with abundant bacteria and the development, progression, and even the efficacy of tumor therapy are closely associated with the tumor microbiota [9,10]. For instance, the colonization of specific bacteria in tumor sites has been reported to be able to retard tumor growth and play synergistic antitumor effect with conventional therapy modalities, as these bacteria themselves as well as their metabolites can activate the body's immune system to generate protective immunity [11,12]. Therefore, it is of great significance to positively regulate the balance of the corresponding microbiota for disease treatment [13].

Given their inherent ability to colonize different biointer-

*Corresponding authors (email: jyliu@sjtu.edu.cn; caozhenping@renji.com; xiaolong19891205@126.com)

faces, particularly in the gut and tumor, bacteria have been widely used as living agents to modulate the microbiota [14]. Depending on their species and biological functions, bacteria can be employed as either active delivery vehicles or living biotherapeutics to inhibit pathogenic bacteria and maintain a healthy microbial composition [15]. To achieve satisfactory efficacy, adequate colonization at the sites of interest, which largely relies on the interaction of bacteria with environmental biointerfaces, is essential [16]. To date, surface modification with exogenous functional motifs has been frequently applied to tailor the interaction of bacteria with surroundings [17]. More recently, we and other groups have utilized surface coating of bacteria to control over their interaction with *in vivo* biointerfaces [18–20]. For example, coating with polyphenol-metal networks can promote bacterial colonization at mucous layer basing on an enhanced dually hydrogen bonding- and hydrophobic interaction-mediated adhesion [21]. Moreover, wrapping with tumor cell membranes is able to not only reduce immune clearance of bacteria by macrophages through a specific CD 47 protein/signal regulatory protein α recognition, but also increase tumor accumulation *via* homotypic tropism [22]. Despite these elegant achievements [23–25], alternative modalities capable to manipulate the interaction of bacteria with external environments are highly desirable to expand the feasibility to improve the colonization and corresponding therapeutic effect of living bacterial agents.

Chirality, ubiquitously existing from small-molecular amino acids and polysaccharides to DNA, proteins, teeth, hands, *etc*., is closely related to life [26–30]. Except for chiral proteins and sugars, there are many free short peptides and D-amino acids in the cell membrane and cell wall of bacteria [31], which leads to chiral performance of bacteria themselves in optics. These chiral molecules are essential for bacterial cell structure, growth, and metabolism [32]. In recent years, many studies have shown that chiral nanomaterials have obvious chiral selectivity for cell adhesion [33], proliferation [34], differentiation [35], cell phagocytosis [36], gene editing [37], and even disease diagnosis and treatment [38–40]. Therefore, we speculate that coating bacteria with a chiral surface structure may offer an appealing strategy to tune bacteria-host interfacial interaction for optimal colonization and enhanced treatment efficacy.

Herein, we report the construction of chiral coating on bacterial surface using synthetic polymers through interfacial self-assembly (Figure 1). By means of end-capping with different chiral amino acids, the modified polyethyleneimine (PEI) is able to deposit on bacteria *via* electrostatic interaction, forming a chiral surface coating that is stable in diverse conditions. Interestingly, bacteria coated with chiral coating show surface chirality-dependent selective interactions with mucins, tumor cells, and pathogens. We find that in comparison to those of L- and DL-structures, bacteria wrapped with a D-chiral surface structure display greatly increased adhesion to not only intestinal mucins, but also different types of tumor cells including 4T1, Caco2, B16-OVA, and CT26 cell lines. Furthermore, coated probiotic bacteria can selectively resist pathogenic bacteria including *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA14) by adjusting the chirality of the coating, showing great potential to improve colonization and positively regulate the gut and tumor microbiota. This work discloses surface chirality-dependent interaction of bacteria with different biointerfaces,

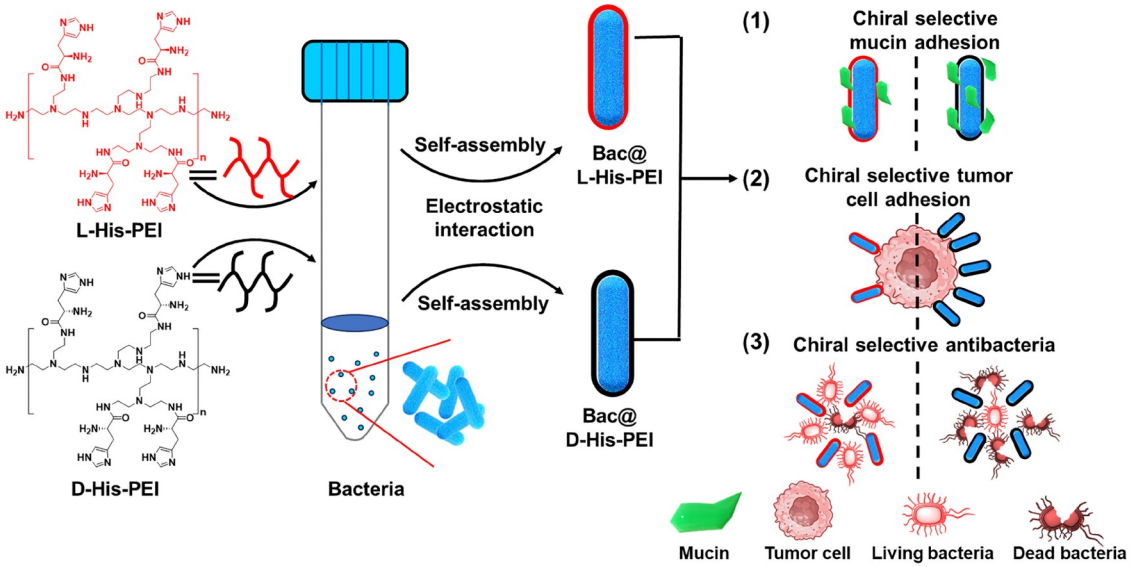

Figure 1 Schematic illustrations of the preparation of self-assembled chiral polymer-coated bacteria and the applications in chiral-selective adhesion to mucin and tumor cells and bacterial competition (color online).

proposing an alternative to develop advanced living bacterial therapeutics.

