Supplementary Information

Facilely tuning bioactivity of orthopedic implant surface based on nanostructured polypyrrole/glycosaminoglycans

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Experimental

Chemicals

All chemicals of analytical grade were purchased from Sigma–Aldrich Co. Ltd, and used without further treatment if not specified otherwise.

Fabrication of NPPy/GAGs

The small electrochemical cell included Ti-6Al-4V (0.5 mm in thickness) as working electrode, platinum wire as counter electrode, saturated calomel electrode (SCE) as reference electrode, a phosphate buffer solution (PBS, pH 7.4) as electrolyte containing 0.02 M Py and 50 mg/mL GAG (*i.e.*, DS, KS and CS). The NPPy/GAGs were obtained through cyclic voltammetry (scanning scale: 0.5 ~ 1.0 V, scanning rate: 20 mV/S, scanning number: 10 cycles) under the control of electrochemical station (Zennium Zahner, Germany). The as-obtained products were rinsed for several times in deionized water, and dried under vacuum.

Wettability of NPPy/GAGs controlled by *in situ* electrical stimulus

To test electrochemical activity and redox potential of NPPy/GAGs, cyclic voltammograms were acquired by scanning the potential from 0.5 to -1.0 V at a rate of 10 mV/s, which were performed in the electrochemical system including NPPy/GAGs on Ti-6Al-4V as the working electrode, platinum wire as the counter electrode and SCE as the reference electrode (PBS with pH 7.4 as an electrolyte containing 50 mg/mL GAG). According to cyclic voltammogram curves of NPPy/GAGs, we herein uniformly applied *in situ* electrical stimulus of 0.4 V (oxidation potential) and -0.6 V (reduction potential) to trigger redox reactions for various times in three-electrode system with PBS (pH 7.4) as electrolyte. Redox states of NPPy/GAGs were reversibly switched by *in situ* applying periodic electrical stimulus (0.4/-0.6 V for 7 min). Static water contact angle (SWCA) measurements were performed on NPPy/GAGs (dried under vacuum) in various states by surface contact angle analyzer (Filderstadt OCA15, Germany) with a 1 μL water droplet at ambient temperature. At least five measurements were taken on each specimen, and the average and standard deviation were calculated.

Protein adsorption on NPPy/GAGs with various and periodic wettability

The specimens with various and periodic wettability were immersed in PBS (pH 7.4) for 30 min prior to protein adsorption. The protein Fn solution of 10 μg/mL was freshly prepared by dissolving Fn in PBS. To study the Fn adsorption behavior in response to wettability of NPPy/GAGs, the surface of NPPy/GAGs with a diameter of 10 mm was exposed to 1 mL protein solution and incubated at 37°C. After 8 h, the specimens were rinsed using PBS for 3 times, followed by shaking in 0.2 mL of 1 wt % sodium dodecylsulfate (SDS) to elute the reversibly

adsorbed protein. The amount of the adsorbed protein on NPPy/GAGs surface was determined by bicinchoninic acid (BCA) assay. The absorbance of the eluent was measured at 562 nm by a microplate reader (Thermo 3001, USA) with at least three repetitions for each group.

MC3T3-E1 osteoblasts culture and seeding on NPPy/GAGs

MC3T3-E1 osteoblasts were cultured in α-modified minimum essential medium (α-MEM) supplemented with 10% foetal bovine serum (FBS) in a humid 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 NPPy/GAGs were sterilized by immersion in 70% ethanol for 5 min, followed by drying under a sterile condition, and exposing 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 3×10^3 cells/cm^2, with the medium changed every 2 days. For the proliferation studies, the numbers of adherent cells on the substrates at day 1, day 4 and day 7 were quantified using Cell Counting Kit-8 (CCK-8) assay at 450 nm.

Morphological observation of MC3T3-E1 osteoblasts by fluorescence staining

To image MC3T3-E1 osteoblasts on specimens after being cultured for predetermined time, 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

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 was added to each well and MC3T3-E1 osteoblasts were incubated for 4, 7 and 15 days. Alkaline phosphatase activity measurement was performed using an alkaline phosphatase assay kit (Abcam). Each replicate was prepared by pooling culture supernatants from three wells. Culture supernatants (30 μL) were combined with alkaline buffer and 50 μL of p-nitrophenyl phosphate and then incubated for 60 min. The reaction was stopped with 20 μL of stop solution (provided in the kit). The absorbance was then measured at 405 nm.

