Supporting information

Topographical regulation of stem cell differentiation by plant-derived micro/nanostructures

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S1. Cytocompatibility Evaluation of Raffia

The raffia was cut to the same size, and the samples were sterilized in 75% ethanol with UV overnight and washed three times with sterile PBS. Then 0.1 mL of cell suspension which contained 2×10^4 cells was seeded on the surface of the samples in 24-well plates, and added 1 mL of complete medium. To investigate the attachment of the cell cultured on the surface of raffia, after 48 hours of culture, the cells were observed by immunofluorescence measurement of F-actin and their nuclei. Typically, hADSCs were fixed by 4% paraformaldehyde solution (SparkJade, Qingdao, China) for 10 min after washed. Then, the cells were permeated with 0.1% Triton X-100 (Solarbio, Beijing, China) for 5 min and blocked with 10% bovine serum albumin (BSA) (Solarbio, Beijing, China) for 30 min. Rhodamine phalloidin (Cytoskeleton, Denver, USA) was used to stain F-actin at a 1:200 dilution for 30 min, and the cells were incubated with DAPI (Thermo Fisher Scientific, Waltham, MA, USA) to stain the nuclei for 5 min. Finally, the cells were washed three times by PBS, and then examined under the laser confocal microscope (LSM 800, Zeiss, Germany).

Live/dead staining was used to obtain the survival of cells cultured on the surface of raffia. After 48 hours of culture in 24-well plates, the cultured medium was replaced with 200 μL serum-free α-MEM medium which counted 4 μM propidium iodide (PI) and 2 μM calcein AM (Thermo Fisher Scientific, Waltham, MA, USA), and then the cells were incubated at 37℃ for 20 min. After washed three times with PBS, hADSCs were examined under the laser confocal microscope.

To further investigate the attachment of the cell cultured on the surface of raffia, after 48 hours of culture in 24-well plates, the cells were washed three times by PBS, and 2.5% glutaraldehyde solution was used to fix the hADSCs overnight. Then a series of alcohol solutions (30%, 50%, 70%, 80%, 90%, 95%, 98%, and 100%) was used to dehydrate cells, and then hADSCs were lyophilized at -60℃ for 6 hours. The cells were observed under the scanning electron microscope after 30 s Au spraying at a current of 10 mA.

To quantitatively measure cell viability and proliferation on the surface of raffia, the cultured medium was replaced with serum-free α-MEM medium containing a 10% CCK-8 solution (MdeChemExpress, Shanghai, China) on the 1, 3, and 5 days, which was used to quantitatively assess cell viability. After 2 hours of incubation at 37℃, the culture medium containing a water-

soluble formazan dye having absorption at a wavelength of 450 nm was measured by a microplate reader (SYNERGY H1, BioTek, USA). Three replicates were used for each sample.

S2. Osteogenic Differentiation of Evaluation of Raffia

In this experiment, after cells cultured for 5 days, the culture medium was replaced with 5% fetal bovine serum α-MEM medium containing 1% penicillin-streptomycin, 10 mM β-glycerol phosphate (Solarbio, Beijing, China), 100 nM dexamethasone (Solarbio, Beijing, China), and 50 μg/mL of L-ascorbic acid (Sinopharm, Shanghai, China). The cells cultured on all the substrates were characterized at the protein, gene, and functional levels to assess the osteogenesis of cells.

S2.1.Alkaline phosphatase (ALP) Activity

After 7, 14, and 21 days of culture in medium, cells cultured on different surface of raffia were lysed with 0.1% Triton X-100 for total intracellular protein content and ALP activity assays. The micro BCA protein assay kit (Solarbio, Beijing, China) was used to assay the total intracellular protein content by measuring the absorbance of the reaction solution at 570 nm. The ALP activity was analyzed using the laboratory-assay ALP activity assay kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions. The relative ALP activity was normalized with the protein content of cells cultured on different samples (n = 3 for each group).

S2.2 Quantitative PCR (q-PCR)

After 7, 14, and 21 days of culture in medium, cells on different surface of raffia were treated with TRIZOL Reagent (Thermo Fisher Scientific, Waltham, MA, USA) to extract total RNA. RNA concentration and purity were measured using a Q-5000 spectrophotometer (Quwell) at 260/280 nm. The real-time PCR system (LightCycler® 96, Roche, Switzerland) was used for q-PCR analysis of one housekeeping gene, β-actin, and three genes of Runx2, osteocalcin (OCN), and osteopontin (OPN) (Table S1 for primer sequence). The relative transcription levels of target gene expression were normalized to β-actin and expressed as the mean ± S.D. (N = 3 / group).

Table S1. Sequence of q-PCR primers

Gene	Forward primers (5'-3')	Reverse primers (5'-3')
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
OPN	TCCTGTCTCCCGGTGAAAGT	GGCTACAGCATCTGAGTGTTTGC
OCN	AAGCCCAGCGACTCTGAGTCT	CCGGAGTCTATTCACCACCTTACT
Runx2	AATGCCTCCGCTGTTATG	TTCTGTCTGTGCCTTCTTG

S2.3 Alizarin Red S (ARS) Staining

After a culture period of 21 days, the cells cultured on the different surface of raffia and TCPs were fixed with 4% paraformaldehyde for 30 min, and then the samples were stained with 2% ARS (Solarbio, Beijing, China) at pH 4.2 for 10 min. After washing the cells with distilled water for three times, the ARS stained samples were observed under a polarizing microscope with camera (CX31-P-OC-1, Olympus, Japan).