2 Results and discussion

Chiral polymers were synthesized by conjugated chiral L- or D-histidine with PEI and abbreviated as L-His-PEI or D-His-PEI, which was based on our previous reported methods [41]. Then, a well-known probiotic strain *Escherichia coli* Nissle 1917 (EcN) was chosen and coated with the as-prepared chiral polymer by vortexing in phosphate buffered solution (PBS) for 30 min, as shown in Figure 2a. By virtue of electrostatic interaction, the positively charged chiral polymers could easily self-assemble onto the negatively charged bacterial surface and form a supramolecular chiral membrane around the bacteria. Considering the cytotoxicity of cationic polymers, the effects of concentrations of chiral polymers on bacteria were tested. The bacteria were coated with three different polymer concentrations including 40, 80, and 160 μg/mL and the coated EcN (termed as EcN@L-His-PEI, EcN@ D-His-PEI, and EcN@DL-His-PEI, respectively) were cultured in 100% Luria Bertani (LB) medium. The growth curve was recorded within 12 h by monitoring the values of optical density at 600 nm ($OD_{600\ nm}$). Encouragingly, the chiral polymers showed negligible effects on the growth of bacteria at a polymer concentration of 40 μg/mL (Figure 2b). The logarithmic phase of bacterial growth curve shifted slightly with the concentration increasing to 80 μg/mL (Figure S1a, Supporting Information online). Upon reaching up to 160 μg/mL, the logarithmic phase of the bacterial growth curve was delayed by 1 to 2 h (Figure S1b). All these concentrations enabled a satisfactory coating efficiency (Figures S2 and S3). The results suggested that although the chiral polymer at high concentrations emerged cytotoxicity, there was no obvious side effect on the growth of bacteria at concentrations that could form a surface coating efficiently. As such, the concentration of 40 μg/mL was selected for all subsequent experiments.

We next characterized the physicochemical properties of the chiral polymer coated bacteria. First, the chirality of the coated bacteria was analyzed by circular dichroism (CD) spectrum. As shown in Figure S4, L-His-PEI appeared a positive CD signal at 220 nm, while D-His-PEI presented a completely opposite signal. As for uncoated bacteria, an obvious CD signal is detected at 230 nm (Figure 2c). After coating with chiral polymer, the CD spectrum of EcN@L-His-PEI showed a negative Cotton effect at 230 nm and a positive Cotton effect at 220 nm, which were attributed to EcN and L-His-PEI, respectively. In terms of EcN@D-His-PEI, it displayed a broad negative CD signal at the range of 210–250 nm and EcN@DL-His-PEI maintained the chirality of EcN (Figure 2c). It was illustrated that the surface chirality of bacteria could be varied by using different chiral polymer coatings. The hydrodynamic size of uncoated EcN was about 1,950 nm, which was increased to 2,100–2,200 nm after coating with chiral polymers (Figure 2d). In addition, the zeta potential of uncoated EcN was about −35 mV, which turned to around −25 mV after coating (Figure 2e). These results indicated that the chiral polymers were successfully self-assembled on the surface of bacteria.

To directly visualize coated bacteria, EcN labeled with red fluorescent protein (EcN-mCherry) was utilized for confocal imaging. The chiral polymers of L-, D- and DL-His-PEI with

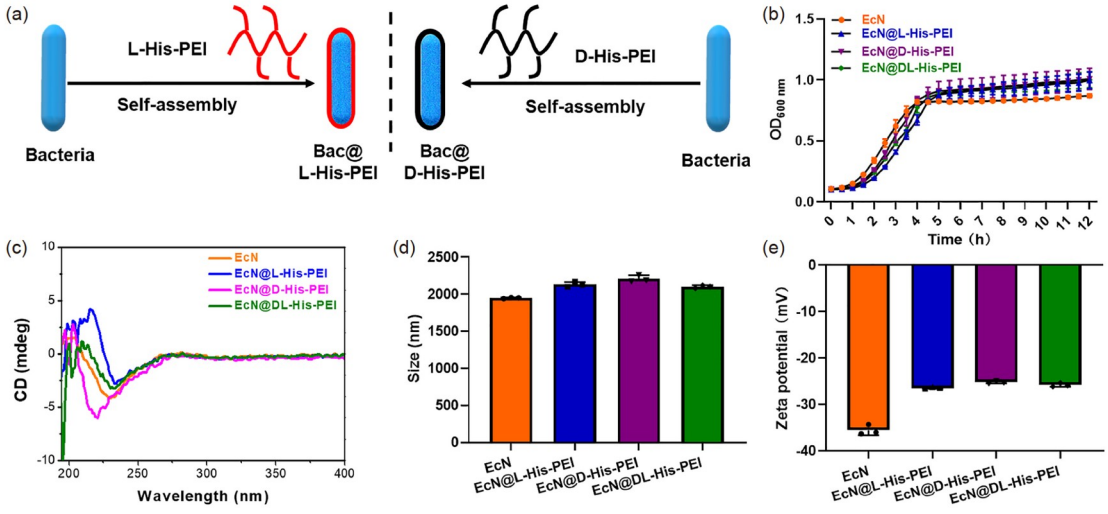

Figure 2 (a) Fabrication of chiral polymer-coated bacteria by self-assembly. (b) Growth curves of uncoated EcN and chiral polymer-coated bacteria in LB medium at 37 °C. $OD_{600\ nm}$ values were recorded at 30 min intervals using a microplate reader (n = 5). (c) CD spectra of EcN, EcN@L-His-PEI, EcN@D-His-PEI, and EcN@DL-His-PEI. (d) Hydrodynamic sizes and (e) zeta potentials of EcN, EcN@L-His-PEI, EcN@D-His-PEI, and EcN@DL-His-PEI measured by DLS (n = 3) (color online).

