Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Experimental

Chemicals

All chemicals of analytical grade were purchased from Aladdin Chem Co. and Sigma–Aldrich Co., and used without further treatment if not specified otherwise. Titanium sheets for biomedical application (0.2 mm in thickness) meeting the standard of ASTM (American Society for Testing & Materials, F67-2002) were obtained from Baoji Qichen New Material Technology Co., Ltd. Biomedical titanium sheets were rinsed and degreased ultrasonically in deionized water, ethanol and acetone, respectively. They were then polished chemically in a mixture of hydrofluoric acid (HF, 0.55 M) and nitric acid (HNO_3, 0.25 M) with a volume ratio of 1:1, followed by multiple rinsing with deionized water.

Fabrication of NAPPy

NAPPy over a large area on the titanium was formed via template-free electrochemical polymerization. Briefly, the small electrochemical cell included a biomedical titanium sheet (effective area of 15 mm × 15 mm) as a working electrode, copper sheet as a counter electrode, saturated calomel electrode (SCE) as a reference electrode, and an electrolyte. A prenucleated film (PNF) was first formed on the titanium. To form PNF, the titanium sheet (*i.e.*, the working electrode) was immersed into the electrolyte (0.2 M KCl solution containing 0.1 M Py). Then the PNF was formed on the working electrode at 0.8 V (*vs* SCE) for 20 s at room temperature under the control of electrochemical station (Zennium Zahner, Germany). Subsequently, the PNF was rinsed in deionized water and dried in a vacuum. To fabricate NAPPy, a phosphate buffer solution (PBS) containing 0.2 M Py and citric acid was used as an electrolyte. NAPPy/citrate was formed on the PNF-coated biomedical titanium (as working electrode) galvanostatically at 0.9 mA/cm^2 for 30 min. The as-obtained products were rinsed in deionized water for several times, and dried in vacuum. Similarly, as a control, 1D NAPPy/NSA was constructed in electrolyte containing NSA instead of citrate, and the formation process of irregular PPy/Cl was the same as PNF with reaction time of 1 min.

In vitro biomineralization in SBF on NAPPy

The NAPPy materials doped with citrate or NSA and biomedical titanium were transferred to 25 mL-conical plastic centrifuge tube containing 25 mL SBF, and then incubated in a constant temperature incubator at 37 ℃ for 3 days. The mineralization solution was refreshed every day. The SBF was

prepared by following a protocol previously reported by Kokubo *et al*.[1] Briefly NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 was mixed in an aqueous solution, followed by adjusting the pH value to 7.4 using TRIS and hydrochloric acid. After soaking, the specimens were washed triply with deionized water and dried under vacuum.

MC3T3-E1 osteoblasts culture and seeding on NAPPy

MC3T3-E1 osteoblasts were cultured in α-modified minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator at 37 ℃ and 5% CO_2. Antibiotics (penicillin/streptomycin, P/S) were added to culture media for all cell growth experiments on the polymers. The NAPPy was sterilized by immersion in 70% ethanol for 5 min, followed by drying under a sterile condition and exposure to UV light for 20 min. Specimens were placed into wells of a 48-well polystyrene cell culture plate. MC3T3-E1 osteoblasts were seeded on the specimens at 2×10^4 cells/mL, with the medium changed every 2 days. For the proliferation studies, the numbers of adherent cells on the substrates at 1, 4 and 7 days were quantified using by Cell Counting Kit-8 (CCK-8) assay.

Morphological observation of MC3T3-E1 osteoblasts by fluorescence staining

To image MC3T3-E1 osteoblasts on specimens after being cultured for 8 h, cells were fixed in ice-cold 4% paraformaldehyde for 30 min and then washed twice in PBS. Nuclei were stained with DAPI (1 μg/mL) for 10 min at room temperature. F-actin of cells was stained with 50 μg/mL Rhodamine-labeled phalloidin in PBS for 20 min at room temperature. Then the cells were washed three times with PBS to remove unbound phalloidin. Images were acquired under a laser scanning confocal microscope (Zeiss LSM 780, Germany).

Alkaline phosphatase (ALP) activity assay

MC3T3-E1 osteoblasts were cultured in osteogenic media (α-MEM containing 10% FBS, 1% P/S, 10 mM β-glycerophosphate, 0.3 mM L-ascorbic acid and 0.1 μM dexamethasone) for 4, 7 and 14 days. An ALP assay kit (Abcam) was used to characterize the ALP activity following the manufacturer's protocol. Briefly, each replicate was taken from the culture supernatants from three wells. The culture supernatants (30 μL) were mixed with alkaline buffer and 50 μL of p-nitrophenyl phosphate and then incubated for 1 h. The reaction was stopped by adding 20 μL of stop solution included in the kit. The absorbance was then measured at 405 nm using a plate reader.