S2.4 Immunofluorescence Staining

After 21 days, cells cultured on the samples were washed with PBS three times and fixed with 4% paraformaldehyde at room temperature for 10 min. Then the cells were permeabilized by using 0.1% Triton X-100 for 10 min and blocked with 10% bovine serum albumin solution for 1 h at room temperature. After blocking, the cells were incubated with primary antibodies at 1:1000 dilution against osteocalcin (mouse monoclonal anti-OCN, Abcam) and osteopontin (rabbit polyclonal anti-OPN, Abcam) at 4℃ for overnight. Goat anti-rabbit and goat anti-mouse secondary antibodies labeled by Alexa Fluor 488 (Thermo Fisher Scientific, Waltham, MA, USA) and Alexa Fluor 594 (Thermo Fisher Scientific, Waltham, MA, USA) at 1:200 dilutions in 1% bovine serum albumin solution were respectively used for staining OPN and OCN for 1 h at room temperature. After rinsing off the second antibody with PBS, the OPN was further stained F-actin by rhodamine phalloidin for 1 h and the OPN and OCN were stained nuclei by DAPI for 10 min . Images of the stained samples were obtained using the laser confocal microscope.

S2.5 Statistical Analysis

Statistics were performed using one-way analysis of variance (ANOVA) in PASW Statistics 18. The data were expressed as mean ± S.D. (standard deviation). The statistical significance of the differences was determined by a one-way ANOVA (* means $p < 0.05$, ** means $p < 0.01$, and

p > 0.05, represents no significance).

S3. Raffia Characterization

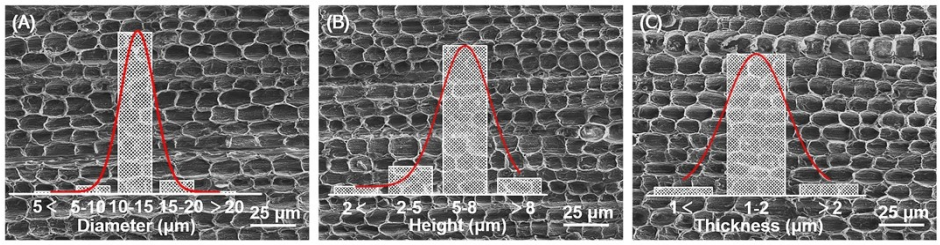

Fig. S1 The statistical analysis of the (A) diameter, (B) height, and (C) thickness of the structures.

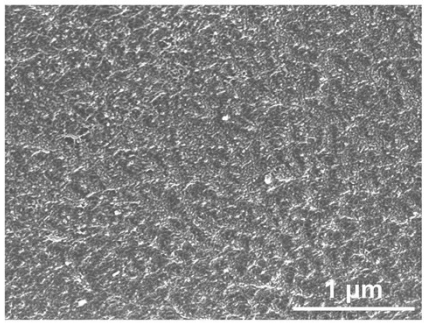

Fig. S2 SEM image at high magnification of the front surfaces of raffia.

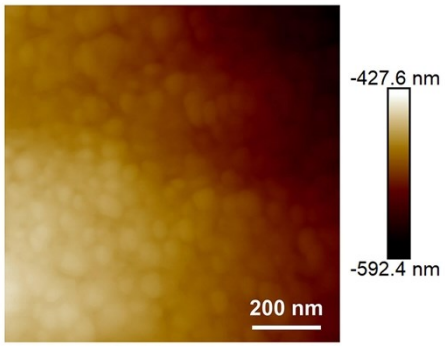

Fig. S3 The AFM morphology image of the front surface of raffia at the higher magnification after OP treatment.

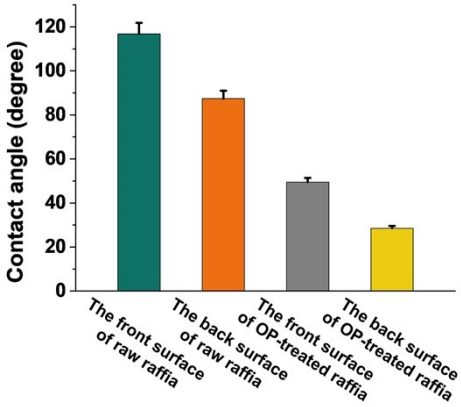

Fig. S4 The statistical analysis about the contact angles of the samples.

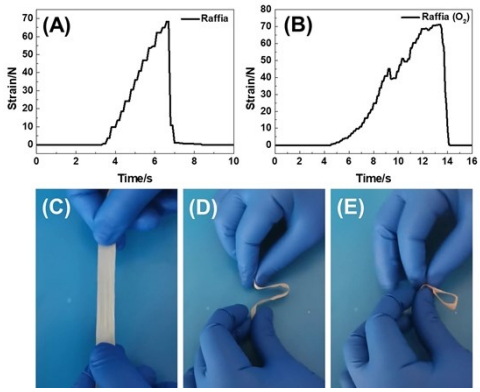

Fig. S5 The mechanical properties experiments about the (A-B) tensile test, (C-E) flexibility test of raffia.