a small amount of fluorescein isothiocyanate isomer (FITC) labeled PEI were added to the PBS suspension of EcN-mCherry, respectively. After half an hour of vortexing, the coated ECN-mCherry samples were centrifuged and washed five times with PBS. The obtained labeled coated bacteria were defined as EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI, respectively. With the help of laser scanning confocal microscope (LSCM), the polymer coated on bacterial surface could be clearly observed. As shown in Figure 3a and Figure S5, bacteria without a chiral polymer coating only showed red fluorescence from EcN-mCherry expressed fluorescent protein. Differently, all the chiral (L- and D-His-PEI) and racemic (DL-His-PEI) polymer coated EcN-mCherry could be detected by both red fluorescence of the bacteria and the green fluorescence of the polymer self-assembled on the surface of bacteria. Histograms obtained from flow cytometric analysis presented a distinct shift of FITC-labeled EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI to higher fluorescence intensity than uncoated EcN-mCherry, further verifying the successful decoration with a chiral polymer coating (Figure 3b). Additionally, the morphologies of uncoated and coated bacteria were also observed by transmission electron microscopy (TEM). The surface of the uncoated EcN-mCherry was clean and bacteria pili could be clearly observed. In contrast, the surface of chiral polymer-coated EcN-mCherry became rough and numerous nanoparticles that might be ascribed to the aggregated PEI were scattered on the surface of the bacteria (Figure 3c–f and Figure S6). To prove the flexibility of this coating method, we tested *Salmonella Typhimurium* VNP20009 (VNP), which could be similarly coated by using the same protocol (Figure S7). Taken together, all these results demonstrate that the chiral polymer could be easily coated on bacterial surface by self-assembly and chiral coatings could be obtained.

To verify the stability of the chiral coating on bacterial surface and study its interactions with the surroundings associated with the intestinal tract and tumor tissue, the chiral polymer-coated bacteria were transferred to LB medium, Dulbecco's modified Eagle media (DMEM) containing 10% fetal bovine serum (FBS), and PBS (pH 2) respectively and vibrated for 1 h. Then, the treated bacteria were centrifuged and washed several times with PBS before LSCM observation (Figures S8 and S9). It could be clearly visualized that the bacteria remained evenly coated with a green chiral coating even after treatment with LB, DMEM + 10% FBS medium or PBS (pH 2). Subsequently, the fluorescence intensity of coated bacteria was analyzed by flow cytometry before and after LB, DMEM + 10% FBS medium, and PBS (pH 2) treatment. It showed that about 80% fluorescence intensity was remained for EcN-mCherry@L-His-PEI and EcN-mCherry@DL-His-PEI groups after treatment with LB medium (Figure S10). Similarly, 70% of retention of fluorescence intensity was observed for EcN-mCherry@D-His-PEI group. After treatment with DMEM + 10% FBS medium, the fluorescence intensity of EcN-mCherry@L-His-PEI group was barely affected (Figure S10). The fluorescence

