Endogenous electric field as a bridge for antibacterial ion transport from implant to bacteria

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ABSTRACT Tissue implant-related infections are among the most serious complications after surgical implantation, including orthopedics and dentistry. Implants with antibacterial ion release systems are efficient and economical antibiotic substitutes to fight against bacterial infections. However, the excessive amount of released antibacterial ions may cause biological toxicity while killing bacteria. This raises a fundamental issue on how to properly control the amounts of ions and their efficacy. Here, we develop low-dose antibacterial ions-incorporated ferroelectric implants (copper-doped potassium sodium niobate, $K_{0.5}Na_{0.5}NbO_3$ -Cu, KNNCu) whose surface potential can be tuned *via* external polarization. The released Cu^{2+} ions can be targeted to bacteria *via* endogenous electric field (EEF) between KNNCu implants and negatively charged bacteria. Intriguingly, the antibacterial efficacy of the implants is determined by the amount of Cu^{2+} ions that reaches bacteria, instead of the total amount of released Cu^{2+} ions. The amount of Cu^{2+} ions reaching bacteria from the high-surface-potential implant is 2.4 times that from the low-surface-potential implant within 12 h, resulting in the increased antibacterial ratio from about 65% to 100%, while remaining low cell toxicity. This work provides insights into the specific role of the EEF in guiding mass transport between charged materials and living organisms, and a new perspective for the design of high-performance antibacterial biomaterials.

Keywords: bacterial infection, endogenous electric field, ion transport, ferroelectric implant

INTRODUCTION

Bacterial infection is a major threat to human health, especially in surgeries involving large exposure of tissue to exogenous implants, such as orthopedics, and in dental and cardiovascular surgeries [1–4]. In order to protect tissue defects from bacterial infection, antibacterial ions and antibiotics are frequently incorporated into implants to enhance their antibacterial performance [5–10]. However, current studies ignore that most of the released antibacterial ions will randomly diffuse to the bacterial surrounding environment instead of penetrating the bacteria to exert an effect [11–14]. In other words, only a small portion of the released antibacterial ions can ultimately target the bacterial cell membrane. The low transport ratio of antibacterial ions hinders the work efficiency of these antibacterial ions-loaded implant materials. A reasonable hypothesis is that manipulation of antibacterial ions to target the bacterial cell membrane would enhance their antibacterial performance. Thus, developing new strategies to promote the transport of antibacterial ions to target bacteria is in urgent need for enhancing the antibacterial performance of implants.

Actually, as bacterial cell membrane is composed of lipids with negative charges [15,16], once they approach charged implant surface, an endogenous electric field (EEF) naturally forms between the implant and the bacterial cell membrane, which can guide electric transport of mass to bacterial surface. However, most previous

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studies did not take the EEF between implant and bacterial cell membrane into consideration, which leads to low antibacterial ion transport ratio [17–19]. It is reasonable to hypothesize that the EEF generated *via* manipulating the surface potential of the implant material will enhance the electric transport of antibacterial ions to target the bacterial membrane to increase the bacteria-killing efficacy.

In this research, we propose to achieve directional and quantitative transport of antibacterial ions to target bacteria *via* modulation of EEF to enhance pathogenic bacterial killing. In order to construct an implant model with regulatable EEF-mediated antibacterial ions, we design a ferroelectric copper-doped implant, potassium sodium niobate ($\text{K}_{0.5}\text{Na}_{0.5}\text{NbO}_3\text{-Cu}$, KNNCu), whose surface potential can be finely tuned *via* external electric field polarization to reverse its dipole direction [20]. Once in an infectious bacterial environment, an EEF will establish between the positively charged implant and negatively charged bacterial cell membrane to guide directional and quantitative transport of antibacterial ions to target the bacterial cell membrane (Scheme 1). Both the polarized implant and charged microorganisms constitute the EEF-controlled Cu^{2+} ion transport system to target bacteria for enhancing antibacterial property.

