Supplementary information

Organoamine-assisted biomimetic synthesis of faceted hexagonal hydroxyapatite nanotubes with prominent stimulation activity for osteoblast proliferation

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Experimental Section

Synthesis of Single-Crystalline HA Nanotubes . In a typical synthesis, calcium dihydrogen phosphate (1.01 g) and calcium chloride (0.629 g) were dissolved in water (80 ml), and then an ethanol solution of dodecylamine and hexadecylamine (8:1 molar ratio) was dropped in very slowly (24 ml for 16 hours). The molar ratio of Ca : P : N was 1.2 : 1 : 2.25 in the final mixture. The mixture was then transferred into a Teflon-lined autoclave and heated statically at 120 °C for varied periods of time. The resulted solid product was separated by centrifugation, washed repeatedly with water and anhydrous ethanol and dried under vacuum at 40 °C for 24 h.

The dodecylamine and hexadecylamine in the precipitate was removed by ions exchange method. Firstly, the dried solid precipitate was dispersed in methylamine chloride aqueous solution (10 %), stirred at room temperature for 2 h and then the solid was recovered by centrifugation. The process was repeated six times. Then the solid was further washed repeatedly with water and anhydrous ethanol. Finally, the solid was calcined at 400 °C for 12 h in air to obtain the white powdered product.

Sample characterization. X-ray diffraction (XRD) analysis was performed on a Philips X'Pro Xray diffractometer with Cu Kα irradiation. The X-ray source was operated at 40 kV and 40 mA. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) measurements were conducted with JEM-100S and JEM-2010 electron microscopes, using an accelerating voltage of 80 kV and 200 kV, respectively. Scanning electron microscopy (SEM) measurements were conducted with an LEO1530 VP instrument.

Cell culture assays. In the assays, murine osteoblasts were cultured with HA nanotubes in vitro. To generate primary murine osteoblast cultures, the calvaria were dissected from 14-hour-old newborn mice, cut to small pieces, and triply digested in the solution of collagenase and trypsin.

The osteoblasts, at a concentration of 2×10^5 cells/ml, were cultured for 2 days in RPMI 1640 culture medium supplemented 10% fetal bovine serum (FBS) and each sample has 6 parallel wells

in the 96-cell culture plates. The powder of HA nanotubes was sterilized and dispersed in the above culture medium. Then it was added in the 96-cell culture plates. In control experiments, no HA nanotubes but culture medium was added. The cells were maintained at 37 °C in a fully humidified atmosphere containing 5% CO_2.

In our another assay for combination of the nanotubes and BMP-7, cells were seeded at a density of 2×10^5 cells/ml and the culture media were changed on alternate days. The dose of the nanotubes is 250 μg/ml and the dose of BMP-7 is 40 ng/ml.

The cells were harvested by trypsinization and viable cells were counted every day using Trypan blue dye exclusion method. The morphology of the cells was evaluated through an inverted microscope in situ.

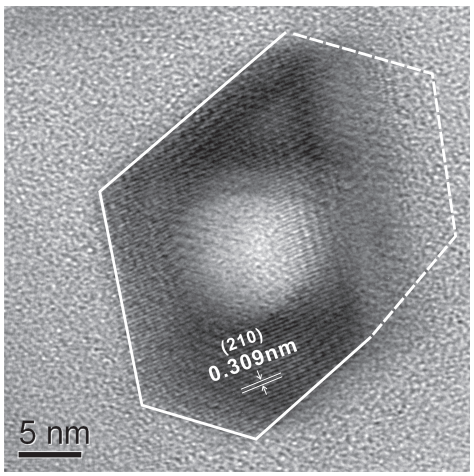

Fig. S1 HRTEM image of the cross section of a single HA nanotube.

