Engineering a favorable osteogenic microenvironment by heparin mediated hybrid coating assembly and rhBMP-2 loading

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Synthesis of HEP-Dopa conjugate

Briefly, HEP and EDC, sulfo-NHS were firstly dissolved in MES buffer (0.05 M, pH=5.3, 0.1 M NaCl) and the reaction mixture was stirred for 3 h at room temperature to activate carboxylic acids groups of HEP. After activation process, Dopa was added and continued to react for 24h. The final molar ratio of HEP:EDC:sulfo-NHS:Dopa is 1:4:4:5. Then the saturated NaCl solution and the cold ethanol were sequentially added, white HEP-Dopa conjugate precipitate was appeared at this moment. After centrifugation at 6000 rpm for 10 min, the precipitated heparin-dopamine conjugate was re-dissolved in ultrapure water, and ethanol was added to allow the conjuate to precipitate again. This purification step was repeated three times. The heparin-dopamine conjugate was dialyzed against deionized water using a dialysis membrane (MWCO:3500) for 2 days under N_2 gas purge to minimize oxidation of catechol groups. The degree of substitution was determined from 1H NMR spectroscopy (400MHz, AVANCE III, Bruker, Switzerland) in D_2O.

Hydroxyapatite nanoparticles preparation

The pH value of 240 mL 0.2 M $(NH_4)_2HPO_4$ aqueous solution was firstly adjusted at about 10~11 by ammonia water, then 400mL 0.2M $Ca(NO_3)_2$ aqueous solution was quickly dumped into the $(NH_4)_2HPO_4$ solution. The reaction mixture was stirred for 12 h in the ice water bath to obtain white products (the pH of mixed solution was controlled at 10~11 by ammonia water during the reaction process). The white products were collected and washed by ultrapure water/ethanol in turn for 3 times, followed by a freeze-drying step. Finally, the freeze-dried powers were heated at 600℃ for 2 h to obtain Hydroxyapatite nanoparticles.

Cell culture

Murine myoblast cells (C2C12) and Rat bone marrow stromal cells (rBMSCs) were used for evaluating osteogenesis vitality of the multilayer-coating. C2C12 cells were purchased from the American Type Culture Collection (ATCC, VA, USA) and rBMSCs extracted from rat bone marrow. C2C12 cells and rBMSCs were grown in Dulbecco's modified Eagle's medium (DMEM) and α-modified Eagle's medium (a-MEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin and 100 μg/mL streptomycin respectively and cultured in 75 cm^2 tissue culture flasks in a humidified 5% CO_2 environmental incubator at 37 ℃ and cultivated at 80% confluency.

Cell implantation

The cultured C2C12 cells and rBMSCs were harvested with 0.05/0.02% trypsin/EDTA, centrifuged at 800 rpm for 4 min and resuspended in the culture medium. Prior to cell seeding, the as-prepared films (1 cm^2) were sterilized soaked in 75% ethanol, and then rinsed with sterilized PBS and DMEM solution with 5 times. Cells were seeded onto the coatings by evenly dropping the cells suspension (0.1 ml, 5×10^4 cells) onto the as-prepared films. After 30 min incubation, 2 ml complete medium was added to per well and the cell/films constructs were incubated for a predetermined period.

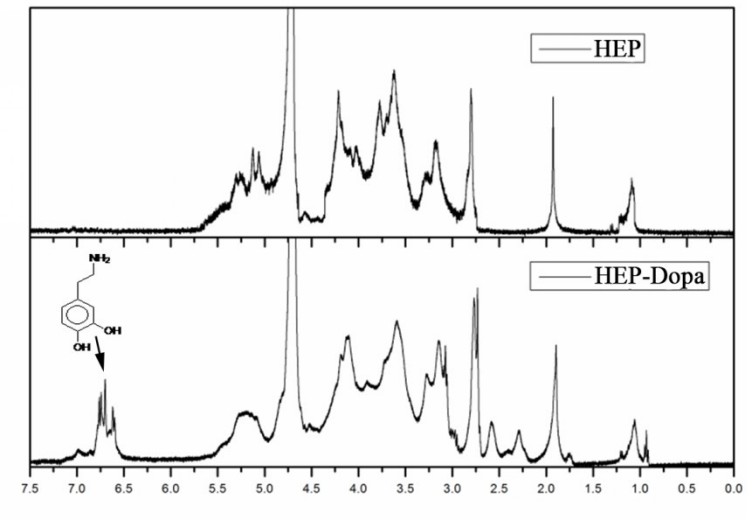

Fig. S11H NMR analysis of HEP and HEP-Dopa. The new peaks present in 6.5-7.0 ppm for HEP-Dopa are characteristic proton peaks of benzene ring ondopamine group. The dopamine substitution degree on heparin molecule is about 34.2 %.

Table S1

The parameters of primers utilized for detecting osteogenic gene expression.

Gene	Direction	Sequence (5'-3')
ALP	Forward	TAT GTC TGG AAC CGC ACT GAA C
	Reverse	CAC TAGCAA GAA GAA GCC TTT GG
RunX2	Forward	ATC CAG CCA CCT TCA CTT ACA CC
	Reverse	GGG ACC ATT GGG AAC TGA TAG G
OCN	Forward	GCC CTG ACT GCA TTC TGC CTC T
	Reverse	TCA CCA CCT TAC TGC CCT CCT G
OPN	Forward	CCA AGC GTG GAA ACA CACA GCC
	Reverse	GGC TTT GGA ACT CGC CTG ACT G
GAPDH	Forward	CAC CCG CGA GTA CAA CCT TC
	Reverse	CCC ATA CCC ACC ATC ACA CC