Supporting Information

The Effect of Chirality on Cell Spreading and Differentiation: From Chiral Molecules to Chiral Self-Assembly

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Author Contributions

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1. Materials and Synthesis

1.1 Materials

\(L/D\)-phenylalanine methyl ester hydrochloride, 1,4-benzenedicarbonyl dichloride and diglycol were purchased from Aladdin Chemicals and used without further purification. Tablets of phosphate buffered saline (PBS) and Hoechst 33258 were purchased from Sigma-Aldrich. Paraformaldehyde (PFA) and Triton X-100 were purchased from VWR. Phalloidin-rhodamine, Trypsin-EDTA, binding buffer for apoptosis assay, Annexin V-FITC, and propidium iodide (PI) were purchased from Invitrogen, Life Technologies. Fluorescein isothiocyanate labeled bovine serum albumin (BSA-FITC) was purchased from Beijing Solarbio Science & Technology Co., Ltd. The cell counting assay kit (CCK8) was purchased from Dojindo, Japan. Throughout the whole study, water was purified using a Millipore Direct Q8 system (Millipore Advantage A10 system, Schwalbach, with Millimark Express 40 Filter, Merck, Germany) affording water with a resistivity of 18.2 MΩ cm.

1.2 Synthesis and Characterization of L(D)PFEG

L(D)PFEG was synthesized following a previously published procedure. 1,4-benzenedicarbonyl dichloride (2.6 g, 13.0 mmol) in dichloromethane (DCM) (20 mL) was added to a solution of \(L/D\)-phenylalanine methyl ester hydrochloride (6.0 g, 26.1 mmol) and triethylamine (Et\(_3\)N) (8 mL, 58.3 mmol) in DCM (100 mL). The solution was stirred at room temperature for 24 h. All the solvents were evaporated under vacuum, and the residue was subsequently dissolved in ethanol (100 mL). After filtration, the undissolved substance was collected and dried to give the dimethyl ester of Phe\(_{L/D\text{-Phe-OH}}\)) (yield: 84%). For the hydrolysis, aqueous NaOH (2.0 M, 10 mL) was added to a
cooled (0 °C) suspension of the dimethyl ester of Phe\(_{\text{L/D}}\)-Phe-OH\(_2\) (3.0 g, 6.1 mmol) in methanol (MeOH) solution (20 mL). The mixture was slowly brought back to room temperature and stirred for 24 h, and a clear solution was obtained. The solution was then acidified with 3.0 M HCl to pH < 3.0, and a gel-like precipitate formed. The gel phase was filtered, washed with deionized water and finally dried to give Phe\(_{\text{L/D}}\)-Phe-OH\(_2\) (yield: 91%). Phe\(_{\text{L/D}}\)-Phe-OH\(_2\) (2.8 g, 6.1 mmol) in diglycol (80 mL) added with concentrated HCl (0.5 mL) was stirred at 145 °C for 3.5 h, then the clear solution was added to a water/ice mixture (350 mL). The gel-like precipitate was collected on a filter, washed with deionized water, and dried to give L(D)PFEG (yield: 77%). Overall yield: 59%.

\(^1\)H NMR (400 M Hz, DMSO-\text{d}_6, \delta): \delta = 3.1 \text{ (m, 4H, CH}_2\text{)}, 3.4 \text{ (m, 16H, CH}_2\text{)}, 4.2 \text{ (q, 2H, OH)}, 4.7 \text{ (q, 2H, CH)}, 7.3 \text{ (m, 10H, Ar H)}, 7.8 \text{ (s, 4H, Ar H)}, 8.9 \text{ (d, 2H, NH)} \text{ ppm. EI-MS for L(D)PFEG calcd. 636.71; found 637.28 [M + H]}.+

**Scheme S1.** Synthesis procedures of L(D)PFEG.
2. Materials Characterization

**H Nuclear Magnetic Resonance (H NMR) Spectroscopy:** H NMR studies were carried out on a Bruker Advance III 400 Instrument (operating at 400 MHz). All spectra were recorded in DMSO.

**Mass Spectra:** Mass spectra were recorded on a Waters Q-Tof Mass Instrument by positive mode electrospray ionization. Methanol was used as solvent.

**Atomic Force Microscopy (AFM) Study:** AFM images were obtained using Vecco NanoScope IIIa Atomic Force Microscope and Mikro Masch NSC11 cantilevers/tips (radius of curvature less than 10 nm). Surface roughness was measured by Nanoscope 5.30r3sr3 software.

**Fourier Transform Infrared (FT-IR) Spectroscopy:** FT-IR spectra of L(D)CA and L(D)UA dried powders were taken using Thermo SCIENTIFIC NICOLET iS5 Instrument. The KBr disk technique was used for the solid-state measurement. The samples were then scanned between the wavelength of 4000 and 400 cm⁻¹ at an interval of 1.9285 cm⁻¹.
3. Supplementary Tables and Figures

**Table S1.** Primers used in RT-qPCR experiments.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tr>
<td>RUNX2</td>
<td>CCCAGAAACTGAGAAAACCTCAAC</td>
<td>TGTGGACTTCTCTGCTCAT</td>
</tr>
<tr>
<td>BMP2</td>
<td>ACCAGAAACGAGTGGGAA</td>
<td>AGCTCTGCTGAGGTGATAA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>CCTCACAGGTTGCCATGTAGA</td>
<td>TGGTACATGACAAGGTGCG</td>
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**Figure S1.** Photographic images of free standing L(D)PFEG hydrogel and MeOH solution of L(D)PFEG.
Figure S2. AFM images of (a) LCA with left-handed helical nanofibers, (b) DCA with right-handed helical nanofibers, (c) LUA, and (d) DUA with unordered aggregates after drying (scan size: 5 μm × 5 μm). Concentrations of all samples were 0.5 mg mL⁻¹.

