Supporting Information.

Improving internal cell colonization of porous scaffolds with chemical gradients produced by plasma assisted approaches

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Supporting information.

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1. Preparation of flat PCL

Flat PCL disks (flat PCL), used as reference materials, were produced by dissolving 1g of PCL in 6 ml CHCl₃. The viscous solution was spin coated (Bruwer CEE200 Spinner Coater, 2 min at 2000 rpm, then 1 min at 3200 rpm) on PolyEthylene Terephthalate discs (Goodfellow, UK; 0.5 mm thick, 12 mm dia.), that were first dipped in ethanol at RT for phase separation and solvent elimination and finally rinsed in dd water.

2. Positioning of the samples in the plasma reactor.
Figure S1. Samples positioned in a) glow and b) downstream during plasma processing between the ground and RF electrode placed on a stainless steel grid. The pumping system is connected to the reactor on the side below the grid.

3. Chemical characterization of native and plasma modified flat PCL.

Table S1. Comparison of chemical composition of native and plasma modified flat PCL samples. The B.E. reported have an error of ±0.2 eV. All the values reported are expressed as % except for O/C ratio.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C (%)</th>
<th>O (%)</th>
<th>O/C</th>
<th>C=O (H) 285.0 eV</th>
<th>C-COOR 285.5 eV</th>
<th>C-OR 286.5 eV</th>
<th>C=O 287.9 eV</th>
<th>O-C=O 289.2 eV</th>
<th>COOR 289.2 eV</th>
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</thead>
<tbody>
<tr>
<td>Native PCL</td>
<td>77.3±0.1</td>
<td>22.7±0.2</td>
<td>0.29±0.03</td>
<td>46.0±0.2</td>
<td>19.0±0.3</td>
<td>19.0±0.1</td>
<td>---</td>
<td>15.0±0.3</td>
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</tr>
<tr>
<td>PEO1</td>
<td>71.3±0.1</td>
<td>28.7±0.2</td>
<td>0.40±0.03</td>
<td>22.5±0.2</td>
<td>---</td>
<td>70±0.2</td>
<td>7.5±0.2</td>
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<tr>
<td>PEO2</td>
<td>77±0.1</td>
<td>23±0.1</td>
<td>0.30±0.01</td>
<td>31±0.1</td>
<td>---</td>
<td>59.7±0.2</td>
<td>9.3±0.2</td>
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<tr>
<td>Ox</td>
<td>67.6±0.2</td>
<td>32.4±0.1</td>
<td>0.48±0.01</td>
<td>56.7±0.2</td>
<td>13.3±0.2</td>
<td>10.8±0.2</td>
<td>8.6±0.2</td>
<td>10.6±0.3</td>
<td></td>
</tr>
<tr>
<td>Ox+PEO1</td>
<td>71.3±0.2</td>
<td>28.7±0.2</td>
<td>0.40±0.01</td>
<td>17.3±0.1</td>
<td>---</td>
<td>72.2±0.2</td>
<td>10.5±0.2</td>
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<tr>
<td>Ox+PEO2</td>
<td>73.5±0.1</td>
<td>26.5±0.1</td>
<td>0.36±0.01</td>
<td>33.8±0.2</td>
<td>---</td>
<td>57.6±0.2</td>
<td>8.6±0.2</td>
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</tbody>
</table>

Figure S2 shows best-fitted C1s and O1s XPS spectra of native and plasma modified flat PCL samples. PEO2 coatings and native PCL materials show a totally different shape of C1s spectra despite the similar O/C ratios acquired on them (Table S1).
Figure S2 XPS C1s spectra of flat materials: native PCL (a), PEO1 (b) and PEO2 (c). The overlap of O1s spectra (d) for native PCL (dark gray, d) and PEO1 samples (light gray, d) is also reported.

The C1s peaks acquired on PEO2 and PEO1 coatings revealed a shift in the maximum, from 285.0 to 286.5 eV. A stronger contribution of this peak, around 70%, is found for PEO1 coatings compared to PEO2 ones (Figure S2b-c and Table S1), in agreement with the higher O/C ratio for the PEO1. The overlap of O1s spectra for native flat PCL (dark grey line, Figure S2d) and PEO1 coating (gray line, Figure S2d) confirms the findings reported: a double peak attributed to the ester (carboxylic) moieties for native PCL, and a single peak centered at 532.8 eV, typical of ether groups for PEO1 coated samples.

No evidence of the PCL underneath (e.g., COOR groups in the C1s spectra) was observed, attesting for the uniformity of the PEO-like coatings on planar PCL.

4. Wettability Measurements
In case of flat PCL the plasma modified samples are more hydrophilic than the native substrate. PEO2 and Ox+PEO2 samples are slightly more hydrophobic than respectively PEO1 and Ox+PEO1 samples. Ox samples are the most hydrophilic substrates among the tested groups.

Figure S3 Advancing and receding WCA values measured on native (flat PCL and 3D-PCL) and plasma modified flat (a) and 3D (b) PCL.

5. Cell adhesion on PEO1 and PEO2 plasma modified flat PCL.

The presence of a coating with PEO-character around 70% on the flat PCL substrates (table S1 and Figure S2) produces a clear cell- repulsive effect against SAOS2 cells, as reported in Figure S4.
Figure S4 Adhesion of SAOS 2 cells (fixed with PFA 4% in PBS after 120h of culture and stained by Comassie blue) on PEO1 (a) and PEO2 (b) coated flat PCL samples. fixed with PFA 4% in PBS after 120h of culture. The cell-repulsive effect of PEO1 flat surfaces is evident, as well as the cell-adhesive character of flat PEO-2 surfaces.

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