Real time PCR Analysis

Real time PCR assay was used to measure the level of the expression of osteoblast mRNA gene markers, including osteocalcin (OCN), osteopontin (OPN), bone sialoprotein (BSP) and collagen I (COL1) in MC3T3-E1 osteoblasts cultured for 7 and 14 days. Briefly, total RNA was extracted from the cells using an RNeasy mini kit (QIAGEN) following the manufacturer's protoocl. RNA (1 μg) was added to a 20 μL reverse transcription reaction mixture. Real time PCR was performed using the following conditions: 45 cycles of 95 ℃ for 30 s, 60 ℃ for 30s, and 72 ℃for 30 s. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene. Quantification of gene expression was determined from the CT value for each sample, which was calculated as the average of three measurements.

Characterization

Field emission scanning electron microscopy (FE-SEM, ZEISS Ultra 55, Germany), transmission electron microscopy (TEM, JEOL JEM-2100, Japan) and atomic force microscopy (AFM, Shimadzu SPM-9600, Japan), were employed to examine the nano-architectures of PPy at various dimensions. Attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR, Bruker Vector 33, Germany), X-ray photoelectron spectroscopy (XPS, Kratos Axis Ultra DLD, Britain) and X-ray diffraction (XRD, Burker D8 Advance, Germany) were utilized to analyze the chemical composition and molecular structure of as-obtained products. The surface potentials of specimens were characterized using Kelvin probe force microscopy (KFM, Shimadzu SPM-9600, Japan). The size of Py nano-droplets in PBS was measured by dynamic light scattering (DLS) zetasizer nanoanalyzer (Malvern Nano-ZS, Britain). Moreover, the water droplet-spreading behavior on the corresponding surface was observed using surface contact angle analyzer (Filderstadt OCA15, Germany) with a 1 μL water droplet at ambient temperature.

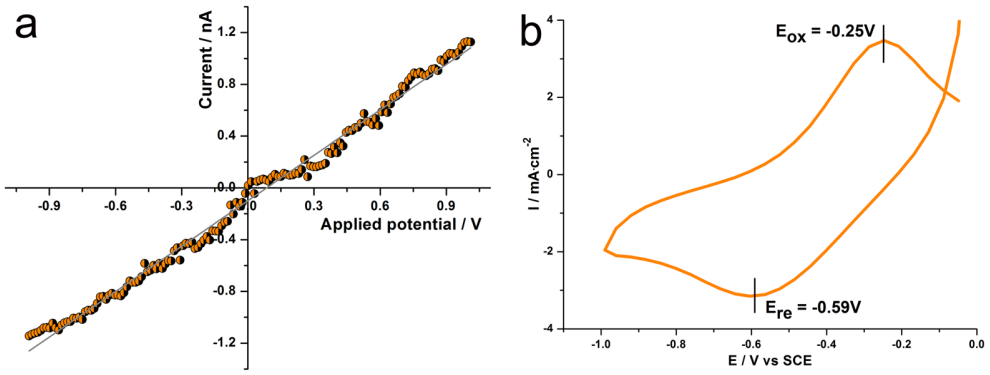

Figure S1. (a) CP-AFM I−V measurement and (b) cyclic voltammogram of 1D NAPPy/citrate obtained in PBS with pH 7.4 containing 0.05 M citrate. The local I−V plot showed linear behavior with conductivity of 44.7 S·cm^{-1}, which was recorded by conductive-probe atomic force microscopy (CP-AFM, PtIr AFM tip). The cyclic voltammogram recorded in Py-free PBS (pH 7.4) containing 0.05 M citrate at a rate of 10 mV/s was characterized by reduction peak at -0.59 V and oxidation peak at -0.25 V, showing good x axis symmetry thus demonstrating the electroactivity and good redox reversibility.