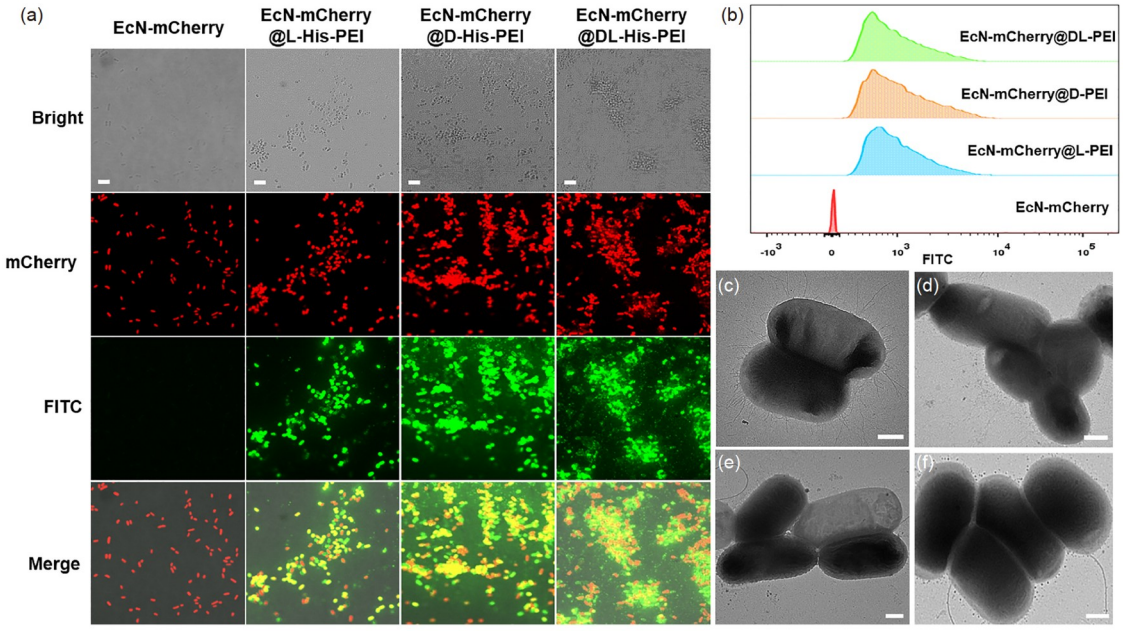

Figure 3 (a) Typical LSCM images of unmodified EcN-mCherry and chiral polymer-coated EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI. Scale bars: 4 μm. (b) Flow cytometry histograms and mean fluorescence intensity (MFI) values of uncoated EcN-mCherry, EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI post votexing with chiral and racemic polymers for 30 min. TEM images of (c) EcN-mCherry, (d) EcN-mCherry@L-His-PEI, (e) EcN-mCherry@D-His-PEI, and (f) EcN-mCherry@DL-His-PEI. Scale bars: 500 nm (color online).

intensity values remained at 80% and over 90% for EcN-mCherry@D-His-PEI group and EcN-mCherry@DL-His-PEI groups, respectively. Similarly, over 90% fluorescence signals remained after incubation bacteria with PBS (pH 2) (Figure S10). The above data illustrated that the chiral polymer coating could exist stably on bacterial surface under different culture media.

Mucous layer, which lines the insides of organs and cavities throughout the body, lubricates and protects these objects from bodily fluids and invasive pathogens [42]. In addition to its involvement in absorption, particularly in the gastrointestinal tract, as the body's largest protective barrier, mucous layer plays a crucial role in maintaining the homeostasis of the gut microbiota [43]. Mucin refers to an important category of large extracellular glycosylated proteins that are the main organic components of mucous layer [44]. As reported, mucin is critical for the colonization of symbiotic bacteria (*e.g.*, *Akkermansia muciniphila*) [45]. To investigate the interaction with mucous layer, we evaluated the binding of mucin with bacteria wrapped by different chiral polymer coatings. Freshly prepared uncoated EcN, chiral and achiral polymer coated EcN were mixed with FITC-labeled mucin in PBS and vibrated for 30 min, respectively. After washing with PBS for five times to remove unbound free mucin, all the samples were analyzed by flow cytometry to quantify the binding efficiency. As shown in Figure 4, there was an obvious shift after incubating the bacteria with mucin, which indicated that bacteria could bind mucin efficiently. More importantly, bacteria coated with a D-chiral polymer coating, namely EcN@D-His-PEI, achieved the strongest fluorescence intensity after incubation with mucin compared with those of uncoated bacteria, EcN-mCherry@L-His-PEI, and EcN-mCherry@DL-His-PEI. This increment indicated that the affinity between D-chiral coating and mucin was stronger than those of L-handed and achiral polymer coatings, disclosing a chirality-dependent selective binding between mucin and bacterial surface.

The tumor microbiota has been reported to be closely related with tumor malignancy [46]. Specific bacteria with ability to target and colonize tumor tissue have been exploited for tumor therapy due to their effectiveness to modulate a beneficial tumor microbial composition and/or elicit antitumor immune responses [47]. EcN, as one of the most widely used bacteria in bacterial therapy, can be easily modified by genetic engineering [48], synthetic bioengineering [49], and chemical approaches [50–52] to improve target accumulation in tumor sites. For example, EcN decorated with aptamers on the surface can facilitate its enrichment in tumor tissue following systemic injection, due largely to the specific interaction between the aptamers on bacteria surface and the receptor on tumor cell membrane [53]. Previous studies have proved that cells have selective adhesion and uptake of chiral nanomaterials [41]. We hence

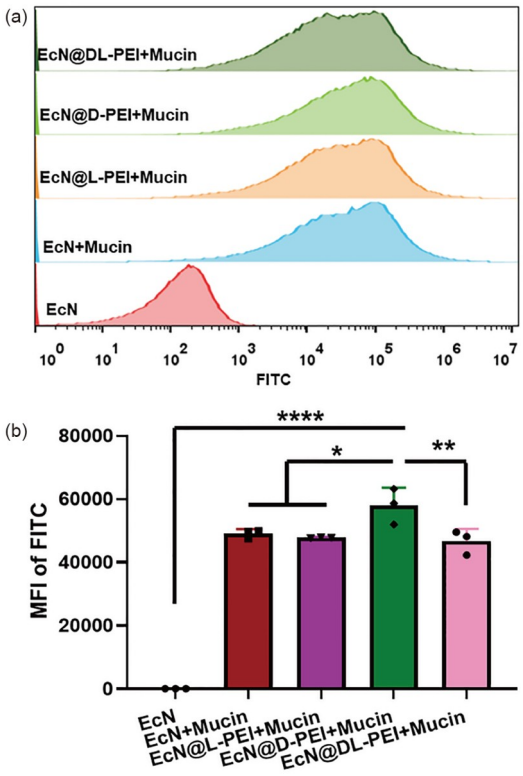

Figure 4 (a) Flow cytometry histograms and (b) MFI values of uncoated EcN-mCherry, EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI post interaction with FITC-labeled mucin for 30 min ($n = 3$). The feed number of bacteria was set at $OD_{600\ nm} = 2$ and the concentration of mucin was set at 0.4 mg/mL. Significance was assessed using one-way analysis of variance (ANOVA) with the Tukey's post-test, giving p-values, *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ (color online).