Real time PCR analysis

The expressions of osteoblast mRNA gene markers, including osteocalcin (OCN), osteopontin (OPN), bone sialoprotein (BSP) and collagen I (COL1), were determined on specimens cultured with MC3T3-E1 osteoblasts for 7 and 15 days. Total RNA was extracted using an RNeasy mini kit (QIAGEN) as per the manufacturer's instructions. RNA (1 μg) was added to a 20 μL reverse transcription reaction mixture. Real time PCR was performed according to the method described by the manufacturer: 45 cycles of 95 ℃ for 30 s, 60 ℃ for 30 s, and 72 ℃ for 30 s. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene. Quantification of gene expression was based on the CT value for each sample, which was calculated as the average of three replicate measurements, and each replicate was prepared by pooling cell lysates from three wells.

Characterization of materials

Scanning electron microscopy (SEM, ZEISS Ultra 55, Germany) was employed to examine surface topography of NPPy/GAGs. The surface potentials of NPPy/KS in various redox states were

characterized using Kelvin probe force microscopy (KFM, Shimadzu SPM-9600, Japan). X-ray photoelectron spectroscopy (XPS, Kratos Axis Ultra DLD, Britain) was utilized to analyze the chemical composition of as-obtained products.

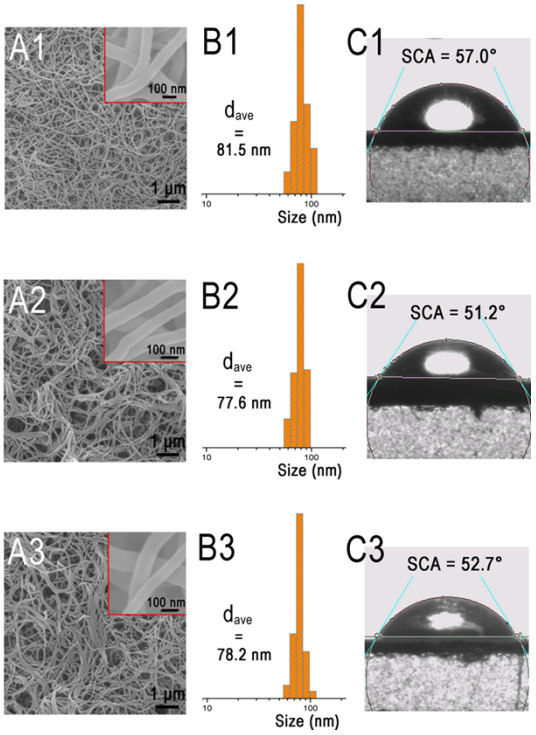

Fig. S1 (A) SEM images, (B) plots of diameter distribution and (C) SWCA images of NPPy/DS (1), NPPy/KS (2) and NPPy/CS (3). Insets in A: higher magnification. A high density of nanowires tangling into tight networks with similar diameter (*ca.* 78 nm) and wettability (*ca.* 52°) could be found in the three NPPy/GAGs.

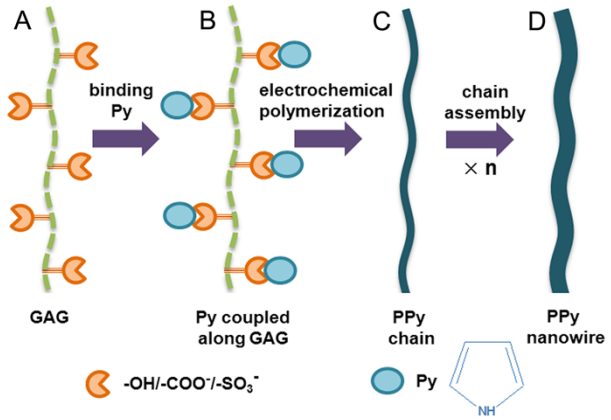

Fig. S2 Schematic illustration of NPPy/GAGs fabricated by the assistance of GAG. (A) Molecular chain of GAG. (B) Py was coupled by -OH/-COO^-/-SO_3^- of GAG. (C) Signal PPy chain was formed by the electrochemical polymerization of Py along GAG. (D) A number of PPy chains assembled to form PPy nanowire. The polysaccharide chains of GAGs are characterized by numerous