EXPERIMENTAL SECTION

Fabrication of KNNCu

KNN doped with CuO was prepared by using a solid-state reaction method. KNN was first synthesized from the potassium carbonate (K_2CO_3 , 99.0%), sodium carbonate (Na_2CO_3 , 99.8%) and niobium oxide (Nb_2O_5 , 99.9%) powders, which were purchased from Aladdin Industrial Inc. Firstly, the mixture of above raw materials was dried after 8 h ball grinding and then calcined at 750°C for 2 h to obtain KNN. Then, 0.5 wt% copper oxide powder (CuO , 99%, Aladdin Industrial Inc.) was doped into KNN to prepare the KNNCu powder. KNNCu samples were prepared by initially compressing (40 MPa), cold isostatic pressing (190 MPa for 5 min) and sintering (1050°C for 2 h). These samples were polarized under the external electric fields of 3 and 5 kV, respectively, for 10 min. The KNNCu samples are named according to their different potentials, H-KNNCu (KNNCu with high surface potential), M-KNNCu (KNNCu with mediate surface potential), and L-KNNCu (unpolarized KNNCu with low surface potential). The thickness of the KNNCu piezoelectric ceramics used in bacterial studies and cell studies was about 2 mm and the surface area was about

Scheme 1 Schematic illustration of the electric transport of antibacterial ions driven by an EEF established between the polarized ferroelectric implant and the negatively charged bacterial cell membrane as a bridge. Under the action of EEF, the amount of antibacterial ions transport to target bacteria increases, which can enhance the antibacterial property.

63.62 mm² (about 9 mm diameter).

Characterization of KNNCu

The morphology and elemental distribution of KNNCu were characterized by using the field emission scanning electron microscopy (FE-SEM) (Zeiss EVO18, Carl Zeiss Company, Germany) equipped with energy dispersive spectroscopy (EDS, Oxford instruments X-Max Extreme). X-ray diffraction (XRD, Bruker D8 advance, Germany) was used for the crystalline phase analysis. The chemical constitution of KNNCu was checked *via* X-ray photoelectron spectroscopy (XPS, Axis Ultra DLD Kratos Company). The surface potentials of the KNNCu samples were characterized by scanning Kelvin probe force microscopy (SKPM, MFP-3D-S, Asylum Research, USA) by averaging the data from randomly selected areas (1 μm × 1 μm) on the substrates. The polarization-electric (*P-E*) hysteresis loop of KNNCu was measured by a TF analyzer (Model 2000E, aixACCT Systems, Germany).

Antibacterial property

The antibacterial activities of the KNNCu samples were examined by using two clinical bacterial species, *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), separated from clinical patients. The clinical bacteria were isolated according to the National Guide to Clinical Laboratory Procedures and characterized by culturing in the specifically appropriate media followed by the matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) VITRK mass spectrometry microbial identifi-

cation system (bioMérieux, France). Bacteria were grown in nutrient bouillon at 37°C at 150 r min⁻¹ for 10–12 h. The nutrient bouillon and nutrient agar medium were supplied by Oxoid Ltd. *E. coli* and *S. aureus* were suspended in phosphate-buffered saline (PBS) to obtain a concentration of 10⁶ colony-forming unit (CFU)/mL for the antibacterial assay, and an extra 1% of the nutrient broth of bacterial suspension was added. The samples ($n=4$) and bacterial suspension were co-incubated in a biochemical incubator at 37°C for 12 h. Then, after co-culture, the bacterial suspension was diluted to a proper concentration, and 50 μ L of the diluted bacterial suspension was uniformly spread on the nutrient agar medium plates. The number of CFUs was counted after incubation at 37°C for 16 h. To quantify the antibacterial ability, the antibacterial ratio was calculated based on the following Equation (1).

$$\begin{aligned} &\text{Antibacterial ratio (\%)} \\ &= (N_{\text{Blank}} - N_{\text{samples}}) / N_{\text{Blank}} \times 100 \%, \end{aligned} \quad (1)$$

where N_{Blank} is the average number of bacteria in the blank group (CFU/sample) and N_{samples} is the average number of bacteria in the evaluated sample (CFU/sample).

