Supporting Information

Surface-Selective Preferential Production of Reactive Oxygen Species on Piezoelectric Ceramics for Bacterial Killing

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Author Contributions

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Experimental Section

Preparation of KNN ($(K_{0.5}Na_{0.5})\ NbO_3$) piezoelectric ceramic and its polarization: KNN was fabricated using a conventional solid-state reaction method. The raw materials were Nb_2O_5 (AR, 99.9%, Shanghai Aladdin), Na_2CO_3 (AR, ≥99.8%, Shanghai Aladdin) and K_2CO_3 (AR, 99%, Shanghai Aladdin). The powders were uniformly mixed in in a molar ratio of 1:1:2 with alcohol for 8 h, then dried and calcined at 750°C for 2 h. The calcined KNN ceramic powder was mixed again to obtain ceramic pellets (10 mm in diameter and 1.0 mm thick) by tableting, isostatic pressing and a sintering process. KNN with piezoelectric constants of 70 pC/N was obtained by polarization under an electric field of 25 kV/cm for 30min.

Characterization of KNN: Field emission scanning electron microscopy (FESEM, Nova Nano SEM 430, Germany) was employed to characterize the morphology of KNN and HA. Energy dispersive spectroscopy (EDS) was adopted for elemental analysis. Grazing incidence X-ray diffraction (XRD, D8 Advance Bruker Company, Germany) was employed to investigate the phase composition and was recorded by Cu Kα radiation (λ = 0.15418 nm, 40 kV and 40 mA) at a scan rate of 0.2°/s. Scanning Kelvin probe microscopy (SKPM, SPM-9600, Shimadzu) was performed with a scan rate of 0.8 Hz in

an area of 500 × 500 nm^2 to analyze the surface potential of KNN, KNN +70 and KNN -70.

Investigation of antibacterial effects: The antibacterial properties of KNN with different piezoelectric constants (KNN, KNN +70, KNN -70) and blank control were evaluated using a plate colony-counting method. *Staphylococcus aureus* (*S. aureus*, ATCC 25923) was obtained from the Microbial Culture Collection Center of Guangdong Institute of Microbiology. Other biological agents such as tryptone and yeast extract were supplied by Oxoid Ltd. *S. aureus* was suspended in phosphate-buffered saline (PBS) to obtain a concentration of 10^6 CFU/mL for the antibacterial assay, and the samples and bacterial suspensions were co-incubated in a biochemical incubator at 37°C for 24 h. Then, each co-cultured solution was diluted 10^4-fold by standard serial dilution to a proper concentration, and 100 μL of suspension was uniformly spread on LB agar plates. The number of viable bacterial colonies was counted after incubation at 37°C for 24 h. To quantify the antibacterial ability, the bactericidal rate was calculated based on the following equation S1:[1, 2]

$$\text{Antibacterial rate (\%)} = (N_{Control}-N_{solution})/ N_{Control}\times 100 \quad (1)$$

where $N_{control}$ is the average number of bacteria in the control sample (CFU/sample) and $N_{solution}$ is the average number of bacteria in the evaluated sample (CFU/sample).

Measurement of reactive oxygen species (ROS): The intracellular ROS level in the bacterial cells was measured using the KeyGEN ROS Detection Kit (Green Fluorescence, KeyGEN BioTECH, China). Bacteria and samples (KNN, KNN +70, KNN -70, blank control) were co-cultured in an incubator at 37°C for 24 h. Then, DCFH-DA (final concentration of 10 μM) was added to the mixed solution and incubated at 37°C for 20 min. To quantify the ROS level, the DCFH fluorescence intensity was detected using a multimode reader at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. F and F_0 indicate the fluorescence intensity of the experimental group and the positive control (Rosup, supplied in the commercial kit), respectively. F/F_0 is the relative ROS intensity of the experimental group.

Cell culture and proliferation: Mesenchymal stem cells (MSCs, CRL-12424, ATCC, USA) were cultured in HDMEM (4.5 g/L glucose, Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA). The medium was replaced every two days. Samples with a thickness of 2 mm and a diameter of 10 mm were settled at the bottom of 48-well polystyrene culture plates with 10^4 cells per well. All cultures were incubated at 37°C in a humidified incubator with 5% CO_2. The cytotoxicity induced by KNN was determined by the CCK-8 assay, which is based on mitochondrial dehydrogenase activity. Briefly, cells were cultured with KNN (non-polarized KNN, KNN+70, and KNN-70) or HA at

37°C for 1, 4 and 7 days. Then, the samples were washed with PBS and incubated with the CCK-8 (Dojindo, Kumamoto, Japan) solution for 1.5 h. Finally, the solution was transferred to 96-well plates to determine the absorbance at 450 nm using a multimode reader (Varioskan Flash, Thermo Scientific).