active/polar groups such as -OH/-COO^-/-SO_3^-. Due to the hydrogen bond between the active groups and N (nitrogen) of Py, the Py monomers were self-assembled on main chains of GAGs. Subsequently, Py monomers were polymerized along the GAGs to form PPy chain in the template-free electrochemical system. A certain number of PPy/GAGs chains self-assembled in order thus harvested PPy/GAGs nanowire.

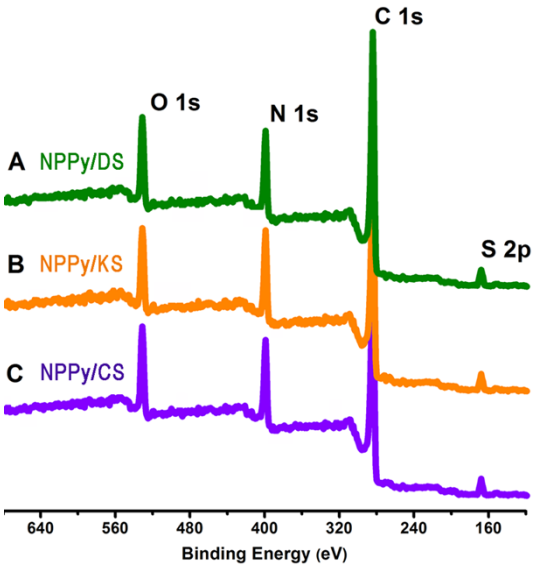

Fig. S3 XPS patterns of (A) NPPy/DS, (B) NPPy/KS and (C) NPPy/CS. The strong peaks of O (oxygen), N (nitrogen) and C (carbon) were attributed to the chemical composition of PPy and GAGs. The peak of S (sulfur) could be assigned to the SO_3^- of GAGs, indicating the presence of GAGs in NPPy.

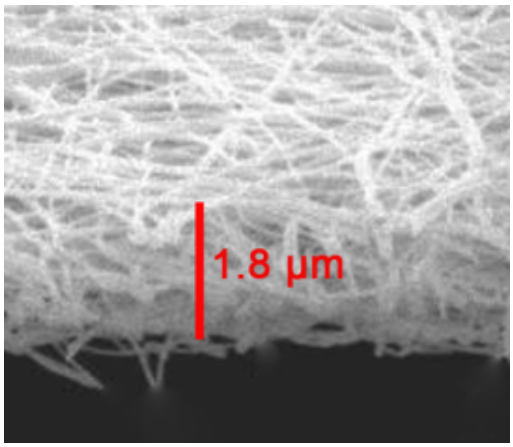

Fig. S4 SEM images of NPPy/KS in cross-section view. The NPPy/KS coating was physically peeled off from the Ti-6Al-4V substrate using a sharp knife, and subsequently its thickness was

measured by SEM. The thickness of NPPy/GAGs obtained in this paper was about 1.8 μm.

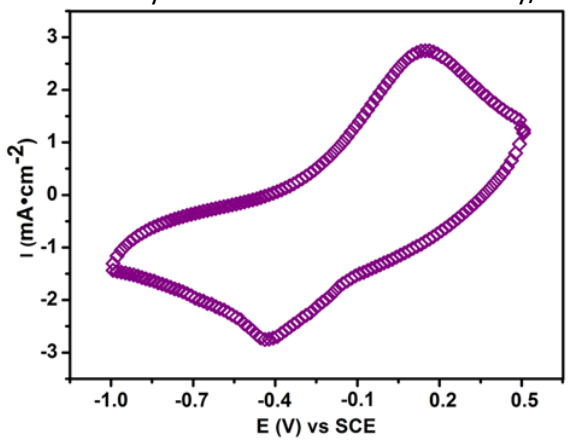

Fig. S5 Cyclic voltammogram of NPPy/KS acquired by scanning the potential from 0.5 to -1.0 V at a rate of 10 mV/s. The oxidation and reduction peaks appeared at 0.11 V and -0.43 V, respectively. The redox peaks showed almost the same values of current thus demonstrated the electrochemical activity and good redox reversibility.