Live/dead bacterial viability assay

To visualize the viable bacterial cells on the KNNCu samples, a LIVE/DEAD BacLight bacterial viability kit (L7012) with two staining agents was used. The SYTO[®]9 is a green-fluorescent nucleic acid stain that penetrates all bacteria. In contrast, the propidium iodide (PI), a red-nucleic acid stain, only penetrates bacteria with damaged membranes. The ratio of red to green fluorescence emitted by bacteria stained with an appropriate mixture of these stains reflects the cellular permeability and viability. Thus, the dead, dying and living bacteria can be distinguished under the confocal laser scanning microscope (CLSM). After treatment with KNNCu ($n=3$), both *E. coli* and *S. aureus* were stained with the dye mixture (combine equal volumes of SYTO[®]9 and PI in a microfuge tube) for 15 min at room temperature in the dark. The stained bacterial cells were observed using a CLSM (Zeiss LSM 780, Germany).

Bacterial morphology evaluation

After treatment of the samples ($n=3$) and bacteria, bacteria were fixed with 3% glutaraldehyde in 0.1 mol L⁻¹ PBS (pH 7.4) at 4°C for 4 h at room temperature. After being washed with PBS three times, bacteria were

dehydrated with a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and 100%, v/v ethanol, for 15 min each treatment). The ethanol was replaced with isoamyl acetate for 20 min twice. Finally, after drying with a CO₂ critical point dryer (Autosamdri-815, Series A, Tousimis Research Corp, Rockville, MD, USA), the treated bacteria were observed by SEM (Zeiss EVO18, Carl Zeiss Company, Germany).

Cellular ROS detection

Intracellular reactive oxygen species (ROS) production was detected with an assay kit using the fluorescent probe dichlorofluorescein diacetate (DCFH-DA). Bacteria treated with samples ($n=4$) were stained with 10 μ mol L⁻¹ DCFH-DA for 30 min in the dark at room temperature. The intracellular ROS levels were recorded in the 500–700 nm emission range with an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

Cytotoxicity assay

Bone marrow mesenchymal stem cells (BMSCs) (CRL-12424, ATCC, USA) were used to evaluate the cytotoxicity of KNNCu with a CCK-8 assay. A cell resuspension was seeded on the KNNCu sample surfaces ($n=4$) in 48-well plates at a density of 1×10^4 cells mL⁻¹ (500 μ L per well) for 1, 3 and 5 days. CCK-8 solution (Dojindo, Japan) was added to each well, and the samples were incubated at 37°C for 2 h. The reacted solution was transferred to a new 96-well plate. The optical density (OD) was measured at a wavelength of 450 nm using a spectral scanning multimode reader (Thermo Scientific Varioskan Flash, USA).

Cytocompatibility assessment

The cytocompatibility of the KNNCu samples was assessed with the use of a live/dead staining assay. BMSCs were seeded on the KNNCu samples ($n=3$) and then cultured. After rinsing with PBS three times, BMSCs were stained with the prepared dyeing fluid (Calcein AM and PI mixed at 2:5) for 15 min at 37°C. Afterwards, the stained BMSCs were observed with an inverted fluorescence microscope (Nikon Eclipse Ti-U, Japan).

Cell spreading behavior observation

Cell spreading behavior was examined for the mouse BMSCs on the KNNCu samples. BMSCs and the samples ($n=3$) cultured for 48 h were used for staining. These materials were washed with PBS three times. Then, cells were fixed with 4% paraformaldehyde for 10 min, and

washed with PBS containing 0.1% Triton X-100 for 5 min. Afterwards, cells were stained with an Actin-Tracker Green (Beyotime, Beijing, China) and DAPI (Beyotime Biotechnology, China). The cell cytoskeleton was observed by the CLSM. The average spreading area of cells was determined by analyzing their Actin-Tracker Green fluorescence images using the open-source software ImageJ.

Copper ions of bacteria absorption test

The KNNCu samples ($n=4$) were cocultured for 2, 4, 8 and 12 h with a bacterial suspension (10^6 CFU/mL), respectively. Then, the bacterial suspension was collected by centrifuging at $8000 \times g$ for 10 min. The supernatant was discarded, and 50 μ L of lysozyme was used to dissolve the bacteria at 37°C for 10 min. Then, 5 mL of ultrapure water was added to dilute the dissolved solution. The filtered solution was used for testing by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo, ICAP RQ) to evaluate the concentration of Cu^{2+} ions.