speculated that coating bacteria with chiral polymer coatings could tune the interaction with tumor cells. Freshly prepared uncoated EcN-mCherry, chiral and achiral polymer-coated EcN-mCherry (1×10^6 colony forming units (CFUs)) were added to mouse breast cancer (4T1) cells and incubated for 1 h under 37 °C. Cells were labeled with Hoechst and then washed with PBS for ten times to remove unbound bacteria before observation by LSCM. As visualized in Figure 5a, it was clearly that bacteria wrapped with chiral polymer coatings presented increased adhesion to 4T1 cells compared with uncoated bacteria. Strikingly, after incubation, bacteria with a D-handed polymer coating showed the highest number on 4T1 cells compared with those of L-handed and racemic counterparts. This highlighted that coated EcN exhibited chiral-selective cell adhesion to 4T1 cells. In addition, the bacteria adhered on cell surface were collected and enumerated by plate counting (Figure 5b–e). Similarly, EcN-mCherry@D-His-PEI group showed near three-time higher adhesion on 4T1 cells than that of EcN-mCherry@L-His-PEI group and more than eight-time higher than those of uncoated EcN and EcN-mCherry@DL-His-PEI groups (Figure 5f). We also studied the adhesion of chiral polymer-

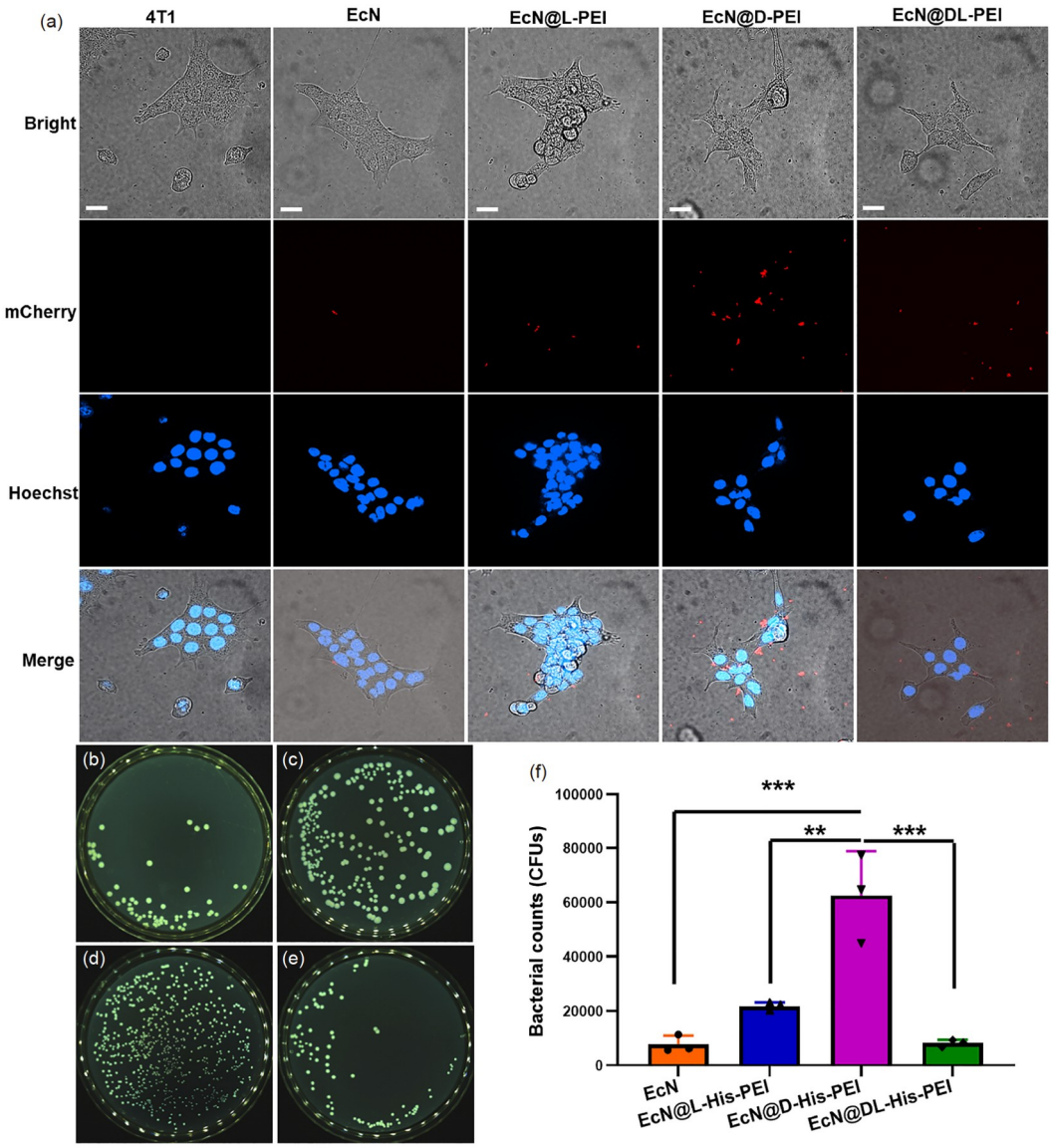

Figure 5 (a) LSCM images of cellular adhesion on 4T1 cells by incubation with PBS, uncoated EcN-mCherry, EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI for 1 h, respectively. Scale bar: 25 μm. Typical digital images of agar plates containing EcN-mCherry collected from (b) uncoated EcN-mCherry, (c) EcN-mCherry@L-His-PEI, (d) EcN-mCherry@D-His-PEI, and (e) EcN-mCherry@DL-His-PEI adhered on 4T1 cells. (f) Statistical results of plate counting of uncoated EcN-mCherry, EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI adhered on 4T1 cells ($n = 3$). Significance was assessed using one-way ANOVA with the Tukey's post-test, giving p-values, $^{**}p < 0.01$, $^{***}p < 0.001$ (color online).

coated bacteria to other tumor cells including Caco2, B16-OVA, and CT26. Both LSCM and plate counting results confirmed that D-handed polymer-coated bacteria achieved a superior adhesion ability to all these tumor cells (Figures S11–S13). Collectively, these results demonstrated that chiral polymer-coated bacteria were endowed with remarkable chiral selectivity for adhesion to tumor cells.