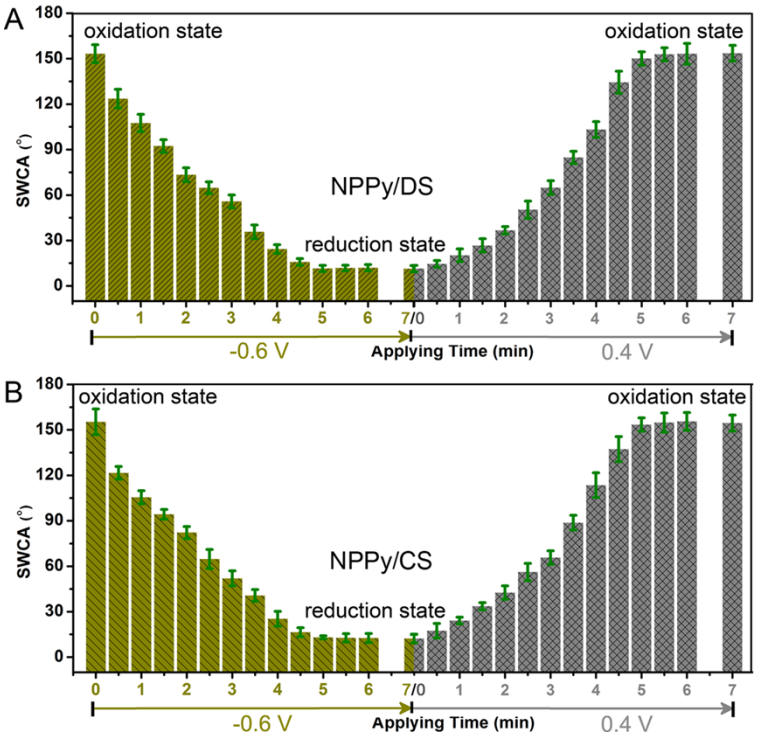

Fig. S6 The plots of SWCA of (A) NPPy/DS and (B) NPPy/CS *versus* applying time of *in situ* electrical stimulus. Both the SWCAs of NPPy/DS and NPPy/CS decreased with applying time of -0.6 V, and stabilized at 5 min (reduction state, highly hydrophilic). When applying 0.4 V, both the SWCAs increased with the applying time, and stabilized at 5 min (oxidation state, superhydrophobic).

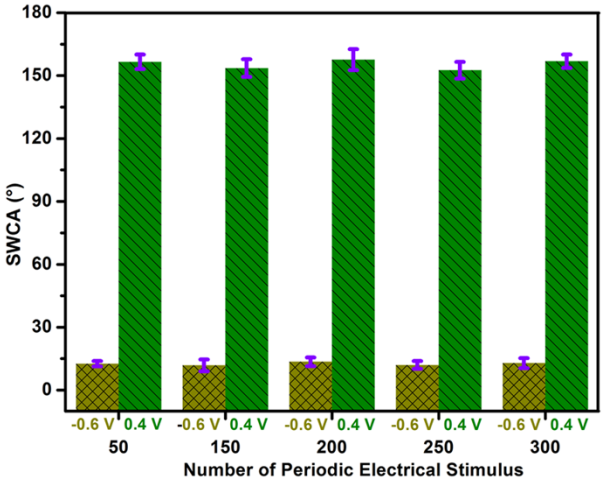

Fig. S7 The plot of SWCA *versus* cycle number of electrical stimulus. We applied various cycles of electrical stimulus (0.4/-0.6 V for 7 min) up to 300 cycles, and it was found that both SWCAs of hydrophilic and superhydrophobic surfaces remained relatively stable.

