Electronic Supplementary Information

Enhanced Bone Morphogenic Property of Parylene-C

Ya-Ting Tsai‡, Chao-Wei Huang‡, Hui-Yu Liu, Mei-Ching Huang, Ting-Pi Sun, Wen-Chien Chen, Chih-Yu Wu, Shih-Torng Ding*, Hsien-Yeh Chen**

Department of Chemical Engineering, National Taiwan University, Taipei, 10617 (Taiwan)

Department of Animal Science and Technology, National Taiwan University, Taipei 10672(Taiwan)

*To whom correspondence should be addressed: hsychen@ntu.edu.tw (H.-Y. Chen);

sding@ntu.edu.tw (S.-T. Ding); surgeonmonster@gmail.com (W.-C. Chen)

Fabrication and characterizations of PEG surface

PEG modified surfaces were prepared following previous reported procedures [Wu et al. *Macromolecular Rapid Communications* 2012, *33*, 922-927]. Briefly, a vinyl-functionalized parylene coating (a polymer system similar to parylene-C) surface was used to react with 50 μg·ml^{-1} thiol-terminated PEG (Sigma Aldrich, USA) via vinyl-thiol coupling reaction at room temperature (25° C) for 6 hrs. A rinse process was performed three times by deionized water to remove the unreacted PEG. The resulting PEG-modified surface formed a rather hydrophilic surface with a water contact angle of 24 ± 0.8 degree, and the PEG layer thickness was approximately 3 nm as characterized by using a ellipsometry (EP^3-SW, Nanofilm Technologie GmbH, Germany). The chemical composition of the PEG-modified surface was further analysed by using IRRAS, and a strong signal of characteristic *O-H* band (3200-3600 cm^{-1}) was detected in the spectrum of such PEG-modified surface, as indicated in Figure S1.

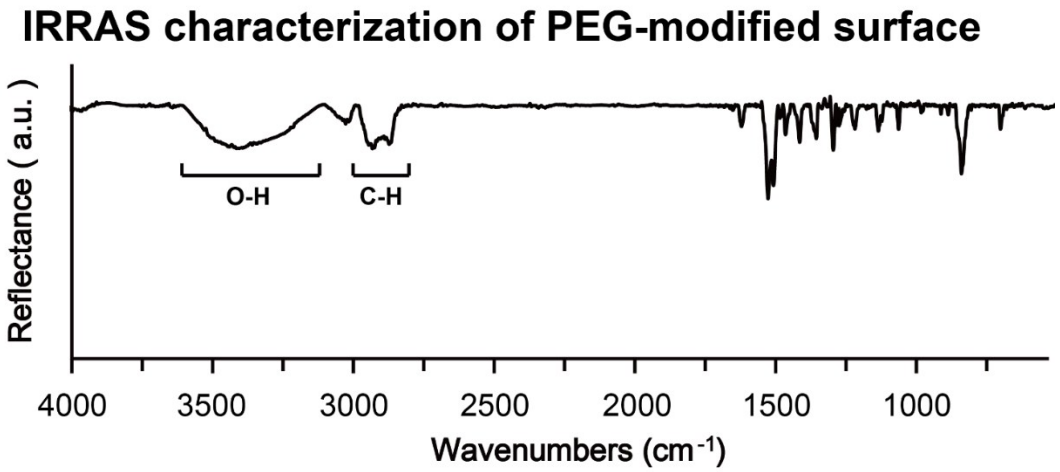

Figure S1. IRRAS spectra of PEG-modified surface. The characteristic bond *O-H* (3200-3600 cm^{-1}) were detected, indicating the presence of PEG on PEG-modified surface.

Surface roughness analysis

The protein surface characterizations by atomic force microscopy (AFM) were conducted using a MultiMode 8 instrument (Bruker, USA) capable of peak force tapping mode in liquids. The data were acquired using silicon nitride tips (Bruker, USA) with a tip radius of 12 nm and a spring constant of 0.04 N/m. Modulus images were recorded using a loading force of 0.3-0.5 nN and were collected on a 500 × 500 nm matrix area. During AFM analysis, the samples were placed in the analysis chamber in PBS (pH 7.4) at 20°C.