Finally, we assessed the interaction between coated probiotics and pathogenic bacteria. As a typical pathogenic strain, SA was chosen given its inherent capability to compete and suppress EcN. The resistant ability of chiral polymer-coated EcN against SA was examined by bacterial plate counting. The as-prepared bacteria were mixed with SA at an OD ratio of 4:1 and vibrated at room temperature. After 1 h incubation, the bacteria were centrifuged and the number of survived bacteria was counted. Notably, D-chiral polymer-coated EcN appeared the lowest number of survived SA (golden yellow colonies, Figure 6a). As expected, the number of living SA after treatment with unmodified bacteria was the highest. The statistical results supported that the residual bacteria in unmodified EcN, EcN-mCherry@L-His-PEI, and EcN-mCherry@DL-His-PEI groups were 208-, 28-, and 7-time higher than those in EcN-mCherry@D-His-PEI group, respectively (Figure 6b). Significantly, the same trend

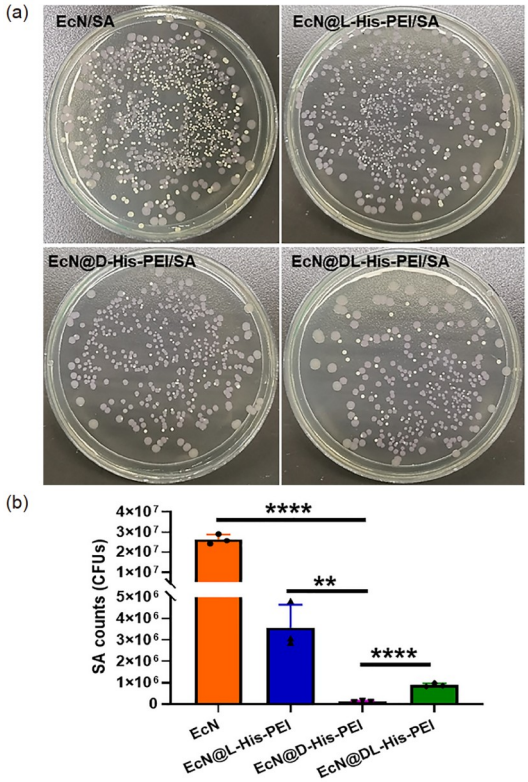

Figure 6 (a) Typical digital images of agar plates containing EcN-mCherry (red) and SA (gold yellow) collected from uncoated EcN-mCherry, EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI after competition with SA for 1 h. (b) Statistical results of survival number of SA after incubation with uncoated EcN-mCherry, EcN-mCherry@L-His-PEI, EcN-mCherry@D-His-PEI, and EcN-mCherry@DL-His-PEI, respectively (n = 3). Significance was assessed using one-way ANOVA with the Tukey's post-test, giving p-values, $^{**}p < 0.01$, $^{****}p < 0.0001$ (color online).

in the resistance of chiral polymer-coated EcN against pathogenic bacteria was observed by using PA14. As depicted in Figure S14, EcN-mCherry@D-His-PEI claimed ~2-fold higher antibacterial efficacy toward PA14 compared with those of EcN-mCherry@L-His-PEI, and EcN-mCherry @DL-His-PEI. All these data certificated that D-His-PEI coating could endow probiotics with superior resistance against some pathogenic bacterial species, demonstrating that chirality-dependent selective bacterial competition.

3 Conclusions

In summary, we have reported the preparation of chiral polymer-coated bacteria *via* interfacial self-assembly. By end-capping with different chiral amino acids, the resulting cationic PEI can self-assemble on bacterial surface through electrostatic interaction, generating a chiral coating. This approach is applicable to different bacterial strains and the formed coating is stable in diverse environments. Bacteria coated with a chiral coating demonstrate surface chirality-dependent selective interactions with mucins, tumor cells, and pathogens. In contrast to L- and DL-structures, bacteria coated with a D-chiral surface structure show significantly increased adhesion to both intestinal mucins and tumor cells (4T1, Caco2, B16-OVA, and CT26 cell lines). Moreover, coated probiotic bacteria are able to selectively suppress pathogenic strains including SA and PA14 by tailoring the chirality of the coating, spotlighting appealing superiority to enhance colonization and positively modulate the gut and tumor microbiota. Our work proposes a concept of surface chirality-dependent interaction of bacteria with different biointerfaces. Given the versatility of chiral coating to modify diverse strains, we anticipate the potential of this approach to develop innovative living bacterial agents with optimal colonization and therapeutic effect for disease treatment.

Acknowledgements This work was supported by the National Key Research and Development Program of China (2021YFA0909400), the National Natural Science Foundation of China (22102097, 52101289, 21875135), the Fundamental Research Funds for the Central Universities (2020JCPT02), the Innovative Research Team of High-Level Local Universities in Shanghai (SHSMU-ZDCX20210900), the Explorer Program of the Science and Technology Commission of Shanghai Municipality (21TS1400400), and the Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support (20181704).

Conflict of interest The authors declare no conflict of interest.

