Electronic Supplementary Information

Built-in Electric Field with Nanoscale Distinction for Cell Behavior Regulation

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Experimental Section

Material Contributions: Unless otherwise noted, all the chemicals were purchased as analytical reagents from Aladdin (China). Niobium pentaoxide (Nb_2O_5), sodiums carbonate anhydrous (Na_2CO_3), potassium carbonate (K_2CO_3) and absolute ethanol were purchased for KNN piezoceramic synthesis. Sodium phosphate dibasic dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$) and potassium phosphate monobasic (KH_2PO_4) were purchased to produced phosphate-buffered solution (PBS). β-Naphthalene sulfonic acid (β-NSA) and pyrrole (Py) monomers were purchased for the PPy polymerization. All chemicals were used without any purification.

Synthesis of the CPNAs: The KNN piezoceramics were prepared with a conventional solid-state reaction method. Nb_2O_5, Na_2CO_3 and K_2CO_3 powders were obtained as raw materials. The chemicals above were mixed in a molar ratio of 2:1:1 with absolute ethanol and ball-milled for 8 h. The mixture was then dried and calcined at 750 °C for 2 h. Afterwards, the calcined KNN powders were obtained and subjected to tableting, isostatic pressing and a sintering process. Finally, the KNN piezoceramics (10 mm in diameter and 1 mm thick) were utilized after rinsing with deionized water and drying in a vacuum. The nanostructured PPy coatings on the KNN piezoceramics were fabricated via a temple-free electrochemical technique. The polymerization process occurred in a

three-electrode system with a KNN piezoceramic as the working electrode, a copper sheet as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode. The electrolyte was composed of PBS, β-NSA as a dopant and Py monomers. The PPy coatings were deposited on the KNN piezoceramics galvanostatically at 0.9 mA/cm^2 for 7 min. The products, after polymerization, were rinsed in deionized water several times and dried in a vacuum. In this work, the piezoelectric constant of the CPNAs after polarization was 70 pC/N. The poled CPNAs were obtained by a polarization process under an electric field of 25 kV/cm for 30 min.

Characterization: Field emission scanning electron microscopy (FE-SEM, Nova Nano SEM 430, Germany) was applied to observe the morphology of the CPNAs on the KNN piezoceramics. Scanning Kelvin probe microscopy (SKPM, Asylum Research MFP-3D-S, USA) was performed to observe the morphology and analyze the surface potential of the samples. The X-ray diffraction (XRD, Bruker D8 advance, Germany) was used for the analysis of KNN crystal structure. A Raman spectrometer (HJY 1720, France) was utilized to analyze the chemical composition of the CPNAs.

Cytocompatibility Assessment: A live/dead stain assay for cell viability was performed to characterize the cytocompatibility of the CPNAs. MC3T3-E1 osteoblasts (1.0×10^4 per well) were cultured in a 48-well plate on the surface of the CPNAs for 24 h. After washing with PBS, the MC3T3-E1 osteoblasts were stained with a live/dead cell viability kit containing Calcein AM (Dojindo, Japan) and propidium iodide (PI, Dojindo, Japan) for 30 min at 37 °C. An inverted fluorescence microscope (Nikon Eclipse Ti-U, Japan) was used to observe the stained live and dead cells.

Cell Viability Characterization: A CCK-8 assay was employed to evaluate the viability of the MC3T3-E1 osteoblasts. The MC3T3-E1 osteoblasts were seeded on the CPNAs in 48-well polystyrene culture plates at a density of 2×10^4 cells/mL (500 μL per well) for 1, 4 and 7 days of co-culture. The specimens were washed by PBS, and the CCK-8 solution (Dojindo, Japan) was added to each well. After a 2-h incubation at 37 °C in a 5% CO_2 atmosphere, the mixed solution was transferred to a new 96-well polystyrene culture plate. The optical density of the mixed solution

in the 96-well polystyrene culture plate was measured at a wavelength of 490 nm using a spectral scanning multimode reader (Thermo Scientific Varioskan Flash, USA).

Protein Absorption Experiment: The CPNAs were immersed in PBS (0.5 M, pH 7.4) for 0.5 h prior to the protein absorption experiment. Bovine serum albumin (BSA) was selected as the model protein. The BSA solution (0.1 mg/mL) was freshly prepared by dissolving the protein in PBS (0.5 M, pH 7.4). The CPNAs were exposed to 500 μL of the BSA solution and were incubated at 37 °C for 0.5 h. Then, the specimens were washed with PBS. The protein solution was removed by adding 0.2 mL of 1 wt% sodium dodecylsulfate (SDS) to the specimens to elute the adsorbed protein. The amount of the adsorbed protein was determined by the bicinchoninic acid (BCA) assay. The optical density of the eluent was measured at a wavelength of 562 nm using a spectral scanning multimode reader (Thermo Scientific Varioskan Flash, USA).