Figure S3. SEM images of (a) LCA with left-handed helical nanofibers, and (b) DCA with right-handed helical nanofibers. Concentrations of both samples were 0.1 mg mL⁻¹.
Figure S4. RMS surface roughness value of L(D)CA and L(D)UA films, respectively. Error bar: standard error (n=5).

Figure S5. FTIR spectra of dried L(D)CA and L(D)UA powders.
Scheme S2. Schematic illustration of LPFEG and DPFEG self-assembling into chiral fibers through π-π stacking and hydrogen bonding in H$_2$O.

Figure S6. (a) CD spectra of L(D)UA (0.5 mg mL$^{-1}$) in ethanol. (b) Solid state CD spectra of L(D)UA films prepared by evaporating ethanol.

<table>
<thead>
<tr>
<th></th>
<th>LCA</th>
<th>LUA</th>
<th>DUA</th>
<th>DCA</th>
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<tbody>
<tr>
<td></td>
<td>24.8° ± 2.6°</td>
<td>27.1° ± 2.0°</td>
<td>26.7° ± 2.9°</td>
<td>24.1° ± 3.0°</td>
</tr>
</tbody>
</table>

Figure S7. Static water contact angles of LCA, DUA, LUA, and DCA dried films.
Figure S8. Optical microscopy images of NIH-3T3 fibroblasts after 5 days culture on LCA, LUA, DUA, DCA films, and TCPS surface, respectively.
**Figure S9.** Fluorescence microscopy images of NIH-3T3 fibroblasts after 1 day, 3 days, and 5 days culture on LCA, LUA, DUA, DCA films, and TCPS surface, respectively. Actin and the cell nuclei were stained red and blue, respectively.
**Figure S10.** SEM images of fixed NIH-3T3 fibroblasts after 5 days culture on (a) LCA, (b) DCA, (c) LUA, and (d) DUA films, respectively.
Figure S11. Optical microscopy images of PaTu 8988t cells after 1 day, 3 days, and 5 days culture on LCA, LUA, DUA, DCA films, and TCPS surface, respectively.
Figure S12. Fluorescence microscopy images of PaTu 8988t cells after 1 day, 3 days, and 5 days culture on LCA, LUA, DUA, DCA films, and TCPS surface, respectively. Actin and cell nuclei were stained red and blue, respectively.
**Figure S13.** SEM images of fixated PaTu 8988t cells after 5 days culture on (a) LCA, (b) DCA, (c) LUA, and (d) DUA films, respectively.

**Figure S14.** (a) Average spreading area per cell for PaTu 8988t cells after 1 day, 3 days, and 5 days culture on LCA, LUA, DUA, DCA films, and TCPS surface, respectively. Error bars: standard error (n = 20). ns means not significant (p > 0.05). *p < 0.05, **p < 0.01, and ***p < 0.001. (b) CCK-8 assay results of PaTu 8988t cells cultured on LCA, LUA, DUA, DCA films, and PS surface respectively. Error bars: standard error (n = 3). ns means not significant (p > 0.05). *p < 0.05, **p < 0.01, and ***p < 0.001. After culture for 5 days, both the cell-spreading area and cell-proliferation
density on LUA films were 1.1~1.2 times as high as those on the DUA films and on LCA films they were 1.4~1.5 times as high as those on DCA films.

**Figure S15.** Calibration curve of BSA.

**Figure S16.** The representative results of flow cytometry analysis for apoptosis of DPSCs at day 3 on (a) LCA, (b) LUA, (c) DUA, (d) DCA, and (e) TCPS surfaces, respectively. (f) Quantitation of apoptosis for different materials from the flow cytometry analysis. Error bars: standard error (n = 3).
Figure S17. Optical microscopy images of DPSCs after 1 day culture on (a) LCA, (b) LUA, (c) DUA, (d) DCA films, and (e) TCPS surface, respectively.
Figure S18. Optical microscopy images of DPSCs cells after 1 day culture in DMEM with serum (10% FBS) and without serum, respectively.
**Figure S19.** Optical microscopy images of DPSCs cells after 3 day culture in DMEM with serum (10% FBS) and without serum, respectively.
Figure S20. Optical microscopy images of DPSCs cells after 5 day culture in DMEM with serum (10% FBS) and without serum, respectively.
Figure S21. Average spreading area per cell for DPSCs after 1 day culture in DMEM without serum.

Error bars: standard error (n = 20). ns means not significant (p > 0.05).

Figure S22. Optical microscopy images of T47D cells after 1 day culture in DMEM with serum (10% FBS) and without serum, respectively.
Figure S23. Optical microscopy images of T47D cells after 3 day culture in DMEM with serum (10% FBS) and without serum, respectively.
Figure S24. Optical microscopy images of T47D cells after 5 day culture in DMEM with serum (10% FBS) and without serum, respectively.
Figure S25. ALP staining images of DPSCs after 7 days culture on (a) LCA, (b) LUA, (c) DUA, (d) DCA films, and (e) TCPS surface, respectively.