Electronic Supplementary Information

Comparative study of resazurin reduction and MTT assays for cytocompatibility evaluation of nanofibrous materials

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Part of experimental details:

Materials

PLGA (50:50, M_W = 81 000 g/mol) was purchased from Jinan Daigang Biotechnology Co., Ltd. (Jinan, China). n-HA and HNTs were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China) and Zhengzhou Jinyangguang China Clays Co., Ltd. (Zhengzhou, China), respectively. MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide) and resazurin were purchased from Sigma-Aldrich (St. Louis, MO). Tetrahydrofuran (THF) and N,N-dimethylformamide (DMF) were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L929 were obtained from Institute of Biochemistry and Cell Biology (the Chinese Academy of Sciences, Shanghai, China). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and antibiotic-antimycotic were purchased from Hangzhou Jinuo Biomedical Technology (Hangzhou, China). All chemicals were used as received. Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with resistivity higher than 18 MΩ.cm.

Cell culture, cytocompatibility evaluation, and MTT assay

Mouse fibroblasts (L929) were cultured in a humidified incubator with 5% CO_2 at 37 ^oC using Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomycin. Nanofibrous mats were first placed in a 24-well tissue culture plate (TCP) and fixed with stainless steel rings, TCPs and cover slips were used as controls. Then they were all sterilized with ethanol for 2 h and soaked with medium overnight before cell seeding.

For MTT assay, L929 cells were seeded at a density of 1×10^4 cells/well and cultured for 1, 2, 3, and 4 days and washed with PBS, respectively. MTT solution (40 μL MTT and 360 μL medium) was added to each well at the corresponding time points, followed by incubation for another 4 h. After that, the medium was taken out and each well was added with DMSO (400 μL) to dissolve the purple MTT formazan crystal for 15 min. The solution of dissolved formazan in each well (100 μL) was then transferred to separate wells of a 96-well plate to test the OD value at 570 nm using a microplate reader (MK3, Thermo, USA). Mean and standard deviation from the triplicate wells for each sample were reported.

SEM observation of L929 cells

To further examine the cytocompatibility of the nanofibers materials, the morphology of L929 cells grown onto nanofibers, TCPs, and cover slips was observed using SEM. Before analysis, cells grown onto different substrates were washed with PBS, fixed with 2.5% glutadialdehyde for 2 h at 4 ºC, dehydrated with a series of gradient ethanol solutions (30, 50, 70, 90, 95, and 100% ethanol, respectively), and air-dried. Then the samples were sputter coated with gold film with a thickness of 10 nm and observed by SEM (JEOL JSM-5600LV, Japan) with an operating voltage of 15 kV.