Statistical analysis: Data are expressed as means ± standard deviations. Statistical analysis was performed using the Graph Pad Prism statistical software Origin. The statistical significance of the difference was measured using one-way analysis of variance. $P < 0.05$ was considered statistically significant.

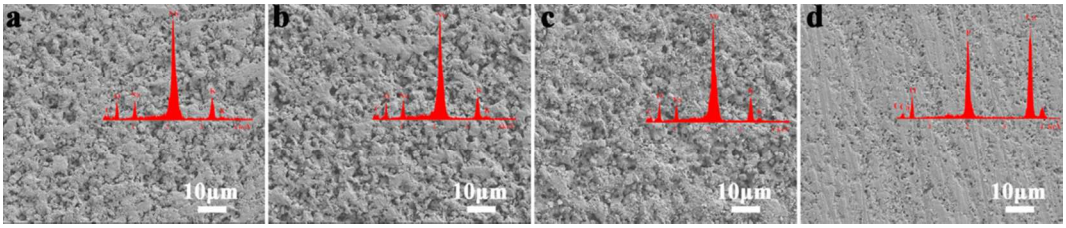

Figure S1. Evaluation of the morphology and composition of KNN and HA. SEM and EDS of KNN samples ((a) non-polarized KNN, (b) KNN+70 (positively polarized KNN) and (c) KNN-70 (negatively polarized KNN)) and (d) HA indicated that KNN with different piezoelectric constants had the same morphology and chemical composition as non-polarized KNN.

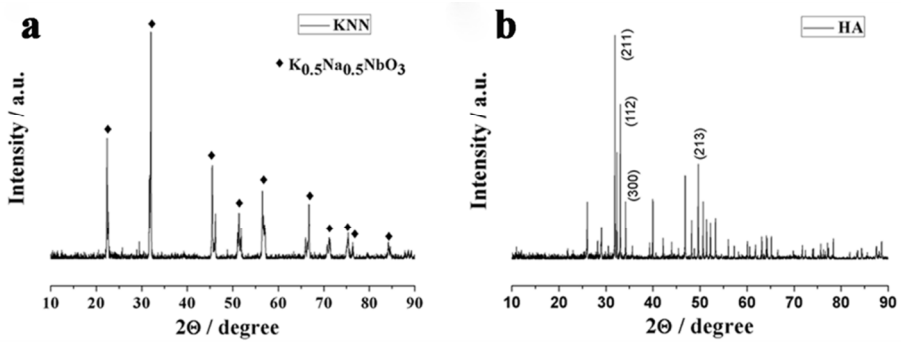

Figure S2. Evaluation of the phase composition of KNN and HA. XRD of non-polarized (a) KNN and (b) HA, confirming the formation of KNN and HA.

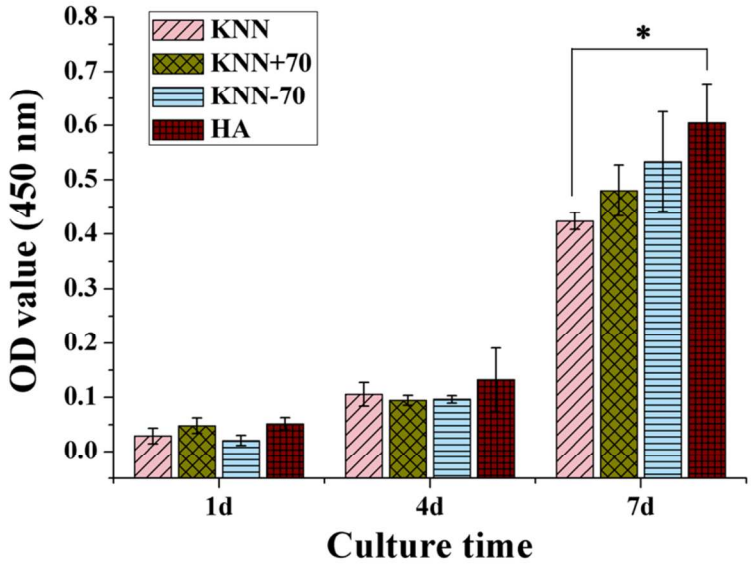

Figure S3. Analysis of the cell proliferation (CCK-8 assay) on the surface of different substrates (KNN, KNN+70, KNN-70 and HA) after incubation for 1, 4 and 7 days (n = 4). There was no significant difference in cell proliferation between the polarized KNN and

HA, suggesting that the polarized KNN has good biocompatibility. *, $p < 0.05$ compared with pure HA.

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