Electronic Supplementary Information

Analytical Investigation of Nano-Bio Interfacial Protein Mediation for Fibroblast Adhesion on Hydroxyapatite Nanoparticles

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Scheme S1



Scheme S1 Illustration of the QCM-D measurement procedure for the FBS protein adsorption, cell adhesion and subsequent other characterization methods in this study.

Scheme S2



Scheme S2 (a) Illustration of the interfacial measurement height (*I*) of the cell adhesion and protein adsorption layers on HAp or Pox using a QCM-D technique. (b) Illustration of the measurement parts of the inner (red-color square mark) and outer (blue-color square mark) surfaces of the cell using a localized FT-IR spectroscopy, which were captured as the aperture size of $50 \times 50 \ \mu\text{m}^2$ and screen size of $359 \times 476 \ \mu\text{m}^2$.



Fig. S1 Δf (red line) and ΔD (blue line) curves with the adsorption of (a, c) Anti-BSA and (b, d) Anti-VN on (a, b) FBS-HAp and (c, d) FBS-PSox and subsequent buffer wash by PBS.



Fig. S2 AFM (a) topographic and (b) phase-shift images of the FBS-PSox.



Fig. S3 (a, b) Δf (red line) and ΔD (blue line) curves with the cell adhesion on (a) HAp and (b) Psox, and (c, d) $\Delta D - \Delta f$ plots with the cell adhesion on (c) FBS-HAp and HAp and (d) FBS-Psox and Psox (cell seeding concentration: 10.0×10⁴ cells/mL).



Fig. S4 (a) $\Delta f - \Delta N$ and (b) $\Delta D - \Delta N$ plots of the cells adhered on FBS-HAp (•) and FBS-PSox (\circ). There were linear relationships between Δf and ΔN , and ΔD and ΔN . The values of $\Delta f / \Delta N$ and $\Delta D / \Delta N$ were -6.3×10^{-3} (*R*=0.93) and 6.1 × 10⁻³ (*R*=0.99), and -10.8×10^{-3} (*R*=0.99) and 16.3 × 10⁻³ (*R*=0.98) for FBS-HAp and FBS-PSox, respectively.



Fig. S5 (a) ΔN , (b) area and (c) volume changes of the cells adhered on FBS-HAp (•) and FBS-PSox (\circ) with the culture time.



Fig. S6 CLSM images of the morphological observation of the cells adhered on (a, b) FBS-HAp and (c, d) FBS-PSox in the different positions at the culture time of 120 min.



Fig. S7 CLSM images of the morphological observation of the cells adhered on (a) FBS-HAp, (b) FBS-PSox, (c) HAp and (d) Psox at 120 min. (e) ΔN and (f) area of the cell adhered on the surfaces. ** indicates P < 0.01.

Table S1

Table S1 The ascription of the localized FT-IR spectral absorption bands of the cells adhered onFBS-HAp.

Wavenumber (cm ⁻¹)	Ascription
3500, 3300 cm ⁻¹	Hydrogen bonded N-H, N-H stretching of amide A
2960, 2925, 2853 cm ⁻¹	Alkyl chain C-H stretching
1745 cm ⁻¹	Stretching vibrations of -COOH or H2O
1658 cm ⁻¹	Amide I (C=O stretching)
1546 cm ⁻¹	Amide II (N-H bending/C-N stretching)
1460, 1395 cm ⁻¹	Scissoring of CH_2 wag, $\upsilon_s COO^-$ stretching
1262 cm ⁻¹	Amide III (N-H deformation)
1235 cm ⁻¹	Phospholipids in cell membrane (P=O)
1175 cm ⁻¹	Presence of alcohol groups (C-OH)

Table S2

Table S2The ascription of the localized FT-IR spectral absorption bands of the cells adhered onFBS-PSox.

Wavenumber	Attribution
3500, 3300 cm ⁻¹	Hydrogen bonded N-H, N-H stretching of amide A
2960, 2873 cm ⁻¹	Alkyl chain C-H stretching
1660 cm ⁻¹	Amide I (C=O stretching)
1540 cm ⁻¹	Amide II (N-H bending/C-N stretching)
1395 cm ⁻¹	υ _s COO ⁻ stretching
1262 cm ⁻¹	Amide III (N-H deformation)
1170 cm ⁻¹	Presence of alcohol groups (C-OH)
1080 cm ⁻¹	Psox (C-O stretching)



Fig. S8 FLM images of the cells adhered on (a) FBS-HAp and (b) FBS-PSox at the culture time of 120 min. The cells were stained with the polyclonal rabbit anti-mouse collagen type I α_2 chain as a primary antibody and the FITC-labeled IgG as a secondary antibody.