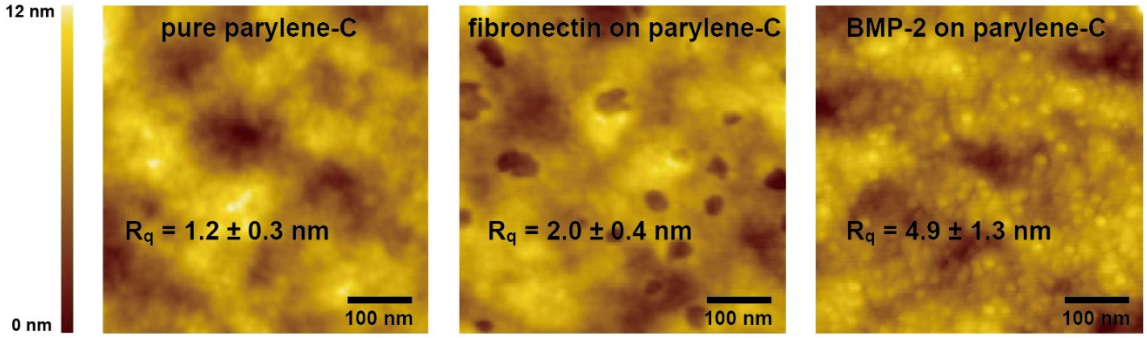

Figure S2. Surface roughness analysis by AFM for pure parylene-C surface, adsorption of fibronectin on parylene-C and adsorption of BMP-2 on parylene-C. The root-mean-square roughness (Rq) of 1.2 ± 0.3 nm, 2.0 ± 0.4 nm and 4.9 ± 1.3 nm was measured for parylene-C, fibronectin and BMP-2, respectively.

Stability of adsorbed protein layer

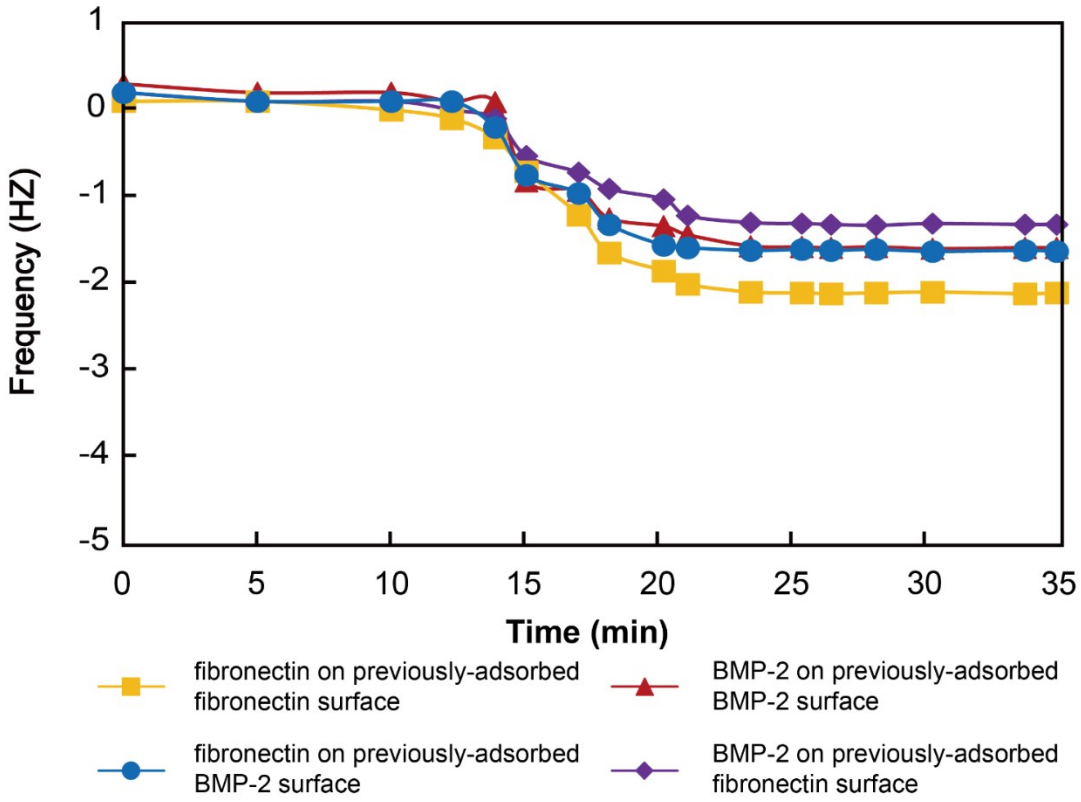

Figure S3. QCM analysis of adsorption stability by introducing secondary BMP-2 or fibronectin proteins to the first adsorption surfaces of BMP-2 or fibronectin. Low frequency changes were detected irrespective to the protein types on the adsorption surfaces of BMP-2 and fibronectin.