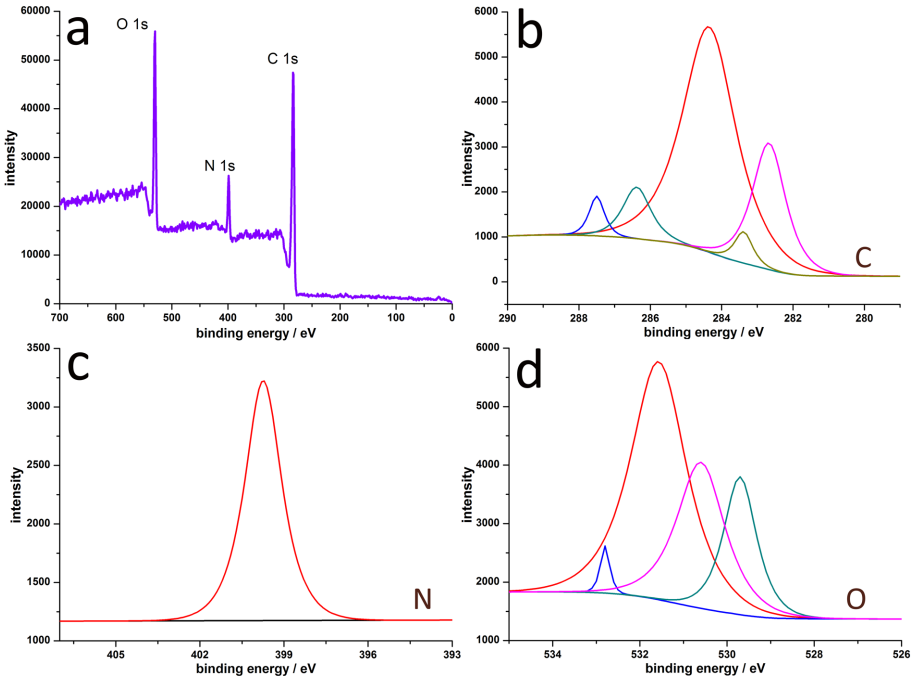

Figure S2. XPS general scan (a) of 1D NAPPy/citrate obtained in PBS with pH 7.4 containing 0.05 M citrate, and the corresponding XPS C 1s (b), N 1s (c) and O 1s (d) core-level spectra. Figure S2a shows

only three peaks from C, N and O elements of the PPy and citrate. The s1b-red peak: pyrrole α-carbons and hydrocarbon contamination; s1b-pink peaks: pyrrole β-carbons; s1b-green peak: C-N, C-N^+, C-O, C=N, C=O and C=N^+ species; s1b-blue peak: COOH and COO^- from citrate. The s1c-red peak: C=N, N-H, C-N^+ and C=N^+ species. The s1d-red peak: C=O of citrate and overoxidation of PPy; s1d-pink peak: C-O of citrate; s1d-green peak: C-O^- of citrate; s1d-blue peak: C-OH of citrate.

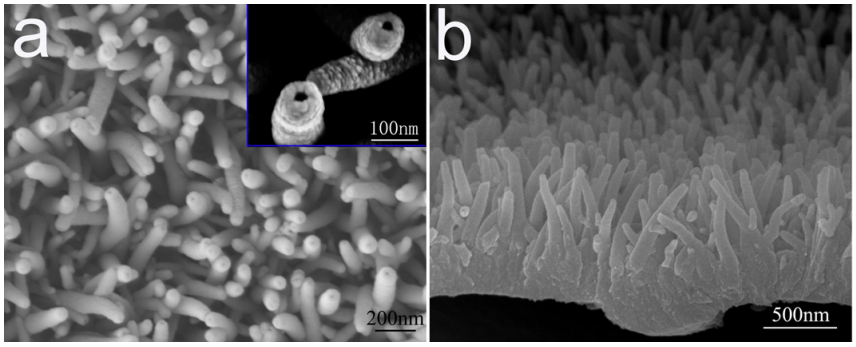

Figure S3. FE-SEM images of 1D NAPPy/NSA obtained in PBS (pH 6.8) containing 0.2 M Py and 0.01 M NSA by template-free electrochemical polymerization at a galvanostatical mode. (a) Top view. (b) Cross-sectional view. Inset in (a): nano-tubular structure imaged at a higher magnification.