Data analysis

All data are presented as the means $\pm$ standard deviation (S.D.). A two-tailed Student's t -test was used for two-group comparisons and the statistical significance testing. $p < 0.05$; $**p < 0.01$; $***p < 0.001$.

RESULTS AND DISCUSSION

Preparation and characterization of KNNCu

The material model of ferroelectric Cu-doped implant, KNNCu, was constructed *via* a solid-state sintering process [21]. The SEM image of KNNCu displays a uniform microstructure (Fig. 1a). EDS mapping confirms that Cu element uniformly distributes on the KNNCu surface (Fig. 1b). To analyze the valence states of the doping copper element in KNNCu, XPS analysis was carried out (Fig. S1a in Supplementary information). The Cu 2p spectrum shows satellite peaks at 931.9 and 952.0 eV (Fig. S1b1), indicating that copper element is doped into the KNN. There is a slight shift towards high binding energy of Nb atoms at 205.97 and 208.58 eV in KNNCu (Fig. S1b2). This can be attributed to B-site replacement of Nb^{5+} ions by Cu^{2+} ions, which is in accordance with the similar ionic radii between Cu^{2+} (0.73 Å) and Nb^{5+} (0.64 Å) [22,23]. XRD analysis was performed to examine the crystal phase of KNNCu, which indicates that KNNCu possesses two peaks near 45° with a perovskite structure (ABO_3) (Fig. 1c). The relative intensity of the (002)/(200) peaks of the KNNCu is 1.5, which indicates the coexistence of orthorhombic and tetragonal phases in the KNNCu sample, whose crystalline phase is a typical piezoelectric phase composition [24]. To examine the

Figure 1 Characterization of KNNCu. (a) SEM image; (b) Cu elemental mapping of KNNCu surface; (c) XRD pattern; (d) P - E hysteresis loop. The results confirm the construction of KNNCu with ferroelectric properties.

ferroelectric property of KNNCu, the P - E hysteresis loop of KNNCu was detected. The results show that KNNCu possesses a representative P - E hysteresis loop. The remnant polarization (P_r) of KNNCu is $6.50 \mu\text{C cm}^{-2}$, which confirms that KNNCu possesses good ferroelectric property (Fig. 1d) [25–28].

To modulate the implant surface potential, and thus the EEf, KNNCu was polarized using corona polarization. The SEM images indicate that KNNCu morphologies remain unchanged before and after polarization (Fig. S2). The SKPM results confirm that the KNNCu samples polarized under different external electric fields exhibit different surface potentials (Fig. 2). The SKPM results of KNNCu indicate that the surface potentials of M-KNNCu (Fig. 2c) and H-KNNCu (Fig. 2d) increase by approximately 54 and 110 mV compared with L-KNNCu (Fig. 2b), respectively. Meanwhile, as the bacterial cell membrane potential can be regarded as constant, the intensity of the EEf is proportional to the surface potential of KNNCu.

Antibacterial property under EEf

After the EEf was constructed between the implant and the negatively-charged bacterial cell membrane, the an-

tibacterial efficacy of KNNCu with the EEf-mediated Cu^{2+} ion transport was examined. The KNNCu samples and control were cocultured with *E. coli* and *S. aureus* for 12 h, respectively [29]. The antibacterial ratio was first quantitatively examined by using a plate counting method (Fig. 3a). Compared with the other three groups (KNN, L-KNNCu, and M-KNNCu), the H-KNNCu group (the highest EEf intensity group) shows a remarkable antibacterial activity (100% antibacterial ratio with both *E. coli* and *S. aureus*). The M-KNNCu and L-KNNCu with relatively low polarization electric potentials and lower EEf intensities suffer decreased antibacterial ratios (Fig. 3b). These results confirm the proposed mechanism that enhanced Cu^{2+} ion transport to target bacteria under EEf will promote the bacterial killing efficacy. Furthermore, the bacteria were stained with live/dead cell-staining kits, wherein green fluorescent SYTO*9 enters live cells, and red fluorescent PI only enters cells with damaged cell membranes [30,31]. Schematic diagrams of the relative EEf intensity between the membrane and polarized KNNCu are shown in Fig. 4. The fluorescent images (Fig. 4) show that the H-KNNCu group has the largest number of dead *E. coli* and *S. aureus* cells, followed by the M-KNNCu group, while most