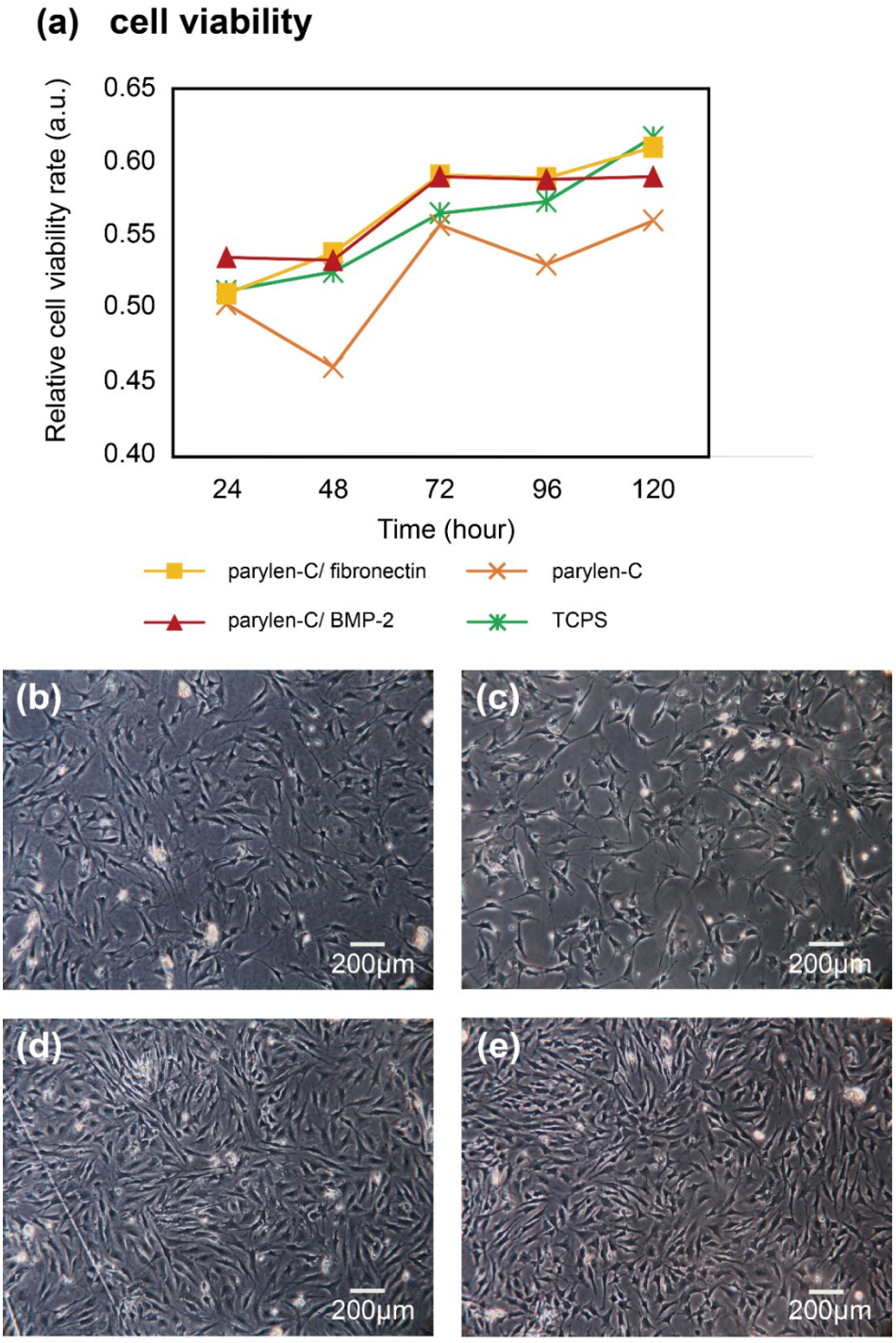

Figure S4. (a) The viability of pADSCs cultured for up to 120 hours on the experimentalfibronectin- and BMP-2-modified surfaces and the control TCPS and parylene-C surfaces. The cell growing number was recorded and monitored every 24 hours by using MTT assay to determine viable cells on the studied surfaces. Images of pADSC growth patterns after 120 hours of culture on (b) TCPS, (c) pure parylene-C, (d) fibronectin, and (e) BMP-2.