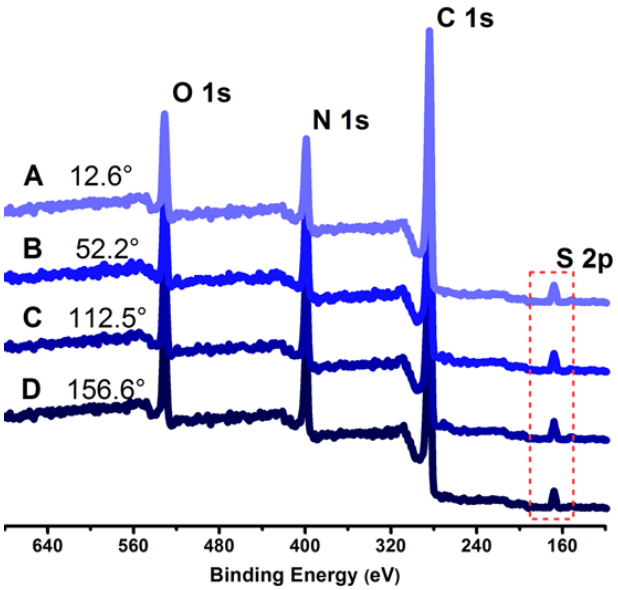

Fig. S8 XPS patterns of various SWCAs of NPPy/KS. (A) 12.6 ± 1.4°, (B) 52.2° ± 2.7°, (C) 112.5° ± 2.2°, (D) 156.6 ± 3.5°. The four SWCAs of NPPy/KS were obtained by applying various times of *in situ* electrical stimulus. The peak heights of S were the same in the four patterns of XPS, implying that the amount of KS remained unchanged after undergoing electrical stimulus.

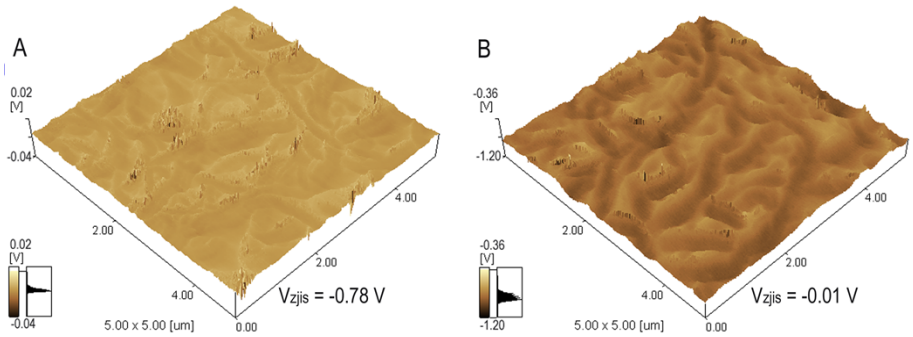

Fig. S9 KFM surface potential imagings on the NPPy/KS with SWCA of (A) 12.6 ± 1.4° and (B) 156.6 ± 3.5°. SWCA 12.6 ± 1.4° possessed V_{zjis} of -0.78 V while -0.01 V for SWCA 156.6 ± 3.5°. The negative potentials were derived from negatively charged groups ($-COO^-$/$-SO_3^-$) of KS chain. That was to say, the concentration of KS (a hydrophilic molecule) on the hydrophilic surface of NPPy (12.6 ± 1.4°) was much higher than that on superhydrophobic surface (156.6 ± 3.5°).