Formation mechanism for 1D NAPPy/citrate

The free-Py and Py nano-droplets with a diameter of about 70 nm (Figure 4a#), which were in dynamic equilibrium depending on the pH value of PBS,[2] were dispersed in PBS containing citric acid (Figure 4a). Because the free electron pairs in the C=O of citrate[3, 4] was prone to interacting with the N-H of Py through hydrogen bonding (Figure 4b), citrate could capture and stabilize either the free-Py to form a complex (designated as free-Py/citrate) or the surface of Py nano-droplet to form another complex (termed Py nano-droplet/citrate). Under the triggered potential applied vertically from the biomedical titanium with PNF, Py nano-droplet/citrate migrated to pre-nucleated form (PNF) and subsequently electrochemically polymerized to form initial PPy nano-architecture (Figure 4c). Meanwhile, free-Py/citrate was polymerized on the outer surface of nano-architecture, thus increasing the size of the initial nano-architecture. Due to the edge effect, the edge of PPy nano-architectures possessed a higher electric field[5] and thus promoted ordered self-assembly polymerization of Py nano-droplets/citrate,

along with the outer surface polymerization of free-Py/citrate. The polymerization of free-Py/citrate on the surface of 1D nanostructures continued while the 1D nanostructures were elongated through the self-assembly polymerization of Py nano-droplets/citrate. Thus the diameter of the earlier formed segments of the 1D nanostructure was larger, leading to the formation of conical structures (Figure 4d). Namely, the nano-cones were formed as a result of synchronized self-assembly polymerization of Py nano-droplets and free-Py.

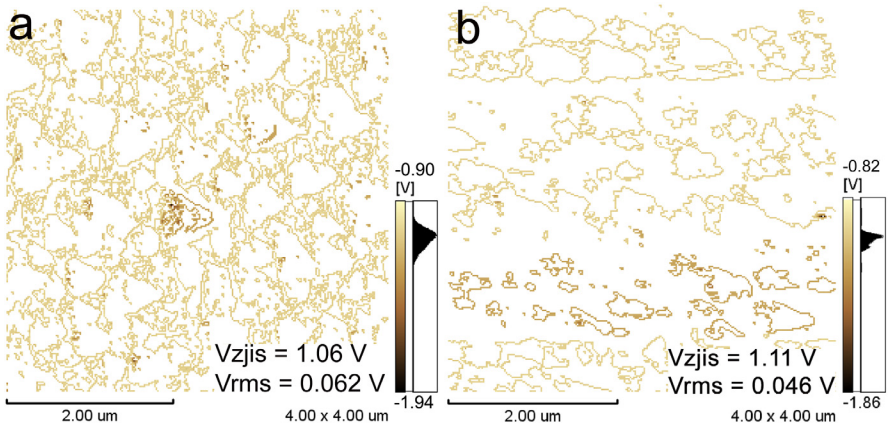

Figure S4. KFM surface potential imaging (potential contour display) on the 1D NAPPy/citrate (a) and 1D NAPPy/NSA (b). Vzjis: 10-point average potential (absolute value), Vrms: root mean square potential. 1D NAPPy/citrate and 1D NAPPy/NSA possessed a very closed Vzjis value. The surface potential distribution of 1D NAPPy/NSA was ralatively uniform.

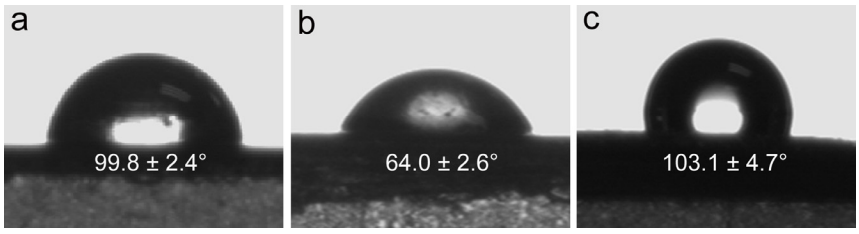

Figure S5. The water contact angle images on biomedical titanium (a), 1D NAPPy/citrate (b) and 1D NAPPy/NSA (c)

Reference:

1. Kokubo, T.; Takadama, H. *Biomaterials*, **2006**, *27*, 2907-2915.
2. Liao, J.; Ning, C.; Tan, G.; Ni, G.; Pan, H. *ChemPlusChem*, **2014**, *79*, 524-530.
3. Ni, T.; Nagesha, D. K.; Robles, J.; Materer, N. F.; Müssig, S.; Kotov, N. A. *Journal of the American Chemical Society*, **2002**, *124*, 3980-3992.
4. Samia, A. C.; Schlueter, J. A.; Jiang, J. S.; Bader, S. D.; Qin, C.-J.; Lin, X.-M. *Chemistry of Materials*, **2006**, *18*, 5203-5212.
5. Akahane, Y.; Asano, T.; Song, B. S.; Noda, S. *Nature*, **2003**, *425*, 944-947.