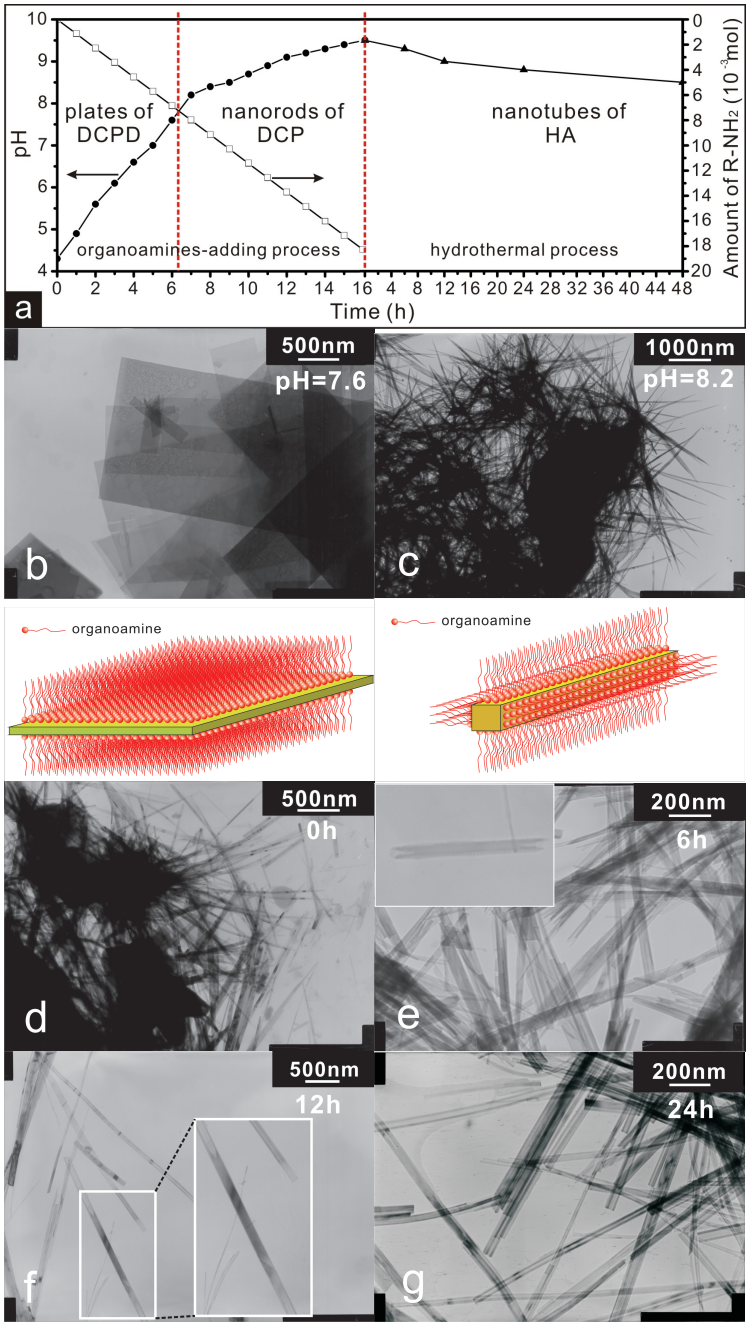

Fig. S2 pH value and morphology of the products in the synthetic process. (a) pH evolution. (b) and (c) TEM images of the solids produced from the reactions among the organoamines, calcium dihydrogen phosphate and calcium chloride at (b) pH=7.6 and (c) pH=8.2. (d)-(g) TEM images of the samples obtained at different hydrothermal treatment stages: 0, 6, 12 and 24 h, respectively. The inset of (e) shows a nanofiber with pits at both ends and the inset of (f) shows a magnified part of a fiber with deep holes.

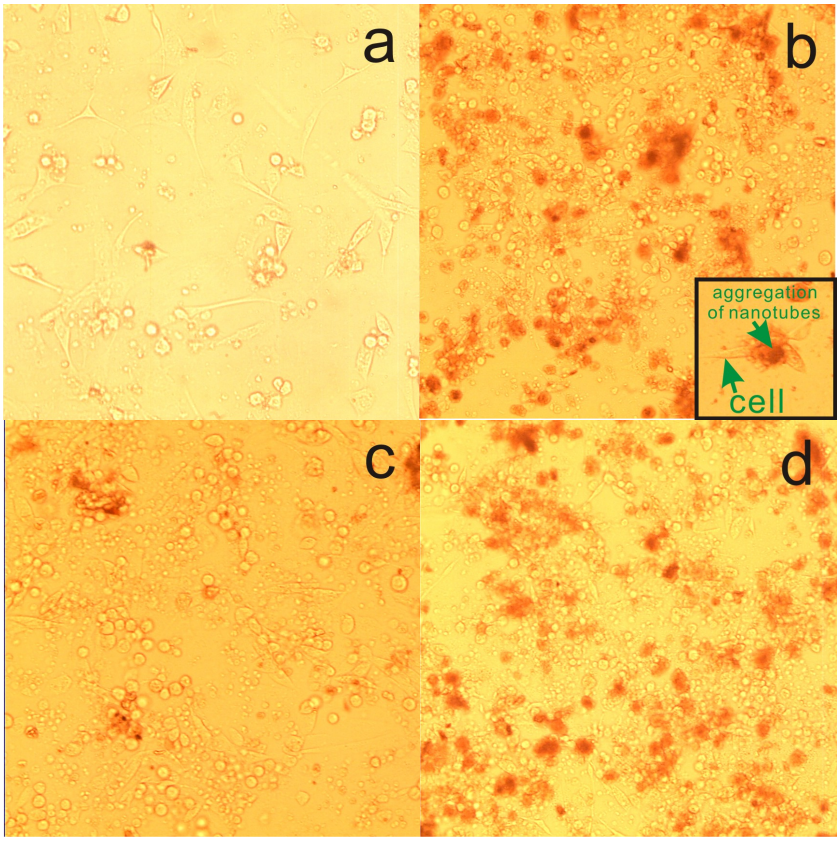

Fig. S3. Inverted microscope images in situ (magnification times: 200×). a) cells in control. b) cells cultured with HA nanotubes (250 μg/ml) for 5 days and inset is an aggregation of the nanotubes with the cells proliferate radially around. c) cells cultured with BMP-7 (40 ng/ml) for 5 days. d) cells cultured with HA nanotubes (250 μg/ml) and BMP-7 (40 ng/ml) for 5 days.