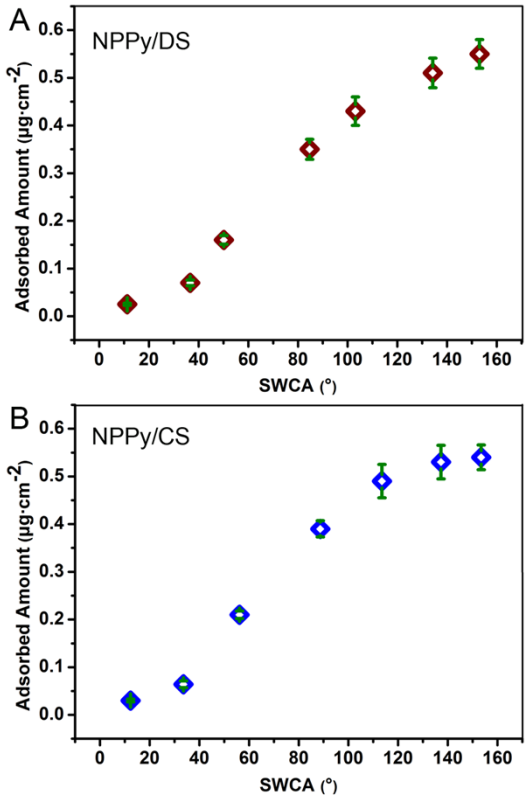

Fig. S10 The plots of adsorbed amount of Fn on (A) NPPy/DS and (B) NPPy/CS *versus* SWCA. The adsorbed amount of Fn on NPPy/DS and NPPy/CS was in direct proportion to the SWCA of NPPy/DS and NPPy/CS, that was, the adsorbed amount was increased with the SWCA of NPPy. The adsorbed amount on highly hydrophilic surface of NPPy was in a negligible level, while a relatively high adsorbed amount was on superhydrophobic surface.

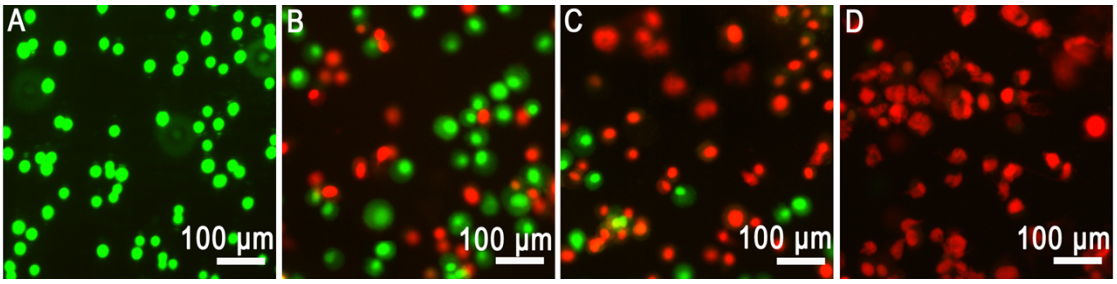

Fig. S11. Acridine orange–propidium iodide (AO/PI) staining fluorescence images of MC3T3-E1 osteoblasts on NPPy/KS after applying 40 cycles (cycle: positive electrical stimulus for 7 min and follow by negative stimulus for 7 min) of periodic electrical stimulus with different magnitudes. (A) 0.4/-0.6 V, (B) 1.4/-1.6 V, (C) 2.4/-2.6 V, (D) 3.4/-3.6 V. Live cells were stained green (AO) while dead cells were colored red (PI). It was showed that under the 0.4/-0.6 V, which were applied to *in situ* control wettability in this work, all the cells remained alive. However, when the magnitudes of periodic electrical stimulus were increased to 1.4/-1.6 V, 2.4/-2.6 V and 3.4/-3.6 V, more and more cells were found dead, suggesting that relatively high electrical stimulus was biologically unacceptable for MC3T3-E1 osteoblasts or bone tissue.

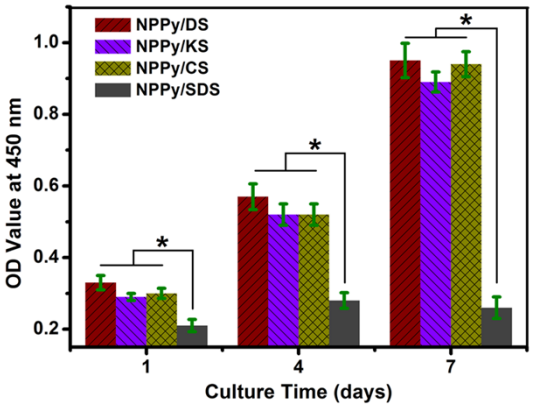

Fig. S12 The plot of OD values of MC3T3-E1 osteoblasts seeded on NPPy doped various GAGs and SDS *versus* culture time. The * indicated significant difference ($p < 0.05$). The OD values of the three NPPy/GAGs were gradually increased with the culture time, whereas no positive change in OD value could be found in NPPy/SDS (sodium dodecylsulfate, non-biomolecular dopant), implying that innate advantage of GAGs in biocompatibility rendered NPPy/GAGs ideal candidate for **o**rthopedic implant surface.