Cell Spreading Behavior Observation: After the incubation on the CPNAs, the cell spreading behavior was observed via immunofluorescence staining of the cytoskeleton. After a 24-h incubation on the surface of the CPNAs, the BMSCs were fixed using 4% paraformaldehyde for 10 min. Then, the fixed cells were washed with PBS three times (5 min for each) to remove the residual 4% paraformaldehyde. PBS containing 0.1% Triton X-100 was then added for cell permeation. After washing an additional three times, the F-actin in the cells was stained using Actin-Tracker Green (Beyotime, China) in PBS with 1% BSA and 0.1% Triton X-100 for 30 min at room temperature. After F-actin staining, the cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma, USA). After nuclei staining, the samples were washed in PBS three times and were then stored at 4 °C before observation. The images were acquired under a confocal laser scanning microscope (CLSM, Zeiss LSM 780, Germany).

Osteogenic Differentiation Evaluation: To characterize the effect of the CPNAs' surface properties on osteogenic differentiation, MC3T3-E1 osteoblasts were selected as the model for ALP activity evaluation. ALP activity was determined quantitatively by measuring the amount of the p-nitrophenol converted from p-nitrophenol phosphate. MC3T3-E1 osteoblasts were seeded on the

CPNAs at a density of 2×10^4 cells/mL (500 μL per well) without osteogenesis induction media. After 7 days of incubation, the cell lysate was obtained using 0.2% Triton X-100 at 4 °C. A total of 50 μL of cell lysate was mixed with 50 μL of p-nitrophenyl phosphate for 30 min at 37 °C. After the termination of the reaction, the absorbance of the mixture was read in a spectral scanning multimode reader (Thermo Scientific Varioskan Flash, USA) at 405 nm.

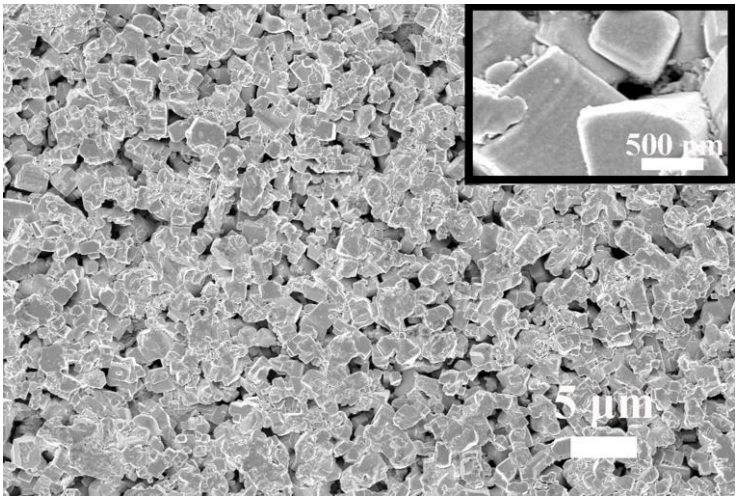

Figure S1 SEM observation of a KNN piezoceramic.

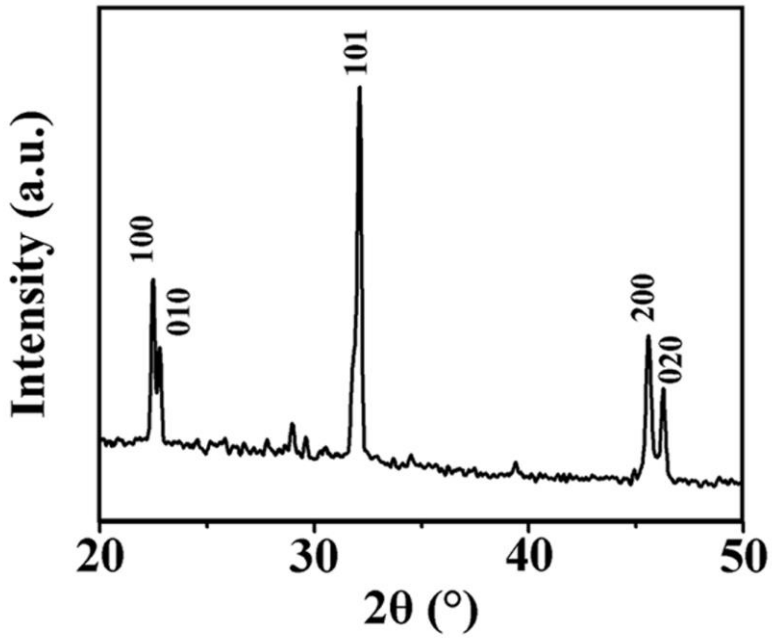

Figure S2 XRD pattern of a KNN piezoceramic. The result showed the ABO_3-type perovskite phase of the KNN piezoceramic. The perovskite phase was one of the crstal structure exibiting piezoelectricity.