Supporting information The supporting information is available online at chem.scichina.com and link.springer.com/journal/11426. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

1 Dekaboruah E, Suryavanshi MV, Chettri D, Verma AK. *Arch Microbiol*, 2020, 202: 2147–2167
2 Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, Zhu D, Koya JB, Wei L, Li J, Chen ZS. *Sig Transduct Target Ther*, 2022, 7: 135
3 Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, Littmann E, van den Brink MRM, Jenq RR, Taur Y, Sander C, Cross JR, Toussaint NC, Xavier JB, Pamer EG. *Nature*, 2015, 517: 205–208
4 Knip M, Siljander H. *Nat Rev Endocrinol*, 2016, 12: 154–167
5 Zheng DW, Dong X, Pan P, Chen KW, Fan JX, Cheng SX, Zhang XZ. *Nat Biomed Eng*, 2019, 3: 717–728
6 Nakatsuji T, Hata TR, Tong Y, Cheng JY, Shafiq F, Butcher AM, Salem SS, Brinton SL, Rudman Spergel AK, Johnson K, Jepson B, Calatroni A, David G, Ramirez-Gama M, Taylor P, Leung DYM, Gallo RL. *Nat Med*, 2021, 27: 700–709
7 Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. *Nat Rev Gastroenterol Hepatol*, 2017, 14: 356–365
8 Eom T, Kim YS, Choi CH, Sadowsky MJ, Unno T. *J Microbiol*, 2018, 56: 189–198
9 Fu A, Yao B, Dong T, Chen Y, Yao J, Liu Y, Li H, Bai H, Liu X, Zhang Y, Wang C, Guo Y, Li N, Cai S. *Cell*, 2022, 185: 1356–1372.e26
10 Galeano Niño JL, Wu H, LaCourse KD, Kempchinsky AG, Baryiames A, Barber B, Futran N, Houlton J, Sather C, Sicinska E, Taylor A, Minot SS, Johnston CD, Bullman S. *Nature*, 2022, 611: 810–817
11 Kalaora S, Nagler A, Nejman D, Alon M, Barbolin C, Barnea E,