Figure 2 SKPM characterization of the KNNCu samples. (a) KNN (142.85 ± 23.89 mV); (b) L-KNNCu (149.51 ± 11.28 mV); (c) M-KNNCu (202.95 ± 7.05 mV); (d) H-KNNCu (261.81 ± 7.20 mV). These results indicate that the surface potential of KNNCu, and thus the EEf, can be modulated via external electric field polarization.

Figure 3 Evaluation of the antibacterial performances of the KNNCu samples under EEf. (a) Bacterial culture plate colony units incubated with KNN, L-KNNCu, M-KNNCu, and H-KNNCu. (b) Antibacterial ratios of *E. coli* and *S. aureus* after incubation with KNN, L-KNNCu, M-KNNCu, and H-KNNCu. These results indicate that the H-KNNCu group with the highest EEf intensity achieves 100% antibacterial ratio. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Figure 4 Schematic diagrams of the relative EEF intensity between bacteria membrane and polarized KNNCu and confocal fluorescence microscopy images of the live/dead staining assay of *E. coli* and *S. aureus* incubated with KNN, L-KNNCu, M-KNNCu, and H-KNNCu.

bacterial cells are still live for the other two groups, KNN and L-KNNCu. Thus, H-KNNCu shows strong antibacterial abilities against *E. coli* and *S. aureus*. And the number of dead bacteria increases with the increase of EEF intensity. These results are consistent with the results of the CFU analysis.

Antibacterial mechanism under EEF

To verify the antibacterial mechanism of EEF-mediated antibacterial ion transport, the KNNCu samples were firstly cocultured with *E. coli* and *S. aureus* for 2, 4, 8 and 12 h, and then the Cu^{2+} ions transport to target bacteria were extracted and measured via ICP-MS, respectively. Intriguingly, the amount of Cu^{2+} ions transport to target bacteria increases with increasing time and EEF intensity (Fig. 5a). The amount of Cu^{2+} ions from the H-KNNCu group transport to target *E. coli* bacteria under EEF increases by only about 35% compared with that of L-KNNCu group at the first 2 h, respectively. With co-culture time increasing to 12 h, the amount of Cu^{2+} ions to target bacteria from H-KNNCu group increases by 140% compared with that of L-KNNCu group. In short, the amount of Cu^{2+} ions targeting bacteria increases with

the increase of EEF intensity and the increase of time, respectively. Meanwhile, the cumulative release profiles of Cu^{2+} ions from the L-KNNCu and H-KNNCu groups in a PBS solution were conducted via ICP-MS, which are shown in Fig. S3. Firstly, the release profiles indicate that Cu^{2+} ions continuously release from the L-KNNCu and H-KNNCu surfaces, with a similar release rate. To further explicate the role of EEF in bacterial killing, we compared the amounts of Cu^{2+} ions that release from L-KNNCu and H-KNNCu and the amounts of Cu^{2+} ions that transport to target bacteria. As shown in Table S1, the results indicate that after 12 h culture, the amount of Cu^{2+} ions released from H-KNNCu increased by about 14.90% compared with that from L-KNNCu. Nevertheless, after 12 h culture, we found that the amount of Cu^{2+} ions transport to target bacteria from H-KNNCu was up to 140% more than that from L-KNNCu. The above results indicate that the increase of EEF intensity has a slight effect on the release of Cu^{2+} ions, but a more significant effect on the increase of the amount of ions target to bacteria, which demonstrates the significant role of EEF in promoting Cu^{2+} ions to transport to target bacteria. Actually, in a bacterial environment, antibacterial ion will