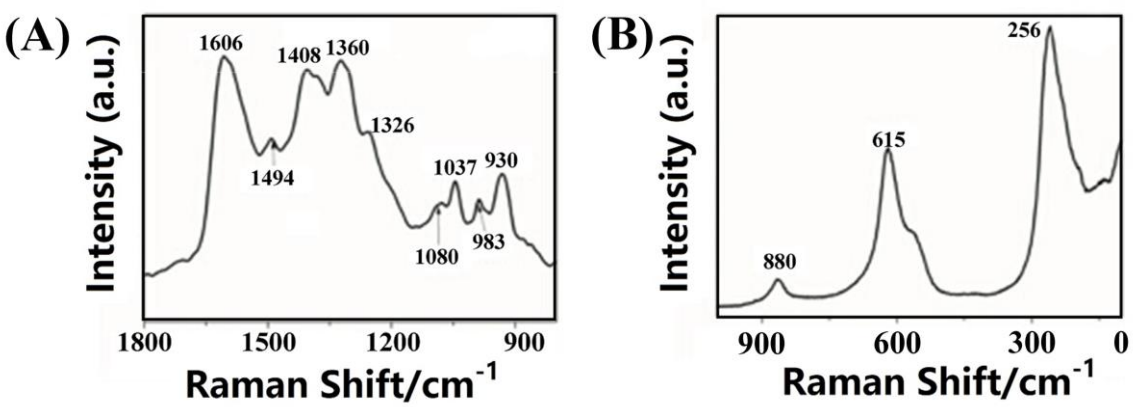

Figure S3 Raman spectra were utilized to characterize the chemical structure of the CPNAs. (A) In the range of wavenumbers between 900 cm^{-1} to 1800 cm^{-1}, peaks assigned to the PPy coating were obtained. The strongest peak, at 1606 cm^{-1}, was assigned to the C = C stretching vibration. The peaks at 1408 cm^{-1} and 1326 cm^{-1} were assigned to the Py ring stretching vibration, while the peaks at 983 cm^{-1} and 930 cm^{-1} were attributed to the Py ring deformation vibration. The peak at 1360 cm^{-1} was attributed to the C – N stretching vibration. (B) In the range of wavenumbers between 0 cm^{-1} to 900 cm^{-1}, peaks assigned to the KNN substrate were obtained. The peaks at 256 cm^{-1} and 615 cm^{-1} were assigned to the F_{2g} and A_{1g} vibration of the NbO_6 octahedron, respectively.

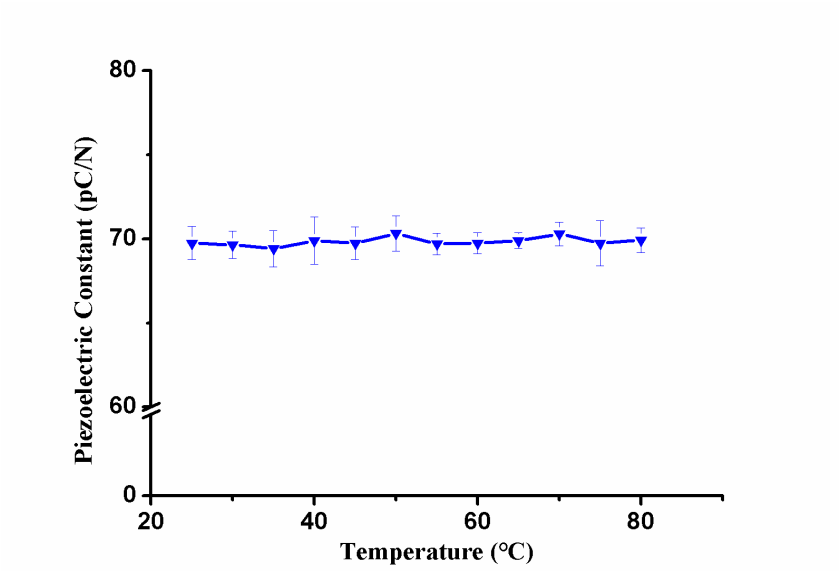

Figure S4 The measurement of piezoelectric constant after the CPNAs were heated from 30℃ to 80℃. The values represent the mean±s.d.. (n=4)