Ketelaars SLC, Cheng K, Vervier K, Shental N, Bussi Y, Rotkopf R, Levy R, Benedek G, Trabish S, Dadosh T, Levin-Zaidman S, Geller LT, Wang K, Greenberg P, Yagel G, Peri A, Fuks G, Bhardwaj N, Reuben A, Hermida L, Johnson SB, Galloway-Peña JR, Shropshire WC, Bernatchez C, Haymaker C, Arora R, Roitman L, Eilam R, Weinberger A, Lotan-Pompan M, Lotem M, Admon A, Levin Y, Lawley TD, Adams DJ, Levesque MP, Besser MJ, Schachter J, Golani O, Segal E, Geva-Zatorsky N, Ruppin E, Kvistborg P, Peterson SN, Wargo JA, Straussman R, Samuels Y. *Nature*, 2021, 592: 138–143
12 Chen Y, Du M, Yuan Z, Chen Z, Yan F. *Nat Commun*, 2022, 13: 4468
13 Houghteling PD, Walker WA. *J Pediatr Gastroenterol Nutr*, 2015, 60: 294–307
14 Zheng D, Liwinski T, Elinav E. *Cell Res*, 2020, 30: 492–506
15 Raman V, Van Dessel N, Hall CL, Wetherby VE, Whitney SA, Kolewe EL, Bloom SMK, Sharma A, Hardy JA, Bollen M, Van Eynde A, Forbes NS. *Nat Commun*, 2021, 12: 6116
16 Li SS, Zhu A, Benes V, Costea PI, Hercog R, Hildebrand F, Huerta-Cepas J, Nieuwdorp M, Salojärvi J, Voigt AY, Zeller G, Sunagawa S, de Vos WM, Bork P. *Science*, 2016, 352: 586–589
17 Ya'ari S, Halperin-Sternfeld M, Rosin B, Adler-Abramovich L. *Int J Mol Sci*, 2020, 21: 7370
18 Wang L, Cao Z, Zhang M, Lin S, Liu J. *Adv Mater*, 2022, 34: 2106669
19 Chen QW, Liu XH, Fan JX, Peng SY, Wang JW, Wang XN, Zhang C, Liu CJ, Zhang XZ. *Adv Funct Mater*, 2020, 30: 1909
20 Chen WH, Chen QW, Chen Q, Cui C, Duan S, Kang Y, Liu Y, Liu Y, Muhammad W, Shao S, Tang C, Wang J, Wang L, Xiong MH, Yin L, Zhang K, Zhang Z, Zhen X, Feng J, Gao C, Gu Z, He C, Ji J, Jiang X, Liu W, Liu Z, Peng H, Shen Y, Shi L, Sun X, Wang H, Wang J, Xiao H, Xu FJ, Zhong Z, Zhang XZ, Chen X. *Sci China Chem*, 2022, 65: 1010–1075
21 Luo H, Chen Y, Kuang X, Wang X, Yang F, Cao Z, Wang L, Lin S, Wu F, Liu J. *Nat Commun*, 2022, 13: 7808
22 Cao Z, Cheng S, Wang X, Pang Y, Liu J. *Nat Commun*, 2019, 10: 3452
23 Cao Z, Wang X, Pang Y, Cheng S, Liu J. *Nat Commun*, 2019, 10: 5783
24 Kwiecień I, Kwiecień M. *Gels*, 2018, 4: 47
25 Lin L, Zhang J. *BMC Immunol*, 2017, 18: 2
26 Kumar A, Capua E, Kesharwani MK, Martin JML, Sitbon E, Waldeck DH, Naaman R. *Proc Natl Acad Sci USA*, 2017, 114: 2474–2478
27 Nemati A, Shadpour S, Querciagrossa L, Li L, Mori T, Gao M, Zannoni C, Hegmann T. *Nat Commun*, 2018, 9: 3908
28 Bonner WA. *Origins Life Evol Biosphere*, 1991, 21: 59–111
29 Feringa BL, van Delden RA. *Angew Chem Int Ed*, 1999, 38: 3418–3438
30 Ma B, Bianco A. *Nat Rev Mater*, 2023, 8: 403–413
31 Miyamoto T, Homma H. *J Biochem*, 2021, 170: 5–13
32 Suzuki M, Sujino T, Chiba S, Harada Y, Goto M, Takahashi R, Mita M, Hamase K, Kanai T, Ito M, Waldor MK, Yasui M, Sasabe J. *Sci Adv*, 2021, 7: eabd6480
33 Liu F, Cheng Z, Jiang Y, Gao L, Liu H, Liu H, Feng Z, Lu P, Yang W. *Angew Chem Int Ed*, 2022, 61: 7789–7793
34 Zhao X, Xu L, Sun M, Ma W, Wu X, Xu C, Kuang H. *Nat Commun*, 2017, 8: 2007
35 Jiang S, Zeng Q, Zhao K, Liu J, Sun Q, Huang K, He Y, Zhang X, Wang H, Shi X, Feng C, Deng X, Wei Y. *Adv Mater*, 2022, 34: 2105136
36 Yeom J, Guimaraes PPG, Ahn HM, Jung BK, Hu Q, McHugh K, Mitchell MJ, Yun CO, Langer R, Jaklenec A. *Adv Mater*, 2020, 32: 1903878
37 Sun M, Xu L, Qu A, Zhao P, Hao T, Ma W, Hao C, Wen X, Colombari FM, de Moura AF, Kotov NA, Xu C, Kuang H. *Nat Chem*, 2018, 10: 821–830
38 Hou K, Zhao J, Wang H, Li B, Li K, Shi X, Wan K, Ai J, Lv J, Wang D, Huang Q, Wang H, Cao Q, Liu S, Tang Z. *Nat Commun*, 2020, 11: 4790
39 Xu L, Wang X, Wang W, Sun M, Choi WJ, Kim JY, Hao C, Li S, Qu A, Lu M, Wu X, Colombari FM, Gomes WR, Blanco AL, de Moura AF, Guo X, Kuang H, Kotov NA, Xu C. *Nature*, 2022, 601: 366–373
40 Liang K, Xue Y, Zhao B, Wen M, Xu Z, Sukhorukov G, Zhang L, Shang L. *Small*, 2023, 2304857
41 Jiang H, Liu R, Wang L, Wang X, Zhang M, Lin S, Cao Z, Wu F, Liu Y, Liu J. *Adv Mater*, 2023, 35: 2208157
42 Leal J, Smyth HDC, Ghosh D. *Int J Pharm*, 2017, 532: 555–572
43 Paone P, Cani PD. *Gut*, 2020, 69: 2232–2243
44 Johansson MEV, Sjövall H, Hansson GC. *Nat Rev Gastroenterol Hepatol*, 2013, 10: 352–361
45 Zhang T, Li Q, Cheng L, Buch H, Zhang F. *Microb Biotechnol*, 2019, 12: 1109–1125
46 Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, Rotter-Maskowitz A, Weiser R, Mallel G, Gigi E, Meltser A, Douglas GM, Kamer I, Gopalakrishnan V, Dadosh T, Levin-Zaidman S, Avnet S, Atlan T, Cooper ZA, Arora R, Cogdill AP, Khan MAW, Ologun G, Bussi Y, Weinberger A, Lotan-Pompan M, Golani O, Perry G, Rokah M, Bahar-Shany K, Rozeman EA, Blank CU, Ronai A, Shaoul R, Amit A, Dorfman T, Kremer R, Cohen ZR, Harnof S, Siegal T, Yehuda-Shnaidman E, Gal-Yam EN, Shapira H, Baldini N, Langille MGI, Ben-Nun A, Kaufman B, Nissan A, Golan T, Dadiani M, Levanon K, Bar J, Yust-Katz S, Barshack I, Peeper DS, Raz DJ, Segal E, Wargo JA, Sandbank J, Shental N, Straussman R. *Science*, 2020, 368: 973–980
47 Zhou S, Gravekamp C, Bermudes D, Liu K. *Nat Rev Cancer*, 2018, 18: 727–743
48 Hurt RC, Buss MT, Duan M, Wong K, You MY, Sawyer DP, Swift MB, Dutka P, Barturen-Larrea P, Mittelstein DR, Jin Z, Abedi MH, Farhadi A, Deshpande R, Shapiro MG. *Nat Biotechnol*, 2023, 41: 919–931
49 Lin L, Song J, Du Y, Wu Q, Gao J, Song Y, Yang C, Wang W. *Angew Chem Int Ed*, 2020, 59: 11923–11926
50 Zhao E, Chen Y, Chen S, Deng H, Gui C, Leung CWT, Hong Y, Lam JWY, Tang BZ. *Adv Mater*, 2015, 27: 4931–4937
51 Wu M, Wu W, Duan Y, Liu X, Wang M, Phan CU, Qi G, Tang G, Liu B. *Adv Mater*, 2020, 32: 2005222
52 Li Y, Hu X, Ding D, Zou Y, Xu Y, Wang X, Zhang Y, Chen L, Chen Z, Tan W. *Nat Commun*, 2017, 8: 15653
53 Geng Z, Cao Z, Liu R, Liu K, Liu J, Tan W. *Nat Commun*, 2021, 12: 6584