Figure 5 EEF-mediated Cu^{2+} ion transport and its effect on *E. coli* and *S. aureus*. (a) Amounts of Cu^{2+} ions transport to bacteria after coculture with L-KNNCu, M-KNNCu, and H-KNNCu for 2, 4, 8 and 12 h, respectively. (b) Quantification of ROS levels in *E. coli* and *S. aureus* after cocultured with KNN, L-KNNCu, M-KNNCu, and H-KNNCu for 12 h with the ROS assay kit. F/F_0 represents the ratio of the fluorescence intensities of the experimental group to that of the blank control. (c) SEM images of *E. coli* and *S. aureus* treated with KNN, L-KNNCu, M-KNNCu, and H-KNNCu for 12 h, respectively. *E. coli* and *S. aureus* are pseudo-colored. The arrows indicate bacteria with damaged cell membranes. These results confirm that EEF can mediate directional and quantitative transport of antibacterial ions to target bacteria. A two-tailed Student's *t*-test was used for the statistical significance testing. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

exhibit diffusion, convection and electric migration. As the temperature and ion concentration gradient are relatively stable in the surrounding bacterial environment, the diffusion- and convection-driven transport of antibacterial ions can be ignored. Thus the enhanced Cu^{2+} ion transport from KNNCu to bacteria could be attributed to the electric migration under the EEF to transport Cu^{2+} ions along the tangential direction of the electric field line to reach the bacterial surfaces. The migration rate of antibacterial ions under an EEF is proportional to the potential gradient and ionic mobility of the electric field. The relationship among migration rate, potential gradient and ionic mobility can be expressed by Equation (2) [32]

$$v = -zuF \nabla \Phi, \quad (2)$$

where v is the migration rate, z is the number of electronic charges of the ion, u is a proportionality factor that relates how fast the ion moves in response to an electric field, which is called ionic mobility; F is the Faraday's constant; and Φ is the potential gradient of EEF in the

solution, which can form electric field. Thus, under the guidance of EEF, directional and quantitative transport of antibacterial ions to target bacteria is achieved.

According to the above proof, different EEF intensities can mediate the amount of antibacterial ions transport to bacteria, which might enhance the bacteria-killing efficacy. As antibacterial ions mainly kill bacteria *via* intracellular ROS enrichment, we then detected the effect of EEF-mediated ion transport on ROS generation. Intracellular ROS generation was measured using DCFH-DA as a fluorescent staining probe after bacteria were incubated for 12 h with the KNNCu samples and control group [33]. The ROS production results (Fig. 5b) indicate that there is more ROS production in bacteria cocultured with H-KNNCu because more Cu^{2+} ions are targeted to the bacterial cell membrane. This result may be due to the fact that the amount of Cu^{2+} ions transport to bacteria under the highest EEF intensity is relatively large, which leads to the abnormal ion exchange between the intracellular and extracellular surrounding, and thus causes

abnormal respiration process to produce more ROS [34–36]. To further investigate the antibacterial behavior at different EEF intensities, the morphologies of *E. coli* and *S. aureus* adhering to the KNNCu samples and control were observed by SEM (Fig. 5c). As shown in the SEM images, bacteria on KNN show a clear edge and appear smooth. For bacteria on L-KNNCu, M-KNNCu and H-KNNCu, some cell membranes are damaged, which shows a rough fold. In particular, seriously collapsed and split membranes are observed in the group treated with H-KNNCu. The bacteria gradually become irregular in shape and wizen due to extensive collapse of the cell membranes, followed by massive leakage of cell substances, such as proteins and DNA, similar to the previous reports [37–39]. These results suggest that the EEF transport of Cu^{2+} ions to target bacterial cell membrane would disrupt cell membranes, and induce intracellular

ROS generation to damage the bacterial cell membranes, resulting in the leakage of intracellular contents and eventually the death of bacteria.