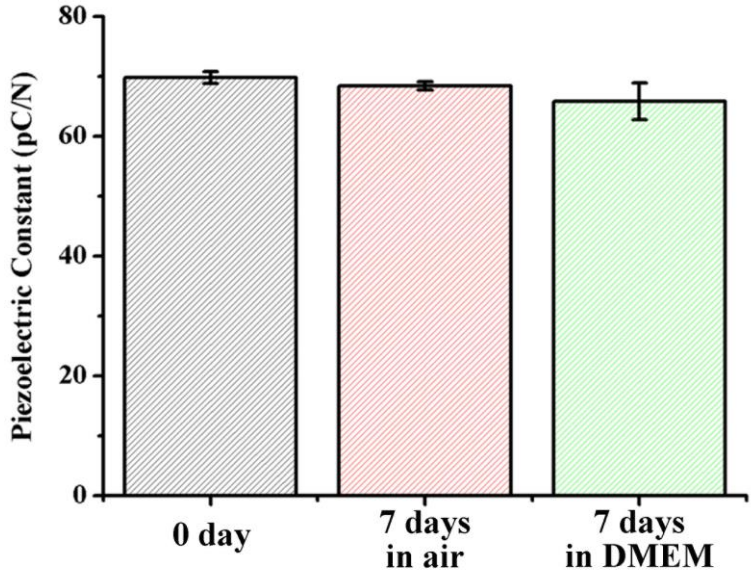

Figure S5 The measurement of piezoelectric constant of CPNAs after standing in air for 7days or immersed in DMEM cell culture for 7days. The values represent the mean±s.d.. (n=4)

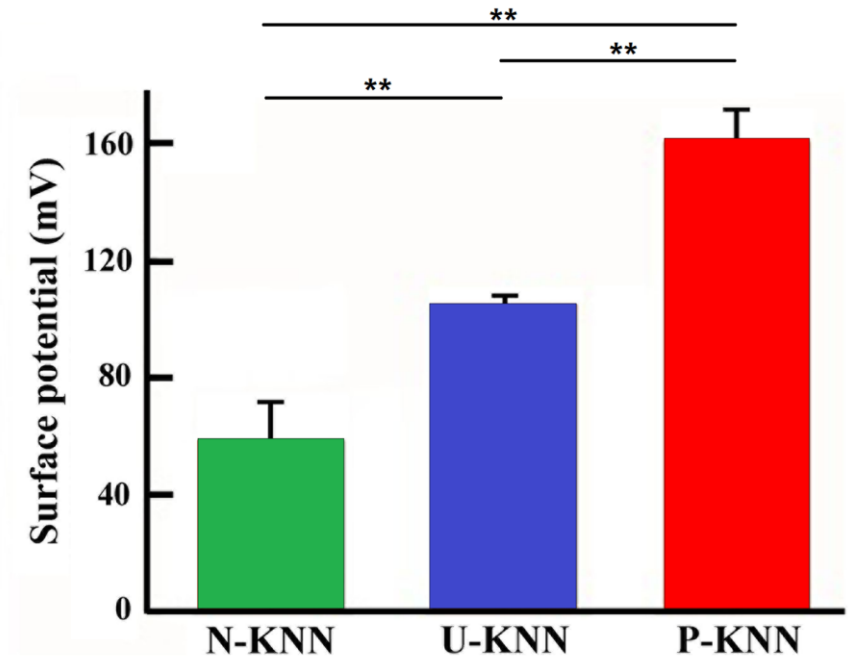

Figure S6 Surface potential of N-KNN (negatively poled surface of the KNN), U-KNN (surface of the unpoled KNN) and P-KNN (positively poled surface of the KNN). **Significant difference of $P<0.01$. (n=4)

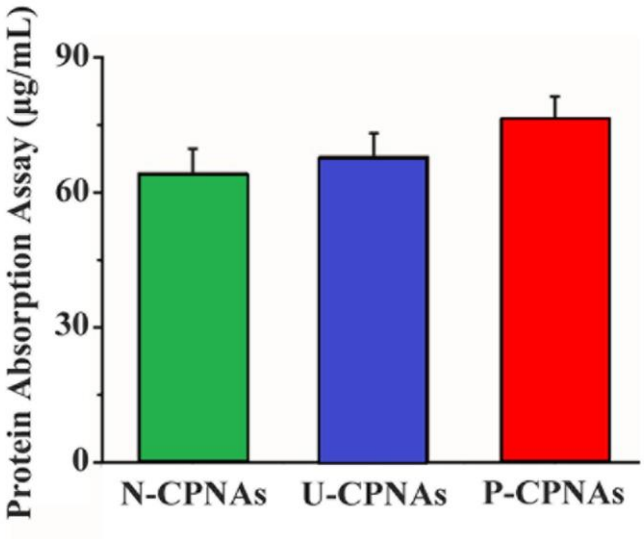

Figure S7 BSA was selected as research subject for the protein absorption assay. The CCK-8 and protein absorption results showed that the P-CPNAs promoted MC3T3-E1 cell proliferation and BSA absorption among the three samples. The values represent the mean±s.d.. (n=4)