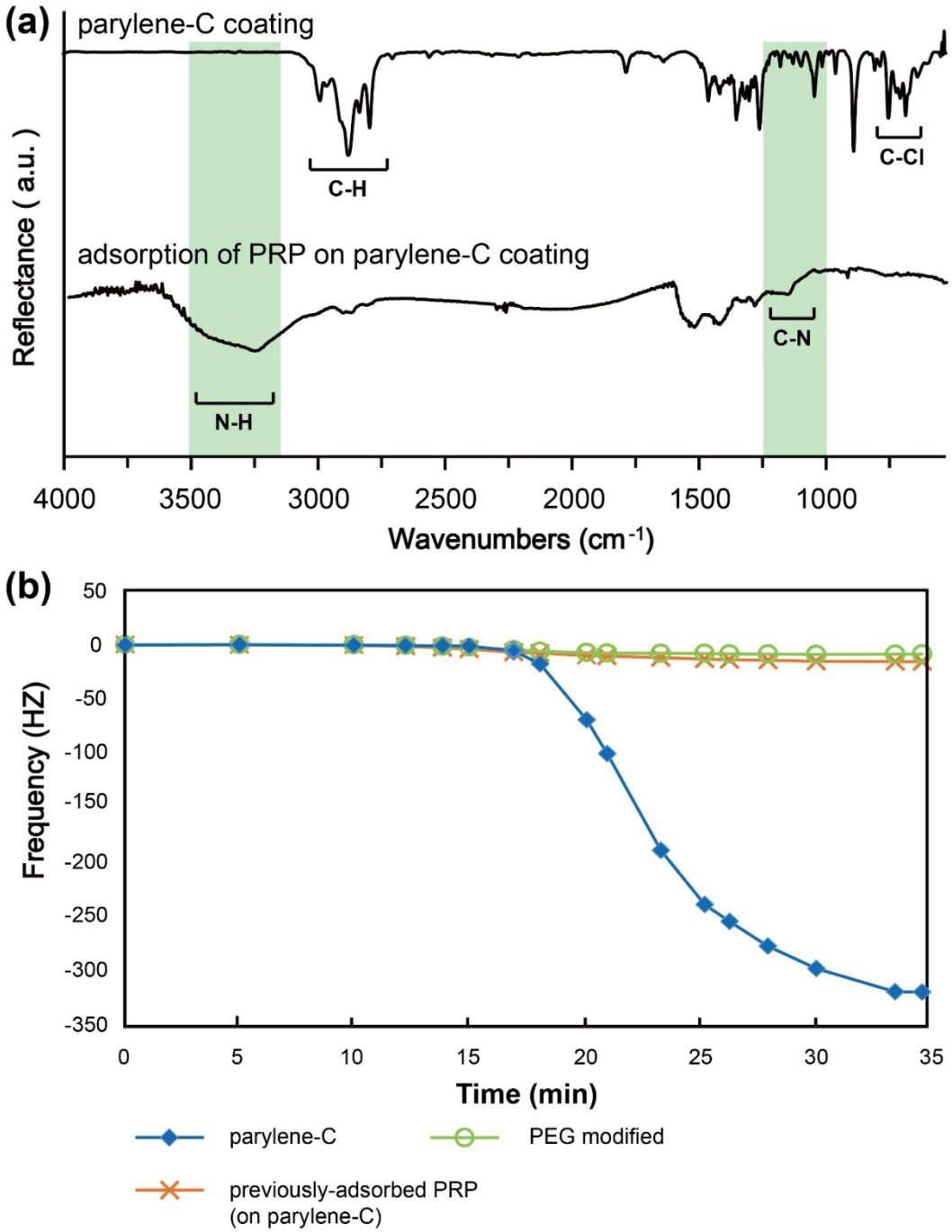

Figure S5. (a) IRRAS spectra of adsorbed PRP on parylene-C surfaces. Characteristic N-H and C-N bands were detected at 3300-3500 cm^{-1} at 1000-1250 cm^{-1}, respectively, indicating the presence of immobilized PRP on the parylene-C surfaces. (b) Dynamic QCM analysis of PRP adsorption onto parylene-C surfaces. Pronounced frequency changes were observed, stabilizing at 340.4 ± 11.9 Hz and indicating the saturated and irreversible adsorption of PRP onto the surface.