Biocompatibility of KNNCu

To examine the potential toxicity of the KNNCu samples, CCK-8 test was first performed with mouse BMSCs (Fig. 6a). Cell activity can be assessed by the optical density (OD) values. The higher the OD value, the higher the cell activity. The OD values for the KNNCu samples are slightly lower than that for pure KNN after 1 day of incubation. Encouragingly, after the 3rd and 5th days of incubation, no statistic difference can be observed between the viabilities of BMSCs on KNN and the KNNCu samples. Live/dead cell staining was also conducted to examine the influence of the KNNCu samples on the BMSC viability (Fig. 6b). Hardly any dead cells on the

Figure 6 Biocompatibility assessment of the KNNCu samples. (a) Cytotoxicity of BMSCs incubated on KNN, L-KNNCu, M-KNNCu, and H-KNNCu for 1, 3, and 5 days assessed using a CCK-8 assay, respectively. (b) Live/dead cell stain of BMSCs incubated on KNN, L-KNNCu, M-KNNCu, and H-KNNCu for 48 h, respectively. The green fluorescence represents live cells and the red fluorescence represents dead cells. (c) Cytoskeletal stain of BMSCs incubated on KNN, L-KNNCu, M-KNNCu, and H-KNNCu for 48 h, respectively. (d) Average spreading areas of cytoskeleton in (c). * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

KNNCu samples demonstrate excellent biocompatibility of these materials [40–42]. The good biocompatibility of KNNCu groups on BMSCs can be attributed to the relatively low-dose copper element incorporated into the KNNCu samples and appropriate surface electric properties after polarization. The relatively low-dose copper element incorporation ensures a relative low ion release level to maintain the bioactivity of KNN, which is in accordance with previous research that the low-dose Cu^{2+} ions are biocompatible to cells [43,44]. And various previous studies also demonstrate the biocompatibility of polarized piezoelectric biomaterials, such as biocompatible ferroelectric KNN nanofibers [45], ferroelectric LiNbO_3 [46,47] and barium titanate biomaterial [48,49]. Moreover, the cytoskeleton staining images show that the BMSCs are spreading out on the surfaces of KNN and the KNNCu samples (Fig. 6c). The statistical data (Fig. 6d) indicate there is no significant difference in the spreading areas among KNN and the KNNCu samples, which indicates that the KNNCu samples are favorable for cell spreading. Therefore, the polarized ferroelectric implant with the antibacterial ion transport system to target bacteria can not only enhance the antibacterial property, but also have good biocompatibility.

CONCLUSIONS

In summary, this work provides a ferroelectric copper-doped implant model to construct EEF with bacterial cell membrane with the polarization parameters conveniently as a bridge for studying the effect of EEF on transport ratio of antibacterial ions to target bacteria. Interestingly, in an infectious bacterial environment, directional and quantitative transport of antibacterial ions to bacteria can be achieved *via* modulation of EEF. The high EEF-mediated ion transport can achieve a 100% antibacterial ratio. This research demonstrates that EEF as a bridge heavily impacts the antibacterial performance, and concludes the significance of manipulating the implant surface charging property to control EEF-mediated antibacterial ions to target bacteria when designing antibacterial biomaterials.

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Supplementary information Supporting data are available in the online version of the paper.

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内源性电场调控抗菌离子传输以提高植入材料抗菌性能

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摘要 组织植入材料相关感染是临床治疗的一大顽疾, 基于抗菌离子释放系统的抗菌植入材料是经济高效的抗生素治疗替代手段. 但过量释放的抗菌离子在杀灭细菌的同时也可能引起生物毒性. 因此, 如何提高释放离子的抗菌效率是急需解决的根本问题. 本研究设计了一种负载低剂量抗菌离子的铁电植入材料, 该植入材料表面电势可在不改变自身成份的前提下通过外源电场极化进行调控. 研究发现上述铁电植入材料释放的抗菌离子将在植入材料自身与带电细菌之间形成的内源性电场作用下定向运输到达细菌. 研究结果表明在12 h内, 高表面电势植入材料向细菌运输的离子量达到低表面电势植入材料输运量的2.4倍, 从而使抗菌效率从65%提高到100%, 并展现出较低的细胞毒性. 本研究表明植入材料与生命体间的内源性电场在介导物质传输中发挥重要作用, 为设计高性能抗菌生物材料提供了一个新的视角.